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Research

The stress response of 6-month-old horses to abrupt weaning is influenced by their sex

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ABSTRACT

In this study, we investigated differences in the immediate response of male and female foals to weaning. A total of 22 foals (11 male and 11 female) of 6 months of age were weaned abruptly from their dams but remained in their familiar foal groups. Body weight was determined. Behavior of foals was observed from 2 days before to 7 days after weaning. Saliva samples for determination of cortisol concentration were collected from 2 days before to 7 days after weaning at 6 AM, 0:30 PM, and 7 PM. On the day of weaning, saliva was collected from 1 hour before until 4 hours after weaning at 30-minute intervals. Heart rate and heart rate variability were monitored. Weaning resulted in higher weight loss in female than in male foals ($P < 0.05$) until day 5 after weaning but not thereafter. Weaning was followed by a marked increase in vocalization of foals with more frequent vocalization events in colts than in fillies (day, $P < 0.001$; sex, $P < 0.05$). The frequency of defecation changed over days ($P < 0.001$) with a higher frequency in colts. Lying of foals decreased after weaning (day $P < 0.001$) but was not influenced by sex. The time spent on feed intake was reduced in response to weaning (day $P < 0.001$) but was not affected by foal sex. Weaning caused an immediate increase ($P < 0.001$) in salivary cortisol concentration that did not differ between sex groups. However, the daily pattern of salivary cortisol concentration over the experimental period was affected by day ($P < 0.001$) and sex ($P < 0.05$). A day × sex interaction existed ($P < 0.05$) with fillies having a higher overall cortisol concentration in saliva than colts in the postweaning period. Weaning increased heart rate which peaked immediately after removal of the dams, but sex of foals did not influence heart rate or heart rate variability. In conclusion, weaning of foals is a stressful event with male and female foals displaying stress differently. Results demonstrate early sex differences in behavioral and physiological responses to stress for prepubertal horses.

Introduction

Heretofore, there has not been much information available about whether differences in the behavior between male and female horses are inherited or acquired or at what age and under which conditions such differences develop (Murphy et al., 2004; Wulf et al., 2013). Developmental programming studies in other species demonstrate that male and female offspring exhibit different phenotypes that are influenced by conditions and insults experienced during intrauterine life (Aiken and Ozanne, 2013). It is therefore possible that sex-related differences are also present in horses before puberty. Puberty in Warmblood horse breeds is reached at approximately 80 weeks of age (Naden et al., 1990). Analysis of sex differences before puberty largely excludes the effects of gonadal steroids and, in females, estrous cyclicity on the animals' responses to environmental challenges. In vertebrates, sexual differentiation of the brain is regulated by organizational and activational effects of endogenous hormones. In mammals, the organizational period is characterized by a surge of sex steroid hormones during differentiation of specific neural circuits; however, activational effects depend on later increases in the same hormones at sexual maturation (reviewed by Rosenfeld et al., 2017). To the best of our knowledge, information on sex steroid hormone

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concentrations in equine fetuses before day 100 of pregnancy is not available. However, fetuses of both sexes are exposed to high estrogen and testosterone concentrations between approximately 100 and 300 days of pregnancy (for review, see Ginther, 1992). In both sexes, steroid hormone concentrations are high at birth but decrease immediately thereafter and stay low until puberty (Dhakal et al., 2012). Male and female prepubertal horses therefore have been exposed to a similar steroid hormone background. Several studies on sex differences in postpubertal horses indicate lower anxiety (Duberstein and Gilkenson, 2010), less manageability (Schmidt et al., 2010b), and superior visual-spatial ability (Murphy et al., 2004) in male compared with female horses, whereas others did not find any sex effects (Wolff et al., 1997; Kedzierski and Janczarek, 2009).

Weaning is widely recognized as one of the most stressful events in a foal's life (Fraser et al., 1975; Apter and Householder, 1996; Heleski et al., 2002; Waran et al., 2008). In domesticated horses, almost all foals will be exposed to the weaning process which is often followed by changes in housing and management. The procedure has been associated with the development of unwanted behavior in horses (Nicol, 1999; Heleski et al., 2002); however, "stable vices" have a higher prevalence in stallions (Tadich et al., 2012). A different stress response of male and female foals to weaning might contribute to differences in behavior in their future life. Behavioral, physiological, and immune response indicators have been used for determining the intensity of the foals' stress response to weaning. Apart from maternal deprivation, changes in housing and feeding, the type of weaning management, and the social structure in the investigated horse groups affect the stress response of foals to weaning (Houpt, 1984; McCall et al., 1985; Waran et al., 2008; Dubcova et al., 2015). One study showed that fillies have higher cortisol levels in response to weaning for stable and forest raised Konik Polski foals (Górecka-Bruzda et al., 2015). Apart from this study, no clear sex-related differences in foal behavior to weaning were found, but they may have been masked by a more pronounced effect of other relevant factors such as weaning method and diet (Hoffmann et al., 1995).

The aim of this study was to investigate if foal sex influences the behavioral and physiological stress response to weaning. For this reason, we compared the stress reaction of male (colts) and female foals (fillies) to weaning in warmblood foals kept and weaned under identical conditions.

Material and methods

Animals

A total of 22 Warmblood foals (11 female and 11 male) born at the Brandenburg State Stud in Neustadt (Dosse), Germany, were available for this study. Before weaning, all foals were kept with their dams in groups on pastures night and day. Every morning, the groups of mares and foals were brought into straw-bedded group stables for feeding grain and minerals. All foals were familiar with handling for routine procedures such as deworming, grooming and physical examinations as well as wearing heart rate girths and saliva sampling.

Experimental design

The foals had lived in groups of 8-10 foals with their dams from an age of 2-4 days. From birth until the pasture period, they were kept in spacious group stables (winter stable, 12 × 14m) on straw and had access to outdoor paddocks for several hours per day. The pasture period started in May when the groups were gradually accustomed to grazing. From mid-May onward, they had access to pasture during daytime but were housed in spacious group stables during night (pasture stable, 14 × 23m). For feeding concentrates, mares were tethered. Foals were gradually accustomed to foal concentrate which was offered in a part of the stable only accessible to the foals. Foals were weaned at an age of approximately 6 months. To ensure a comparable age at weaning, this was performed in 3 subgroups of animals of mixed sex born either in February (n = 10), March (n = 9), or April (n = 3) that were weaned in August, September, or October, respectively. Two days before weaning, the group of dams and foals was brought to the winter group stable. On the day of weaning, foals remained in their familiar foal groups in the stable, whereas the mares were led away to a distant pasture out of the foals' visual and auditory range. Starting on day 3 after weaning, the foals were turned out into sand paddocks for 3.5 hours per day (8:30 until 12:00 AM). From 2 days before the day of weaning until 7 days after the day of weaning, the following parameters were assessed: body weight, cortisol concentration in saliva, heart rate, heart rate variability, and the behavioral traits—vocalization, defecation, lying, and feeding.

Bodyweight

Bodyweight was measured on the day before weaning and on days 1 through 7 as well as 14 and 28 after weaning with an electronic horse scale (Model TWI; Bosche, Damme, Germany). A weight up to 1500 kg could be measured with a resolution of 1 kg.

Behavior

Behavior of the foals was observed by 2 experienced observers for 1 hour in the morning (7 AM-8 AM) and afternoon (1 PM-2 PM) from 2 days before to 7 days after weaning. On weaning day, the foals were observed for 4 consecutive hours, starting when the foals were separated from their dams (10:30 AM). Vocalization events (n), defecation events (n), and lying time (minutes) were recorded for each foal during the morning and afternoon observation periods. The mean value per hour was calculated. In contrast, feeding behavior was scored from 1 to 4 per every 15 minutes (1: no interest in hay, pasture, or straw; 2: interest in hay, pasture, or straw nearly absent, i.e., feeding less than 1 minute in 15 minutes; 3: reduced interest in hay, pasture, or straw, i.e., feeding between 1 and 10 minutes per 15 minutes; and 4: constant or nearly-constant eating when hay, straw, or pasture was available, i.e., more than 10 minutes per 15 minutes) as described previously (Erber et al., 2012).

Cortisol concentration

For cortisol analysis, saliva was collected with a cotton-based swab (Salivette; Sarstedt, Nümbrecht, Rommelsdorf, Germany) as described (Erber et al., 2012). The Salivette was inserted at the angle of the lips into the mouth of the foal and placed gently onto the tongue for 1 minute until it was well soaked. After centrifugation for 10 minutes at 1000 g, saliva was aspirated and frozen at −20°C until analysis. A single person was able to obtain samples without restraining the foals. Saliva samples were taken from 2 days before to 7 days after weaning in the morning (6 AM), at noon (0:30 PM), and in the evening (7 PM). On the day of weaning, saliva was collected in the morning and evening and at 60 and 30 minutes before weaning, immediately after weaning, and then every 30 minutes until 240 minutes after weaning.

Concentrations of cortisol were determined with an enzyme immunoassay without extraction (Demeditec Diagnostics, Kiel-Wellsee, Germany) validated for equine saliva in our laboratory (Kuhl et al., 2016). The intraassay coefficient of variation was 5.76%;