### Research

**Effects of prompt versus stepwise relocation to a novel environment on foals’ responses to weaning in domestic horses (Equus caballus)**

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**Abstract**

Artificial weaning is often highly stressful for both mother and offspring. We investigated the effect of 2 different weaning and relocation schedules on growth rate and saliva cortisol concentrations in a group of loose-housed domestic horses. We predicted higher acute stress (cortisol concentrations), but a lower long-term effect on weight gain, which would indicate lower chronic stress in foals, if the foals were moved to the new environment immediately after separation from the mothers ("prompt relocation" [PR]) compared with relocation that was adjourned for a week ("stepwise relocation" [SWR]). Within 2 seasons, 56 foals weaned at age of 165-250 days were regularly weighed up to 140 days after weaning. Growth rate significantly differed between SWR and PR foals. The PR weanlings revealed relatively stable increase in their weights, whereas SWR foals experienced a significant drop in growth during the first 3 weeks after weaning. The weight differences were still apparent 5 months after weaning (predicted increase in their weights, whereas SWR foals experienced a significant drop in growth during the first 3 weeks after weaning). Cortisol concentrations changed significantly between and within different weaning procedures and were highest in PR foals after weaning and relocation. Compared with pre-weaning values, cortisol levels increased in PR but not in SWR foals, either after weaning or deferred moving. We found large individual variability in foals’ growth and in cortisol concentrations for both weaning procedures. There was also a long-term effect on weight gain, which would indicate lower chronic stress in foals, if the foals were moved to the new environment immediately after separation from the mothers ("prompt relocation" [PR]) compared with relocation that was adjourned for a week ("stepwise relocation" [SWR]). Within 2 seasons, 56 foals weaned at age of 165-250 days were regularly weighed up to 140 days after weaning. Growth rate significantly differed between SWR and PR foals. The PR weanlings revealed relatively stable increase in their weights, whereas SWR foals experienced a significant drop in growth during the first 3 weeks after weaning. Cortisol concentrations changed significantly between and within different weaning procedures and were highest in PR foals after weaning and relocation. Compared with pre-weaning values, cortisol levels increased in PR but not in SWR foals, either after weaning or deferred moving. We found large individual variability in foals’ growth and in cortisol concentrations for both weaning procedures. There was also a year effect in SWR foals. Stepwise changes of the physical and social environment (deferred removal to the remote facility) within a short period after abrupt weaning resulted in lower acute stress but induced long-term negative effects on foals’ growth rate compared with joint weaning and relocation. Our results support rather prompt moving of the weanlings to the new facility, rather than to a stepwise location on horse breeding farms.

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### Introduction

The breaking of the mother-offspring bond (whether natural or forced) often constitutes a highly stressful event for both mother and offspring, especially in the case of the artificial weaning of domestic animals (Weary et al., 2008). Recent pressure to improve the quality of welfare in animal management systems invites further discussion about weaning as a process and emphasizes a need for scientific investigations of the welfare consequences of alternative weaning methods to better inform future management practice.

Natural weaning in equids is a gradual process that starts at about 8 months of age. Weaning termination depends mostly on the mare’s body condition and her reproductive status. Pregnant mares usually wean their foals before 1 year of age when the next delivery approaches, whereas mares who failed to conceive in a subsequent season may commonly continue to nurse their yearlings up to 2 years of age (Berger, 1986; Cameron et al., 2000; Crowell-Davis and Weeks, 2005; Duncan et al., 1984; Pluháček et al., 2007; Rutberg and Keiper, 1993). Cessation of milk support, however, does not usually break the mother-offspring bond, and it frequently persists for several years in many mammalian species (reviewed in Newberry and Swanson, 2008), including horses. The bond between mother and offspring remains strong

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until the foal leaves the natal group at an age from 1 to 4 years (Khalil and Kaseda, 1997; Monard and Duncan, 1996; Tyler, 1972; Waring, 2003).

In contrast, foals born to domestic horses experience artificial weaning at much younger age than those born to wild horses. The attendant nutritional, social, and environmental changes may have long-lasting consequences for both mother and foal (Apter and Householder, 1996; Henry et al., 2012). Weaning of foals under farm conditions may occur as early as immediately after birth or is more commonly undertaken at the age of 4-8 months (Apter and Householder, 1996; McCall et al., 1985; Rogers et al., 2004; Weeks et al., 2000). Time of weaning on horse farms reflects the tradeoff between the 2 opposite concerns for the breeders: the condition of the mare which could result in earlier weaning (Ladewig et al., 2005), and the effect on developmental and behavioral aspects for the foal, which results in a tendency to delay weaning. The allocation of maternal care in pregnant mares may have an additional effect on concerns about the condition of the mare (Bartosová et al., 2011).

In the most widespread weaning practice, abrupt weaning, the mare and foal are suddenly and completely separated by enough distance to prevent them seeing, hearing, or smelling each other (Apter and Householder, 1996; Ladewig et al., 2005; McCall et al., 1985). Besides loss of mother and milk (Coleman et al., 1999; McGee and Smith, 2004), abrupt weaning process typically imposes additional change on the foal including a new unfamiliar environment or mixing with new social partners (Houpt and Hintz, 1983; Newberry and Swanson, 2008; Nicol et al., 2005). These changes can elicit various behaviors in foals that may include increased locomotion and vocalization, and stereotypic or self-destructive behavior (Houpt and Hintz, 1983; McCall et al., 1985; McGee and Smith, 2004; Nicol and Badnell-Waters, 2005). Abrupt change may increase the incidence of health problems associated with growth curve deviations that usually occur immediately after weaning (Houpt et al., 1984; Rogers et al., 2004; Warren et al., 1998).

Given these potential repercussions of abrupt weaning, various alternative methods have been subjected to detailed research (reviewed in Apter and Householder, 1996; Waran et al., 2003), including gradual weaning (Rogers et al., 2004); partial separation allowing fence-line contact between mothers and foals (McCalt et al., 1985); weaning in pairs (Hoffman et al., 1995; Malinowski et al., 1990) or groups (Heleski et al., 2002), weaning in paddocks with the possibility of grazing (Heleski et al., 2002; Nicol et al., 2005), weaning with a presence of some unrelated adult horse(s) (Henry et al., 2012), and feeding different diets at the time of weaning (Coleman et al., 1999; McCall et al., 1985; Nicol et al., 2005). These more weaning techniques that mimic parts of natural weaning appear to be associated with lower levels of stress in both mother and foal, but the various studies undertaken are not easily comparable owing to different methodologies and differences in measures recorded.

Evaluation of stress levels in foals has been based on various indicators, including the foals’ behavior [including locomotion, vocalization, or abnormal behavior (Heleski et al., 2002; Henry et al., 2012; Houpt et al., 1984)]; activity (McCall et al., 1985); blood cortisol levels (Berger et al., 2013; Hoffman et al., 1995; Houpt et al., 1984; Malinowski et al., 1990; McCall et al., 1987); salivary levels of cortisol and other compounds (Erber et al., 2012; Henry et al., 2012; Moons et al., 2005); growth rates (Coleman et al., 1999; Erber et al., 2012; Rogers et al., 2004; Warren et al., 1998); and weanlings’ trainability, tractability, and responses to a novel object (Nicol et al., 2005). Stress affects normal biological functions (e.g., growth) through biological responses that are used to redress stressful situations (Moberg, 1987; Morgan and Tromborg, 2007).

Resources (energy) allocated to the activities assisting the animal to cope with stress, such as vocalization, locomotion, stereotypic behavior, increased heart rate and respiration, or increased secretion of various isomers of adrenal glucocorticoids, are unavailable for biological needs such as growth (Moberg, 1987; Morgan and Tromborg, 2007). Body weight reduction or growth rate disruption as a consequence of the stress has been documented in multiple species [laboratory mice (Bartolomucci et al., 2004; Laugero and Moberg, 2000a, 2000b, 2000c), rats (Konikle et al., 2003; Marti et al., 1994), and domestic pigs (Hemsworth et al., 1981; Pluske et al., 1997)]. Increases in cortisol level and reduced weight gain have been used as indicators of exposure to stressful situations including weaning (Erber et al., 2012; Malinowski et al., 1990; Rogers et al., 2004).

Despite the potential negative repercussions of abrupt weaning, it is still common because it is considered easier and more cost-effective than gradual separation of mother-foal pairs (Apter and Householder, 1996). On some farms, management practices that separate actual weaning (separation from the mother) from other subsequent changes in foals’ life (change of wider social group and change to an unfamiliar environment) have been introduced as an alternative measure to help the foals cope better with loss of the mother within familiar environment. Staying in the home environment after weaning has been found to be less stressful than relocation in weaned piglets (Eklkel et al., 1995; Hoetzel et al., 2011; Puppe et al., 1997), farmed wapiti calves (Church and Hudson, 1999), cattle calves (Lynch et al., 2011), and horse foals (Nicol et al., 2005). Nevertheless, little is known about the effects of later removal of the “home-weaned” offspring to unfamiliar environment. Deferring moving to 35 days post-weaning resulted in a less marked stress response in pastured beef calves compared with simultaneous weaning and moving to the unfamiliar environment (Lynch et al., 2011). In contrast, weaning stress intensifies with increasing numbers of challenges imposed on the young (Enriquez et al., 2011; Newberry and Swanson, 2008; Waran et al., 2008; Weary et al., 2008) so that dividing the weaning-associated changes in the foal’s life into a sequence of partial changes might result in higher overall stress even if each sequence is less stressful.

We investigated the effect of different time schedules of moving to a new environment after abrupt weaning on growth rate and salivary cortisol concentrations, in a group of loose-housed domestic horses.

Materials and methods

Animals and study site

We observed 56 foals of the Kladruby horse, a native Czech warm-blooded horse breed (breed standard: average height at the withers 164 cm, average weight 570 kg) at the National Stud Kladruby nad Labem, Czech Republic (50°3'29"N, 15°29'4.998"E) in 2 consecutive seasons. Numbers of observed foals are shown in Table 1. Age of the foals at abrupt weaning ranged from 165 to 241 days.

Table 1 Numbers of observed foals in the 2 observation years and weaning procedures

<table>
<thead>
<tr>
<th>Number of horses/season</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>11</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>Females</td>
<td>16</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>29</td>
<td>56</td>
</tr>
<tr>
<td>Stepwise relocated foals</td>
<td>14</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>Promptly relocated foals</td>
<td>13</td>
<td>13</td>
<td>26</td>
</tr>
</tbody>
</table>
250 days. The mares were 5-21 years and 8 of them were primiparous.

Each season, 3 herds consisting from 8 to 12 mare-foal pairs were formed and loose housed in barns of the size 10 x 35 m. All horses observed in the study within 1 season were pastured together daily from 9:00 a.m. to 3:00 p.m. so that all horses subjected to this study knew each other from contacts on a regular-daily basis. The mares were individually fed with oats, minerals, and vitamins in the morning and hay was provided twice a day. The foals had free access to the food served to their mothers. The horses had permanent ad libitum access to water. The foals and their mothers were regularly inspected by a veterinarian as a part of management routine and assessed healthy and prospering during both suckling and post-weaning periods.

Data collection

Weaning protocol and weighing

We followed routine weaning practices on the stud farm. The foals born within the same year were abruptly weaned in 2 groups according to their age in mid-October (foals born between February and April) and in mid-December (foals born from late April to June). After weaning (i.e., permanent physical separation from the mother), the group of foals was moved by foot to a remote facility that served for rearing youngsters (“rearing farm”) located 4 km far from home stable. They were moved either immediately (“prompt relocation” [PR foals]) or a week after weaning (“stepwise relocation” [SWR foals]). All SWR foals were weaned during the first weaning period (mid-October), whereas all PR foals were weaned in the subsequent period (mid-December). A cross-over design could not be applied owing to the given horse management and limited space within the stud farm. The only foal, a male, which was taken from the mother and moved to the rearing farm in mid-October together with SWR foals, was excluded from the analysis.

Management of PR foals: In mid-October, the mothers of SWR foals were led away from the home barn and moved to a remote facility about 2 km away so that visual, auditory, and olfactory contact between mothers and foals was prevented. The foals remained in their home barn where they had been kept with the mothers for another week and continued daily grazing together with younger unweaned mother-foal pairs as before their mothers disappeared. The only change in foals’ lives during the first post-weaning week was the loss of their mothers. After moving to the rearing farm, the weanlings were separated by sex and loose housed in 2 barns.

Management of PR foals: Later-born foals maintained the pre-weaning management until mid-December when they were led out from their mothers and immediately moved to the rearing farm. They were mixed there with their earlier weaned SWR herdmates according to sex. The weanlings were individually fed with oats, minerals, and vitamins in the morning and hay was served twice a day around the whole barn so that all horses in the group could reach it. They were kept in the enclosure with a small amount of grass daily from 9:00 a.m. to 3:00 p.m. (sex-separated). The horses had permanent ad libitum access to water.

The foals were weighed at regular intervals: at birth, at the day of weaning from the mother (“weaning weight”), weekly within the first month after weaning (days 8, 14, 21, and 28), and then monthly up to 5 months after weaning (days 57, 85, 112, and 140). In total, 10 weight measurements were available for each foal.

Salivary cortisol

Measurements of salivary cortisol were made only in the second season of the study. Cortisol concentrations were determined in saliva samples collected before and after weaning to investigate what short-term effects the weaning procedure might have had on the foal as used in previous studies (Erber et al., 2012; Henry et al., 2012; Moons et al., 2005). Saliva samples were taken using standardized cotton rolls (Salivette; Sarstedt, Nümbrecht-Rommelsdorf, Germany) put into the foal’s oral cavity for about 1 minute until the swab was sufficiently soaked. Samples were taken (1) in foals 2 hours before weaning from the mother, (2) 2 hours after weaning from the mother (with this period including also the move to the rearing farm in PR foals), and (3) in SWR foals 2 hours after moving to the rearing farm. All samples were taken between 8 a.m. and 12 a.m. to eliminate possible influence of previously reported circadian rhythm of salivary cortisol with morning peak (Böhak et al., 2013).

The saliva samples were centrifuged for 10 minutes at 3500 rpm and at least 1 mL of saliva was obtained and frozen until analysis. Competitive immunoenzymatic colorimetric method for quantitative determination of cortisol concentration in saliva was used (DiaMetrA s. r. l., Segrèt MI, Italy). Analyses were provided with enzyme-linked immunosorbent assay reader ELX 808, BioTek Instruments, Inc (Winooski, VT, USA). The antisera cross-reacted with cortisone and some cortisone metabolites. Thus, the values have to be interpreted as cortisol immunoreactivity. The intra-assay coefficient of variation was ≤8.0%, the inter-assay variation was ≤14.0%, and the minimal detectable concentration was 0.05 ng/mL at the 95% confidence level.

Statistical analyses

Statistical analyses were performed within SAS 9.4 (SAS Institute Inc., Cary, NC, USA) for Windows. All weight variables (birth weight; weaning weight; and weights 8, 14, 21, 28, 57, 85, 112, and 140 days after weaning), as well as weaning age of the foals were normally distributed (Kolmogorov-Smirnov test, procedure UNIVARIATE). General linear models (PROC GLM) were performed to reveal any differences in the characteristics of foals (i.e., sex, year of birth, birth weight, weaning age, and daily weight gain in period from birth to weaning) assigned to different weaning procedures. Partial Pearson correlation coefficients (PROC CORR) were used to detect possible confounding effects of different age at weaning on post-weaning growth.

A general linear mixed model for repeated measures data (PROC MIXED) was fitted to test the effect of weaning procedure on foals’ post-weaning weights following the approach described by Littell et al. (2000), with the foal’s weight at certain weighing day (kilogram) taken as the dependent variable and interaction between weaning procedure and weighing day as fixed factors. The model further included fixed effects of both single factors (weaning procedure and weighing day), and weighing day nested within interaction between weaning procedure and season, and weaning weight as covariate to treat the model for different starting weaning weights of the foals. Repeated measures design of the data was best described by autoregressive (AR1) covariance structure (via REPEATED statement) with random effect for individual foals (for details see Littell et al., 2000). Within-group means presented in results are least squares means appropriately adjusted for the other effects in the model (LSMEANS statement). Linear combinations of fixed effect parameters (defined in ESTIMATE statement) were estimated to acquire specified differences between predicted group means.

Differences in saliva cortisol concentrations before and after weaning in 24 foals born within the second year were analyzed by fitting a general linear mixed model (PROC GLMM) for repeated (pair) measures on the same foals (REPEATED statement); in this case, the dependent variable was cortisol concentration (nanogram/milliliter) in a particular foal and period (before or after...
weaning), fixed effects were the interaction between period and weaning procedure, with cortisol concentration before weaning as a covariate.

Results

Birth weight of the foals ranged from 49 to 78 kg (mean ± standard error of mean: 66.88 ± 0.82 kg) and did not differ between the 2 years of observation (F(1,51) = 1.17, P = 0.28) or weaning procedure (F(1,51) = 0.50, P = 0.48; GLM). Newborn male foals were heavier than female foals (LSMEANS ± SE: 68.62 ± 1.09 vs. 65.13 ± 1.11 kg, F(1,51) = 4.90, P < 0.05) with comparable differences in both years (interaction sex × period: F(2,51) = 0.86, P = 0.43) and weaning procedures (interaction sex × weaning procedure: F(2,51) = 0.32, P = 0.73).

Foals’ age and weight at weaning

The foals born in the first year were on average 16 days younger at weaning than those born in the other year (200.48 ± 3.79 vs. 216.21 ± 3.62 days; F(1,54) = 9.28, P < 0.01), but there was no difference between weaning procedures (F(1,54) = 0.74, P = 0.39) even within different years (interaction weaning procedure × year: F(1,54) = 1.95, P = 0.17). Despite the 2-weeks difference in weaning age, there was no significant difference in weaning weight between the years (288.75 ± 3.27 vs. 291.49 ± 3.20 kg, respectively, F(1,54) = 0.33, P = 0.57).

Weaning weight ranged between 215 and 340 kg (mean: 290.4 ± 3.61 kg). It positively reflected the foal’s age at weaning (slope = 1.001, F(1,54) = 86.59, P < 0.0001) and did not differ between males and females (F(1,55) = 1.57, P = 0.22). The SWR foals were significantly heavier compared with PR foals at weaning (294.73 ± 3.01 vs. 285.57 ± 3.18 kg, F(1,54) = 4.35, P < 0.05), but the absolute difference comprised only 3.15% (9.16 kg) of the overall mean weaning weight. Moreover, daily weight gains in the period from birth to weaning were comparable in SWR and PR foals in both years (year 1: 1.11 ± 0.02 kg vs. 1.06 ± 0.02 kg; year 2: 1.08 ± 0.02 kg vs. 1.07 ± 0.02 kg).

The weight at 140 days after weaning ("weight140") was highly correlated with weight at weaning (r = 0.85, P < 0.0001), as well as with age at weaning (r = 0.71, P < 0.0001, N = 55), and these 2 variables were also highly correlated (r = 0.79, P < 0.0001). Weight140 reflected weight rather than age at weaning because it was still correlated to weaning weight when controlled for weaning age (Pearson partial correlation coefficient: r = 0.67, P < 0.0001) whereas the relationship between weight140 and weaning age disappeared when controlled for weaning weight (partial r = 0.12, P = 0.37). It suggested that age at weaning did not influence the weight 140 days later in studied foals.

In conclusion, the 2 groups of differently weaned foals did not substantially differ in terms of pre-weaning characteristics or breeding conditions. Small differences in weaning weight were handled by including weaning weight as a covariate to the statistical model.

Foals’ growth versus weaning procedure

Measured weights during the observation period are shown in Figure 1. We found significant differences in growth rate on particular weighing days between SWR and PR foals (F(8,408) = 10.58, P < 0.0001, Figure 2) that partially also differed between seasons (F(8,408) = 8.20, P < 0.0001). The PR foals revealed relatively stable increase in their weights over the observed period, whereas foals that spent a week in the familiar environment experienced a significant drop in growth after being moved to the new stables (day 14 and 21 after weaning; Figure 2). The weight differences were even apparent in the end of observation period nearly 5 months after weaning (day 140 after weaning, 374.40 ± 1.75 kg in PR vs. 362.71 ± 1.66 kg in SWR foals, P < 0.0001). The SWR foals reached lower weight at the end of observations compared with PR foals, although SWR foals had started with higher weaning weight.

The SWR foals differed in the course of early post-weaning growth between the 2 seasons. Within the week spent in home
environment, the foals lost their weight in season 1, whereas they prospered well in season 2 (P < 0.0001; Figure 3).

Cortisol concentrations

Saliva cortisol concentrations measured in different periods and weaning procedures are shown in Table 2. Cortisol concentrations changed significantly during weaning procedure between and within different weaning procedures (F(4,31) = 7.26, P < 0.001; Figure 4). The highest cortisol levels were found in PR foals after weaning from the mother and moving to the new environment (Table 2 and Figure 4). Cortisol levels measured 2 hours after weaning increased in PR (P < 0.05) but not in SWR foals (P = 0.19) compared with pre-weaning values. The SWR foals had significantly higher cortisol concentrations after moving to the new environment than a week before after weaning from the mother (P < 0.05) but did not differ from cortisol measured before weaning (P = 0.87; Figure 4).

Discussion

Differences in growth rate

We found significant differences in growth of foals moved to an unfamiliar environment a week or immediately after weaning from the mothers. In accordance to our presumptions, splitting the foals away from the home environment might seem to mimic at least some elements of natural weaning. Such movement may simulate the natural experience of foals leaving the natal herd and forming or joining new herds, although we should recognize that others factors, such as social structure of the group or social relationships among foals may play important role in foals’ perception and coping with weaning.

Table 2

Saliva cortisol concentrations (nanogram/milliliter) in foals moved immediately (PR) or a week after (SWR) separation from the mothers

<table>
<thead>
<tr>
<th>Cortisol concentration</th>
<th>Stepwise relocated foals (N = 15)</th>
<th>Promptly relocated foals (N = 13)</th>
<th>Difference (GLMM), P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before weaning (2 hours)</td>
<td>1.13 ± 0.12 (0.574–2.233, N = 13)</td>
<td>1.38 ± 0.16 (0.453–2.104, N = 11)</td>
<td>0.99</td>
</tr>
<tr>
<td>After weaning (2 hours)</td>
<td>0.67 ± 0.11 (0.05–1.280, N = 12)</td>
<td>2.02 ± 0.29 (0.987–3.645, N = 10)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>After moving to the rearing farm (2 hours)</td>
<td>1.34 ± 0.21 (0.509–2.843, N = 13)</td>
<td>Not measured</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Weaning, separation from the mother; PR, prompt relocation; SWR, stepwise relocation; SEM, standard error of mean; N/A, not available.

Data represented as mean ± SEM (range and number of foals manageable for saliva sampling). Cells in bold indicate measures taken after moving the foals to the rearing farm.

Overall, within the different approaches standardly applied to horse management, our findings do not support strategies that leave the weanlings at the same place in the 1–2-week period after weaning in the presumption that this prevents additional stress (Apter and Householder, 1996; Weary et al., 2008). In our study, staying in the home environment for 1 week resulted in a greater decrease in weight gain. Regardless of whether it happened within the first (season 1) or second (season 2) week after weaning, remaining in the home environment most likely produced chronic stress as has been reported in rats (Marti et al., 1994), mice (Laugero and Moberg, 2000a, 2000b, 2000c), or pigs (Hemsworth et al., 1981).
Cortisol concentrations

Salivary cortisol concentrations showed a clear response to weaning and prompt removal in PR foals. Their saliva contained a 50% higher concentration of cortisol in samples taken shortly after weaning and moving to a new environment by comparison with levels measured before weaning. Such findings indicate acute stress in this group as a result of the weaning process, which is in agreement with previous horse studies (Erber et al., 2012; Henry et al., 2012). In contrast, the SWR foals that stayed in their home environment tended to show even reductions in cortisol levels immediately after the weaning process. Their cortisol concentrations increased after moving to the remote rearing farm but were far from the acute values reached by PR foals after arriving to the rearing farm. The SWR foals thus experienced lower acute stress shortly after both weaning and moving compared with the PR foals.

We found large individual variability in both, body weight and cortisol levels in observed foals exposed to both weaning procedures. This finding agrees with the results of a number of other recent studies, which suggest that individual personal traits may significantly modify behavior and physiological response in wide range of species including horses (Hausberger et al., 2004; Henry et al., 2007; Ijichi et al., 2013; LeScolan et al., 1997).

Conclusion

Stepwise changes of the physical and social environment within a short period after abrupt weaning (deferred removal to the remote facility) resulted in less immediate but higher long-term negative effects on the foals compared with joint weaning and relocation. A week spent in the home environment in which the foals were born and lived with their dams before weaning seemed to help the foals to better cope with loss of the mother only in 1 of the 2 observed seasons. In both seasons, any positive effect was completely eradicated after moving to the new environment. Therefore, as viewed from a long-term perspective, our results support a rather more radical way of moving the weanlings to the new facility than the stepwise one used by horse breeding farms that fail to keep the weanlings in home environment.

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Ethical considerations

This study received approval for animal use and care from The Institutional Animal Care and Use Committee of the Institute of Animal Science and was conducted in accordance with Czech Central Committee for Protection of Animals number 13803/2003-1020.

Conflict of interest

The authors declare no conflict of interest. The study was supported by the Ministry of Agriculture of the Czech Republic (projects No. QH92265 and MZERO0714) and the European Commission’s Seventh Framework Programme (FP7/2007-2013) under grant agreement N266213 (AWIN).

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References
