Veterinärmedizinische Universität Wien

Graf-Lehndorff-Institut für Pferdewissenschaften (Leiterin: Ao.Univ.-Prof. Dr.med.vet. Christine Aurich, Dipl.ECAR)

Peripartum endocrine and cardiac changes in primiparous and pluriparous mares

Diplomarbeit

Veterinärmedizinische Universität Wien

vorgelegt von

Carina Leitner

Wien, Mai 2020

Betreuerin

Priv.-Doz. Dr.med.vet. Christina Nagel, Dipl.ECAR

Graf-Lehndorff-Institut für Pferdewissenschaften

Gutachterin

Ao.Univ.-Prof. Dr.med.vet. Sabine Sykora

Department/Universitätsklinik für Kleintiere und Pferde Universitätsklinik für Pferde Pferdechirurgie Veterinärmedizinische Universität Wien

Table of contents

1. Introduction	1
2. Material and methods	3
2.1. Ethical approval	3
2.2. Animals	3
2.3. Experimental design	3
2.4. Hormone analysis	4
2.5. Heart rate, heart rate variability parameters and cardiac arrhythmias	4
2.6. Statistical analysis	5
3. Results	6
4. Discussion	14
5. Summary	17
6. Zusammenfassung	18
7. References	20

1. Introduction

The sex ratio of the offspring at birth is in part influenced by the uterine environment at conception and during early pregnancy (reviewed by Aurich and Schneider, 2014). Primiparous and aged mares are more likely to produce female foals than middle-aged brood mares (Kuhl et al., 2015). Female horse embryos adapt better to suboptimal uterine conditions which are found in primiparous mares. These suboptimal conditions are less frequent in pluriparous mares (Aurich and Schneider, 2014; Kuhl et al., 2015). In primiparous mares, not only the sex ratio deviated from the expected 50% male and female foals, the foals born to primiparous mares were also lighter and smaller at birth and during their first year of life compared to foals of pluripara (Meirelles et al., 2017; Robles et al., 2019). Thus, substantial differences exist between pregnancies of pluriparous and primiparous mares. These are based on placental structure, maternal metabolism and nutritional supply to the fetus and affect fetal development (Wilsher and Allen, 2003; Robles et al., 2018a; 2019). Placental weight and volume are lower in primiparous than in pluriparous mares (Wilsher and Allen, 2003; Meirelles et al., 2017; Robles et al., 2018a). This is directly correlated to foal birth weight. A one-kg-increase in placental weight increased foal birth weight by 3 kg and foals height by 2 cm (Meirelles et al., 2017). Differences in foal birth weight obviously exist in foals of small and large horse breeds, although foal birth weight is always approximately 10% of the mare's body weight (Beythien et al., 2017; Nagel et al., unpublished). In contrast, foal birth weight in relation to placental weight was lower in small compared to large breed foals, indicating a lower placental efficiency in smaller breeds (Nagel et al., unpublished). This was accompanied by a sympathetic activation suggesting a certain stress response during the last days of gestation in small breed mares (Nagel et al., unpublished). Suboptimal conditions in primiparous pregnancies may also result in suboptimal nutrient supply of the fetus and thus a higher burden of the dam to compensate the unfavourable situation. The autonomous nervous system can be assessed by analysis of heart rate and heart rate variability which provide information on fetal and maternal well-being during pregnancy and parturition (Von Borell et al. 2007; Nagel et al., 2010; 2011a; 2012; 2014; 2015). In addition, increased progestin concentrations during late gestation are a sign of premature enlargement of the fetal adrenal glands initiated by fetal distress (Ousey et al., 2005).

The aim of this study was to investigate antepartum endocrine changes in the mare and fetomaternal cardiac parameters in physiological pregnancies of primiparous and pluriparous Warmblood mares. The study followed the hypothesis that in primiparous mares the maternal

burden during the last weeks of gestation is higher than in pluriparous mares and this is reflected in hormonal and fetomaternal cardiac parameters differences.

2. Material and methods

2.1. Ethical approval

The study was approved by the competent authority for animal experimentation in Brandenburg State, Germany (State Ministry for Rural Development, Environment and Agriculture, license no. 2347-A-5-1-2018).

2.2. Animals

A total of 14 late pregnant Warmblood brood mares with singleton pregnancies of the Brandenburg State Stud in Neustadt (Dosse), Germany, were included into the study. Age of mares at the time of foaling was 4 ± 0 years in primiparous mares (n=7) and 11 ± 2 years in pluriparous mares (n=7). Pluriparous mares had on average 7 ± 1 previous foals (range 3-12) whereas primiparous mares carried their first foal (p<0.001). Mares were housed in straw-bedded group stables and were fed oats, mineral supplements and hay twice daily to their individual requirements and water was freely available. Mares had daily access to an outdoor paddock together with their group mates. Approximately 14 days before the calculated day of parturition, mares were housed in single boxes in the stud's foaling unit where they were observed 24 hours per day. A veterinarian attended parturition, but no obstetrical intervention was needed. All foals underwent a clinical examination immediately after birth and on the first day of life and were classified as healthy and mature. Eighteen hours after birth, all foals had an IgG concentration in blood >800 mg/dl (Snap Test, Idexx, Hoofddorp, The Netherlands). All mares and their foals were healthy throughout the study.

2.3. Experimental design

During the last two weeks before the expected day of foaling, 30 min fetomaternal electrocardiogram (ECG) recordings were made (Televet 100 system; version 5.1.1; Engel Engineering, Heusenstamm, Germany) as described previously (Nagel et al., 2010) and a blood sample was taken daily between 6 and 8 a.m. Blood was collected from one jugular vein into heparinized tubes (Vacuette, Greiner, Kremsm nster, Austria). After collection, blood samples were centrifuged at 1200 g for 10 min, plasma was decanted and frozen at -20°C until analysis. During parturition, fetal and maternal heart rate was recorded continuously with the Televet 100 device. After birth of the foal, maternal ECG recording was continued for two hours and foal heart rate (HR) was recorded with a portable system (V800,

Polar, Kempele, Finland) as described (Erber et al., 2012). Duration of parturition, time to first suckling of the foal and time to passage of fetal membranes were recorded. After foaling and before first suckling colostrum quality was determined by refractometer (Cash, 1999). Placental weight was recorded directly after shedding and the placental surface area was measured as described (Allen et al., 2002). The gestation length, the foal sex ratio and the time to placenta shedding were also analysed. Between 6 and 12 h after parturition, all mares and foals were weighed. In foals, height at withers (determined with a measuring stick and by measuring tape), circumference of the cannon bone, chest circumference, distance fetlock to carpal joint, distance carpal joint to elbow, crown-rump length and poll-to-nose length were determined. All measurements were made as described (Allen et al., 2004). Gestation length was calculated from the day of ovulation to the day of foaling.

2.4. Hormone analysis

Maternal plasma progestin and cortisol concentration were analysed in blood samples taken on days five to one before and day one after parturition. Cortisol was determined as described previously with an enzyme immunoassay (Demeditec Diagnostics, Kiel, Germany) validated for equine plasma in our laboratory (Kuhl et al., 2016). The intra-assay and interassay coefficients of variation were 3.9 and 7.2%, respectively, and the minimal detectable concentration was 1.5 ng/mL. Concentration of progestins was determined with an enzyme immunoassay for progesterone (ADI-900-011, Assay Designs, Ann Arbor, MI, USA) validated for equine plasma in our laboratory as described previously (Nagel et al., 2012). The antiserum cross-reacts 100% with 5- α -pregnane-3,20-dione, thus besides progesterone measuring accurately the most important pregnancy-specific equine progestins. According to the manufacturer's information, cross-reactivity is 3.5% with 17-OHprogesterone and <1% for all other steroids tested. The intra-assay and the interassay coefficient of variation was 5.8 and 8.4%, respectively. The minimal detectable concentration of the assay was 5.8 ng/mL.

2.5. Heart rate, heart rate variability parameters and cardiac arrhythmias

For fetomaternal ECG recordings the Televet 100 device was attached to a belt fixed around the mare's thorax and self-adhesive electrodes were placed on the mares body as described (Nagel et al., 2010). The device was connected to a nearby computer by bluetooth. The Televet 100 ECG device displayed and stored the combined and the individual recordings of

the mare and fetus. The Polar system recorded and stored the data until it was read out at the computer. Both devices recorded beat-to-beat (RR) intervals. For analysis of HR and heart rate variability (HRV) parameters, the Kubios HRV Software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used. Electrocardiogram recordings from days 10, 6, 5, 4, 3, 2 and 1 before parturition were analysed. From all recordings, a five-minute sequence was taken to determine HR and the HRV variables standard deviation of the RR interval (SDRR) and root mean square of successive RR differences (RMSSD) in mares and fetuses. During parturition, HR and HRV were analysed in mares and fetuses for five-minute sequences starting at 120, 90, 60, 30, 5 minutes before and 0, 15, 30, 60, 90 and 120 min after birth of the foal. Additionally, the last 2 hours before and the first 2 hours after parturition were subdivided into 15-minute intervals, and all cardiac arrhythmias e.g. atrioventricular (AV) blocks were counted for each mare as described (Nagel et al., 2014). The ECG recording of one primiparous mare during parturition was not available due to technical problems.

2.6. Statistical analysis

Statistical analysis was made with the SPSS statistic software (version 25.0; SPSS-IBM, Armonck, NY, USA). Data were tested for normal distribution by Kolmogorov-Smirnov test. Occurrence of AV blocks in mares, number of foals and duration of parturition were not normally distributed and were analysed by Mann-Whitney-U-Test. Weight of mares, foals and placentas, gestation length, colostrum quality, time to first suckling, time to placenta shedding and size measurements in foals were normally distributed and analysed by Oneway ANOVA with group as between subject factor. Heart rate and HRV parameters did not show normal distribution and were log-transformed for further analysis. Data of HR, HRV, progestin and cortisol concentration were then analysed by ANOVA with a general linear model (GLM) for repeated measurements with time as within subject factor and group as between subject factor. A p-value <0.05 was considered significant. All values given are means ± standard error of mean (SEM).

3. Results

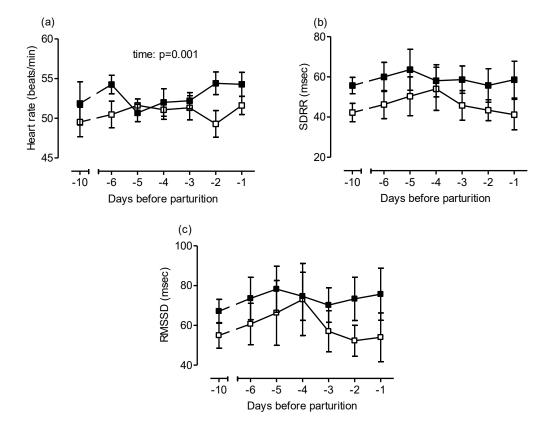


Figure 1: (a) Heart rate, (b) SDRR and (c) RMSSD in pluriparous (\blacksquare ; n=7) and primiparous mares (\square ; n=7) during the last 10 days before parturition. Significant differences are indicated in the figures.

Maternal heart rate changed during the last ten days before parturition but did not differ between pluriparous and primiparous mares (time p=0.001; Fig. 1a). Heart rate variability variables SDRR and RMSSD did neither change over time nor differed between groups (Fig. 1b,c).

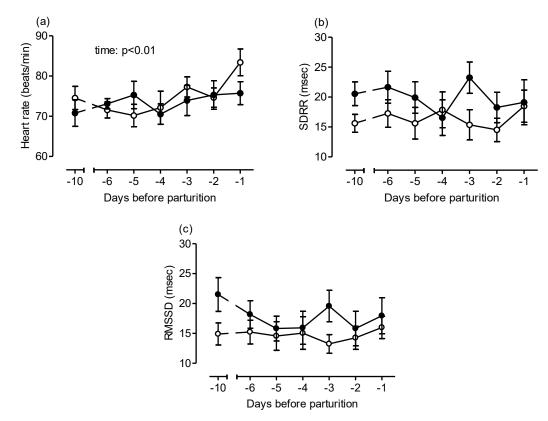


Figure 2: (a) Fetal heart rate, (b) SDRR and (c) RMSSD in pluriparous (\bullet ; n=7) and primiparous mares (O; n=7) during the last 10 days before parturition. Significant differences are indicated in the figures.

In fetuses of both groups, heart rate increased during the last days of gestation (time p<0.01; Fig. 2a). Fetal HRV variables SDRR and RMSSD did neither differ significantly between groups nor changed over time (Fig. 2b,c).

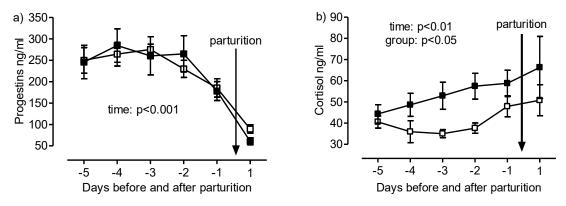


Figure 3: (a) Plasma progestin and (b) cortisol concentration in pluriparous (\blacksquare ; n=7) and primiparous mares (\square ; n=7) during the last 5 days before and on the day of parturition. Significant differences are indicated in the figures.

During the last five days before parturition plasma progestin concentration did not differ between primiparous and pluriparous mares (Fig. 3a). Progestin concentrations decreased one to two days before parturition (time p<0.001). Cortisol concentration in maternal plasma increased towards parturition in mares of both groups (time p<0.01; Fig. 3b). While this increase was continuous in pluriparous mares, cortisol concentration was at all times before parturition lower in primiparous mares and increased markedly only on the last day before parturition (group p<0.05).

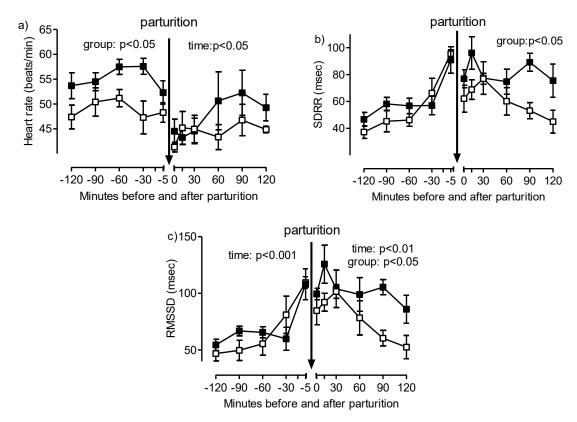


Figure 4: (a) Heart rate, (b) SDRR and (c) RMSSD in pluriparous (\blacksquare ; n=7) and primiparous mares (\square ; n=6) from 120 min before until 120 min after parturition. Significant differences before and after parturition are indicated in the figures.

During the last two hours before parturition maternal heart rate did not change but was at all times lower in primiparous than in pluriparous mares (group p<0.05; Fig. 4a). After parturition heart rate increased (time p<0.05) but did no longer differ between groups. Before parturition, the HRV variable SDRR neither changed over time nor differed between groups while RMSSD increased similarly in both groups (Fig. 4b,c). After birth of the foal, maternal SDRR and RMSSD were lower in primiparous than in pluriparous mares (group: p<0.05). While SDRR did not change over time, RMSSD decreased after parturition (time p<0.01).

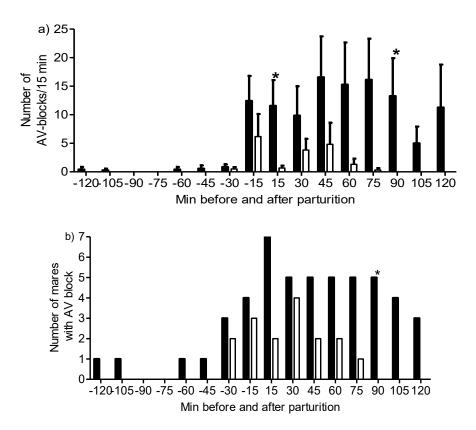


Figure 5: (a) Number of atrioventricular (AV) blocks per 15 min interval and (b) number of mares with AV blocks in pluriparous (\blacksquare ; n=7) and primiparous mares (\square ; n=6) from 120 min before until 120 min after parturition. Significant differences are indicated in the figures *(p<0.05).

From 120 to 45 min before birth of the foal, sporadic AV blocks occurred only in pluriparous mares. In primiparous mares, AV blocks were present during the last 30 min before and the first 75 min after the foals was born. Pluriparous mares showed more AV blocks than primiparous mares 15 and 90 min after the foal was born (p<0.05). During the first 15 min after birth of the foal 7/7 pluriparous mares and 2/7 primiparous mares showed AV blocks (Fig. 5a,b).

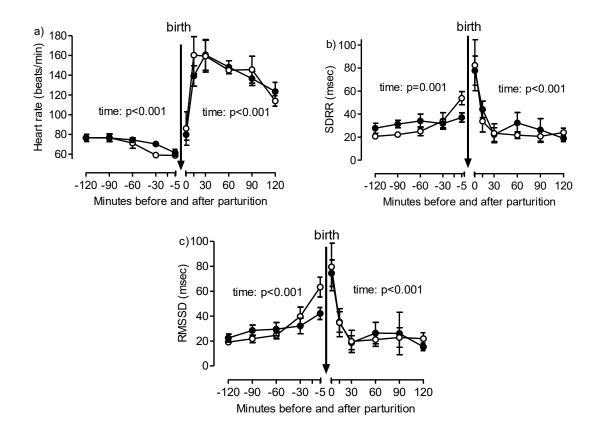


Figure 6: Fetal and neonatal (a) heart rate, (b) SDRR and (c) RMSSD in pluriparous (\bullet ; n=7) and primiparous mares (O; n=6) from 120 min before until 120 min after birth. Significant differences before and after birth of the foal are indicated in the figures.

In the last two hours before birth, fetal HR decreased slightly and then increased after birth in foals of both groups (time p<0.001, Fig. 6a). Neonatal HR reached maximum values during the first 30 minutes after birth and decreased thereafter. Fetal and neonatal SDRR and RMSSD increased towards the end of parturition, reached maximum values immediately after birth and decreased thereafter without differences between the two groups (over time both p=0.001; Fig. 6b,c)

	Pluriparous	Primiparous
Gestation length (days)	338 ± 2	345 ± 7
Foal sex ratio (female/male)	3/4	3/4
Duration of stage 2 of parturition (min)	10.6 ± 2.1	13.3 ± 1.4
Time to first suckling (min)	117.7 ± 11.5	120.0 ± 18.3
Time to placenta shedding (min)	55.0 ± 11.6	33.9 ± 4.4
Colostrum quality (Brix)*	26.4 ± 1.0	29.6 ± 1.0
[*] p<0.05 between groups		

Table 1: Gestation length, sex ratio and birth related parameters in pluriparous and primiparous mares and their newborn foals.

Gestation length, foal sex ratio, duration of the expulsive phase of foaling (stage 2 of parturition), time to first suckling and time to placenta shedding did not differ between primiparous and pluriparous mares. The Brix value of colostrum was higher in primiparous than in pluriparous mares (Table 1; p<0.05).

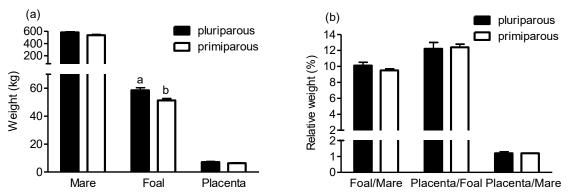


Figure 7: (a) Weight of mares, foals and placenta and (b) relative weight foal/mare, placenta/foal and placenta/mare in pluriparous and primiparous mares. ^{ab}p<0.01 between groups.

Weight of mares and placenta and placental surface area (pluriparous $13129 \pm 592 \text{ cm}^2$, primiparous $12357 \pm 373 \text{ cm}^2$) did not differ between groups but foals born to primiparous mares were lighter than foals from pluriparous mares (p<0.01, Fig. 7a). Relative weight (%) foal/mare, placenta/foal and placenta/mare did not differ between primiparous and pluriparous mares (Fig. 7b).

	Pluriparous	Primiparous
Height at withers (stick; cm)*	106.5 ± 1.1	102.4 ± 1.2
Height at withers (tape; cm)***	113.1 ± 1.1	107.7 ± 0.7
Chest circumference (cm)*	87.5 ± 1.4	83.0 ± 0.9
Circumference of cannon bone (cm)	13.7 ± 0.4	13.1 ± 0.3
Distance fetlock to carpal joint (cm)	27.5 ± 0.6	27.0 ± 0.3
Distance carpal joint to elbow (cm)	33.4 ± 0.7	31.9 ± 0.3
Crown-rump length (cm)**	101.3 ± 2.2	90.5 ± 1.4
Poll-to-nose length (cm)	43.2 ± 0.3	42.3 ± 0.6

Table 2: Size measurement from foals born to pluriparous and primiparous mares.

*p<0.05, **p<0.01, ***p≤0.001 between groups

Foals out of primiparous mares were significantly smaller in height (height at withers measured by stick, p<0.05; height at withers measured by tape, p=0.001; Tab. 2), chest circumference (p<0.05) and crown-rump length (p<0.01) than foals born to pluriparous mares. Foals did not differ in distance from fetlock to carpal joint, distance from carpal joint to elbow, circumference of cannon bone and poll-to-nose length.

4. Discussion

This study demonstrates differences in HR, HRV and AV blocks at foaling between primiparous and pluriparous mares. In mares foaling for the first time, the HRV in the immediate postpartum phase was reduced and AV blocks during and after expulsion of the foal were less frequent than in pluriparous mares. The short, expulsive phase of foaling is characterized by parasympathetic dominance of the autonomous nervous system as indicated by increased HRV and the occurrence of AV blocks (Nagel et al., 2014; 2016). At foaling and in particular directly after expulsion of the foal, the autonomous nervous system of primiparous mares is thus less under parasympathetic dominance than in pluriparous mares. In a recent study, it was demonstrated that increasing sympathoadrenal activity and a prolongation of the expulsive phase of foaling occur, when mares were exposed to a mild external stressor after rupture of the fetal membranes (Melchert et al., 2019). Although, sympathoadrenal activity at foaling was also increased in primiparous mares, the extent of sympathoadrenal activity apparently was too small to significantly prolong foaling.

Compared the phase of foal expulsion, differences between primiparous and pluriparous mares became more pronounced in the immediate postpartum phase, i.e. during the two hours after foaling. Postpartum, HRV decreased in primiparous but not in pluriparous mares and AV blocks occurred frequently in pluriparous but only sporadically in primiparous mares. In the immediate postpartum period, a certain stress response was thus presented in mares giving birth for the first time. This may predispose these mares to complications during the immediate postpartum period such as rejection of her foal or reduced maternal behavior.

The only difference between primiparous and pluriparous mares in the antepartum phase was a delayed increase of plasma cortisol concentration in mares giving birth for the first time. During the last trimester of equine gestation; progestins are synthesized by the fetoplacental unit from pregnenolone originating from the fetal adrenal glands. A few days before foaling, fetal adrenal hormone production changes, pregnenolone is shifted towards cortisol synthesis (Haluska and Currie, 1988) and fetal (Fowden and Silver, 1995) but also maternal (Nagel et al., 2012) cortisol concentration increases. During the last days before parturition, placental transfer of endogenous cortisol from the fetus to the mare is possible. In both groups of the present study, an antepartum cortisol increase occurred but maternal cortisol concentration was at all times before parturition lower in primiparous mares. Cortisol

of fetal origin is part of the cascade which initiates foaling. Stressful situations with increased cortisol release can advance the time of foaling (Nagel et al., 2020). In agreement with this, an increase in fetal cortisol concentration and thus fetal final maturation occurred later in primiparous than in pluriparous mares. Cortisol concentration is also lower in Shetland and Haflinger compared to Warmblood mares (Nagel et al., unpublished). Shetland, Haflinger and primiparous Warmblood mares deliver smaller foals than pluriparous Warmblood mares, suggesting that maternal cortisol concentration is in part influenced by fetal size.

Maternal HR and HRV and thus maternal burden during gestation were not influenced by parity. As reported previously, HR constantly increased without changes in HRV during the last three month of gestation indicating maternal cardiovascular adaptation to the increasing demands of the growing fetus (Nagel et al. 2010, 2016). In small breed pony pregnancies, maternal heart rate increased more during the last three weeks of gestation than in Warmblood mares and was accompanied by a decrease in HRV, indicative of sympathetic dominance (Nagel et al., unpublished). In contrast sympathetic tone of primiparous mares, heart rate and HRV and thus sympathoadrenal activation did not differ between primiparous and pluriparous mares in the last ten days before birth of the present study. Thus, heart rate and HRV are not influenced by parity in mares of the same breed. In contrast, antepartum heart rate was higher and HRV lower in first-calf heifers compared to pluriparous cows (Trenk et al., 2015). Differences in heart rate and HRV between primiparous horse mares and heifers are most probably due to different energetic demands.

During the last five days before foaling progestin concentration did not differ between mare groups and decreased one to two days before parturition. Lower progestin concentrations have been described in maternal blood of Shetland than Warmblood mares during the last days of gestation (Nagel et al., unpublished). This is in agreement with a lower relative placental weight and smaller foals in Shetland pregnancies (Nagel et al., unpublished). Foals of primiparous mares were smaller and lighter due to differences between primiparous and pluriparous pregnancies, but the placenta of primiparous mares is apparently not a limiting factor for progestin synthesis.

Fetal HR and HRV did at no time before, during and within the first two hours after birth differ between fetuses and foals, respectively from primiparous and pluriparous mares. As described previously (Nagel et al., 2015), fetal heart rate increased during the last days

before birth, decreased slightly during birth and then increased after birth in foals of both groups. In contrast to these findings in horses, HRV in fetuses of pluriparous cows increased towards the end of gestation, while an increase in HRV was missing in fetuses of first-calf heifers, indicating sympathetic tone in heifers (Trenk et al., 2015).

Foals of primiparous mares were lighter and smaller than foals born to pluriparous mares. This is largely in agreement with previous studies (Wilsher and Allen, 2003; Meirelles et al., 2017; Robles et al., 2018a). Although placental weight and surface area did not differ between primiparous and pluriparous mares of the present study, more subtle differences in placental histology cannot be excluded. Previous studies have described differences in microcotyledonary connective tissue and reduced placental weight in primiparous mares (Platt, 1984; Wilsher and Allen, 2003, Robles et al., 2018b). Differences in fetal growth are most likely due to long lasting differences in fetal nutritional supply due to placental and maternal metabolic differences (Robles et al., 2019). Differences in fetal growth have also been reported in relation to season with foals born early in the year, being smaller than those born during later (Beythien et al., 2017). Gestation length, sex ratio, duration of the expulsive phase, time to first suckling and to placenta shedding did not differ between primiparous and pluriparous mares.

Unexpectedly, the Brix value of colostrum which correlates with colostral IgG content (Cash, 1999) was higher in primiparous than in pluriparous mares. This suggests that, at least on average colostrum from primiparous mares contains at least the same amount or even more immunoglobulins than colostrum from pluriparous mares.

In conclusion, this study demonstrates a more pronounced sympathetic tone directly after birth in primiparous versus pluriparous mares. Being exposed for the first time to their own foal thus apparently elicits a certain stress response in these mares. Our data confirm that foals born to pluriparous mares on average are bigger and heavier than foals from primiparous mares.

5. Summary

In this study the scientists followed the hypothesis that in primiparous mares the maternal burden during the last weeks of gestation is higher than in pluriparous mares. The aim of the present study was to investigate antepartum and postpartum hormonal changes in mares as well as fetal and maternal cardiac parameters during physiological pregnancies and during and after parturition of primiparous and pluriparous Warmblood horse mares. The scientists also collected birth-related data, colostrum parameters, weight data, placenta-related data, sex ratios and foal-related data.

Foals out of primiparous mares were significantly smaller in height (height at withers measured by stick, p<0.05; height at withers measured by tape, p=0.001), chest circumference (p<0.05) and crown-rump length (p<0.01) than foals born out of pluriparous mares. Furthermore, foals out of primiparous mares were significantly lighter (p<0.01).

During the last five days before parturition the plasma cortisol concentration was at all times lower in primiparous mares (group p<0.05).

In the last two hours before parturition maternal heart rate (HR) was at all times lower in primiparous than in pluriparous mares (group p<0.05).

During the first two hours after parturition heart rate variability parameters were significantly lower in primiparous than in pluriparous mares (group p<0.05). Root mean square of successive RR differences (RMSSD) reflects the parasympathetic activity of the autonomous nervous system. From the beginning of parturition until first 15 min thereafter 7/7 pluriparous mares and 2/7 primiparous mares showed atrioventricular (AV) blocks. Pluriparous mares showed significantly more AV blocks than primiparous mares 15 and 90 min after the foal was born (p<0.05).

Thus, primiparous mares had a higher activity of the sympathetic system than pluriparous mares.

In conclusion, this study demonstrates a more pronounced sympathetic tone directly after birth in primiparous versus pluriparous mares. Being exposed for the first time to their own foal thus apparently elicits a certain stress response in these mares. Our data confirm that foals born to pluriparous mares on average are bigger and heavier than foals from primiparous mares.

6. Zusammenfassung

Die vorliegende Studie folgte der Hypothese, dass es während den letzten Trächtigkeitswochen zu einer größeren Belastung für primipare im Vergleich zu pluriparen Warmblutstuten kommt. Um diese Hypothese zu verifizieren, wurden antepartale und postpartale Hormonunterschiede der Stuten und kardiale Parameter von Stuten und Feten bzw. Fohlen erfasst. Es wurden ausschließlich physiologische Trächtigkeiten untersucht. Auch wurden geburts-, fohlen- und plazentabezogene Daten erfasst. Ebenfalls notiert wurden die Geschlechterverhältnisse der Fohlen, Gewichtsdaten und Kolostrumparameter. Primipare Stuten hatten signifikant kleinere Fohlen, als pluripare Stuten. Diese Fohlen waren nicht nur kleiner in der Höhe (Widerristhöhe gemessen mit einem Zollstock, p<0,05; Widerristhöhe gemessen mit einem Maßband, p=0,001), sondern hatten auch einen kleineren Brustumfang (p<0,05), eine kürzere Scheitel-Steiß-Länge (p<0,01) und sie waren leichter (p<0,01).

In den letzten fünf Tagen vor der Geburt waren die Kortisolkonzentrationen der primiparen Stuten signifikant niedriger, als die der pluriparen Stuten (Gruppe p<0,05).

Die Analysen der letzten zwei Stunden vor der Geburt ergaben, dass die maternale Herzfrequenz permanent niedriger bei primiparen Stuten, als bei pluriparen war (Gruppe p<0,05).

In den ersten zwei Stunden nach der Geburt waren die Herzfrequenzvariabilitäts-Parameter signifikant niedriger bei primiparen Stuten im Vergleich zu pluriparen Stuten (Gruppe p<0,05). Da durch den Quadratischen Mittelwert der Summe aller Differenzen aufeinanderfolgender RR-Intervalle (RMSSD) Aussagen über die parasympathische Aktivität getroffen werden können, spricht das für eine höhere Sympathikus-Aktivität bei primiparen Stuten. Ebenso zeigten sich bei pluriparen Stuten sowohl 15 als auch 90 Minuten nach der Geburt signifikant mehr atrioventrikuläre (AV) Blöcke in den EKGs, als bei primiparen Stuten (p<0,05). Vom Zeitpunkt der Geburt bis 15 min danach zeigten 7/7 pluripare und 2/7 primipare Stuten AV-Blöcke. Dadurch, dass primipare Stuten weniger AV-Blöcke nach der Geburt aufwiesen, kann geschlussfolgert werden, dass ihre sympathische Aktivität in diesem Zeitraum höher war, als die der pluriparen Stuten.

Zusammenfassend ist zu sagen, dass primipare Stuten in dieser Studie eine stärker ausgeprägte sympathische Reaktion direkt nach der Geburt aufwiesen, im Vergleich zu pluriparen Stuten. Der erstmalige Kontakt mit einem eigenen Fohlen löst bei primiparen Stuten offenbar eine gewisse Stressreaktion aus. Unsere Daten bestätigen, dass Fohlen von pluriparen Stuten im Durchschnitt größer und schwerer sind, als Fohlen von primiparen Stuten.

7. <u>References</u>

Allen WR, Wilsher S, Turnbull C, Stewart F, Ousey J, Rossdale PD, Fowden AL. Influence of maternal size on placental, fetal and postnatal growth in the horse. I. Development in utero. Reproduction **2002**;123:445-53.

Allen WR, Wilsher S, Tiplady C, Butterfield RM. The influence of maternal size on preand postnatal growth in the horse: III Postnatal growth. Reproduction **2004**;127:67-77. https://doi.org/10.1530/rep.1.00024.

Aurich C, Schneider J. Sex determination in horses - current status and future perspectives. Anim Reprod Sci. **2014**;146:34-41. https://doi.org/10.1016/j.anireprosci.2014.01.014.

Beythien E, Aurich C, Wulf M, Aurich J. Effects of season on placental, foetal and neonatal development in horses. Theriogenology **2017**;97:98-103. https://doi.org/10.1016/j.theriogenology.

Cash RSG. Colostral quality determined by refractometry. Equine Vet Educ **1999**;11:36-8. https://doi.org/10.1111/j.2042-3292.1999.tb00916.x.

Diel de Amorim M, Montanholi Y, Morrison M, Lopez Rodriguez M, Card C. Comparison of foaling prediction technologies in periparturient Standardbred mares. J Equine Vet Sci **2019**;77:86-92.

https://doi.org/10.1016/j.jevs.2019.02.015.

Erber R, Wulf M, Rose-Meierhöfer S, Becker-Birck M, Möstl E, Aurich J, Hoffmann G, Aurich C. Behavioral and physiological responses of young horses to different weaning protocols: a pilot study. Stress. **2012**;15:184-94. https://doi.org/10.3109/10253890.2011.606855.

Fowden AL, Silver M. Comparative development of the pituitary-adrenal axis in the fetal foal and lamb. Reprod. Domest. Anim. **1995**;30:170-77. https://doi.org/10.1111/j.1439-0531.1995.tb00141.x. Haluska GJ, Currie WB. Variation in plasma concentrations of oestradiol-17 β and their relationship to those of progesterone, 13,14-dihydro-15-keto-prostaglandin F-2 α and oxytocin across pregnancy and at parturition in pony mares. Journal of reproduction and fertility **1988**;84635-46.

https://doi.org/10.1530/jrf.0.0840635.

Klewitz J, Struebing C, Rohn K, Goergens A, Martinsson G, Orgies F, et al. Effects of age, parity, and pregnancy abnormalities on foal birth weight and uterine blood flow in the mare. Theriogenology **2015**;83:721-9. https://doi.org/10.1016/j.theriogenology.2014.11.007.

Kuhl J, Stock KF, Wulf M, Aurich C. Maternal Lineage of Warmblood Mares Contributes to Variation of Gestation Length and Bias of Foal Sex Ratio. PLoS One **2015** https://doi.org/10.1371/journal.pone.0139358.

Kuhl J, Nagel C, Ille N, Aurich JE, Aurich C. The PGF2α agonists luprostiol and dcloprostenol reliably induce luteolysis in luteal phase mares without evoking clinical side effects or a stress response. Anim Reprod Sci **2016**;168:92-9. https://doi.org/10.1016/j.anireprosci.2016.02.031.

Meirelles MG, Veras MM, Alonso MA, de Fátima Guimarães C, Nichi M, Fernandes CB. Influence of maternal age and parity on placental structure and foal characteristics from birth up to two years of age. J Equine Vet Sci **2017**;56:68-79. https://doi.org/10.1016/j.jevs.2017.03.226.

Melchert M, Aurich C, Aurich J, Gautier C, Nagel C. Controlled delay of the expulsive phase of foaling affects sympathoadrenal activity and acid base balance of foals in the immediate postnatal phase. Theriogenology **2019**;139:8-15. https://doi.org/10.1016/j.theriogenology.2019.07.017.

Nagel C, Aurich J, Aurich C. Determination of heart rate and heart rate variability in the equine fetus by fetomaternal electrocardiography. Theriogenology **2010**;73:973-83. https://doi.org/10.1016/j.theriogenology.2009.11.026.

Nagel C, Aurich J, Aurich C. Heart rate and heart rate variability in the pregnant mare and its foetus. Reprod Domest Anim **2011a**;46:990-3. https://doi.org/10.1111/j.1439-0531.2011.01772.x. **Nagel C, Aurich J, Palm F, Aurich C.** Heart rate and heart rate variability in pregnant Warmblood and Shetland mares as well as their fetuses. Anim Reprod Sci **2011b**;127:183-7. https://doi.org/10.1016/j.anireprosci.2011.07.021.

Nagel C, Erber R, Bergmaier C, Wulf M, Aurich J, M tl E, Aurich C. Cortisol and progestin release, heart rate and heart rate variability in the pregnant and postpartum mare, fetus and newborn foal. Theriogenology **2012**;78:759-67. https://doi.org/10.1016/j.theriogenology.2012.03.023.

Nagel C, Erber R, Ille N, von Lewinski M, Aurich J, M tl E, Aurich C. Parturition in horses is dominated by parasympathetic activity of the autonomous nervous system. Theriogenology **2014**;82:160-8. https://doi.org/10.1016/j.theriogenology.2014.03.015.

Nagel C, Erber R, Ille N, Wulf M, Aurich J, M tl E, Aurich C. Heart rate and salivary cortisol concentrations in foals at birth. Vet J **2015**;203:250-2. https://doi.org/10.1016/j.tvjl.2014.11.013.

Nagel C, Trenk L, Aurich J, Wulf M, Aurich C. Changes in blood pressure, heart rate, and blood profile in mares during the last 3 months of gestation and the peripartum period. Theriogenology **2016**;86:1856-64.

https://doi.org/10.1016/j.theriogenology.2016.06.001.

Nagel C, Melchert M, Aurich J, Aurich C. Road transport of late-pregnant mares advances the onset of foaling. J Equine Vet Sci **2020**;86. https://doi.org/10.1016/j.jevs.2019.102894.

Nagel C, Melchert M, Aurich J, Aurich C. Breed-related differences of antepartum endocrine and cardiac changes in horse mares. **unpublished**

Ousey JC, Houghton E, Grainger L, Rossdale PD, Fowden AL. Progestagen profiles during the last trimester of gestation in Thoroughbred mares with normal or compromised pregnancies. Theriogenology **2005**;63:1844-56. https://doi.org/10.1016/j.theriogenology.2004.08.010.

Platt H. Growth of the equine foetus. Equine Veterinary Journal **1984**;16(4):247-52. https://doi.org/10.1111/j.2042-3306.1984.tb01920.x. Robles M, Dubois C, Gautier C, Dahirel M, Guenon I, Bouraima-Lelong H, Viguié C, Wimel L, Couturier-Tarrade A, Chavatte-Palmer P. Maternal parity affects placental development, growth and metabolism of foals until 1 year and a half. Theriogenology 2018a;108:321-30.

https://doi.org/10.1016/j.theriogenology.2017.12.019.

Robles M, Peugnet PM, Valentino SA, Dubois C, Dahirel M, Aubrière MC, Reigner F, Serteyn D, Wimel L, Tarrade A, Chavatte-Palmer P. Placental structure and function in different breeds in horses. Theriogenology **2018b**;108:136-45. https://doi.org/10.1016/j.theriogenology.2017.11.007.

Robles M, Couturier-Tarrade A, Derisoud E, Geeverding A, Dubois C, Dahirel M, Aioun J, Prezelin A, Calvez J, Richard C, Wimel L, Chavatte-Palmer P. Effects of dietary arginine supplementation in pregnant mares on maternal metabolism, placental structure and function and foal growth. Sci Rep **2019**;9:6461. https://doi.org/10.1038/s41598-019-42941-0.

Satué K, Felipe M, Mota J, Muñoz A. Factors influencing gestational length in mares: a review. Livest Sci 2011;136:287–94. https://doi.org/10.1016/j.livsci.2010.09.011.

Trenk L, Kuhl J, Aurich J, Aurich C, Nagel C. Heart rate and heart rate variability in pregnant dairy cows and their fetuses determined by fetomaternal electrocardiography. Theriogenology **2015**;84:1405-10.

https://doi.org/10.1016/j.theriogenology.2015.07.027.

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

Wilsher S, Allen WR. The effects of maternal age and parity on placental and fetal development in the mare. Equine Vet J **2003**;35:476-83. https://doi.org/10.2746/042516403775600550.