



## A two-stage method to approach weaning stress in horses using a physical barrier to prevent nursing



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### ABSTRACT

Many domestic horses are weaned through abrupt physical separation of the foal and mare. Deviations from normal behaviours testify that this abrupt method has an adverse effect on the well-being of both mares and foals. To reduce negative changes in behaviour and physiology induced by weaning, a two-stage approach was tested: the first stage achieved nutritional separation by means of an udder cover to prevent nursing, and the second stage of physical separation occurred four days later. Behaviours (standing, walking, running, eating, drinking, nursing, aggressing, vocalizing) of mares and foals weaned either by abrupt physical separation (control;  $n = 8$ ), or two-stage (TS;  $n = 7$ ) were recorded in real time for 8 h/d, and triaxial accelerometers recorded lying time of mares and foals 24 h/d. Heart rates and global position were recorded on foals during live observations, and fecal samples from mares and foals were collected for cortisol analysis. Behavioural and physiological data were analyzed using a mixed model for repeated measures. No treatment effect was observed in foals, however physical separation induced an increase in vocalizations ( $0.3 \pm 0.13$  versus  $56.9 \pm 39.3$  vocalizations/foal/d  $\pm$  SEM;  $p < 0.0001$ ), observations of running ( $0.31 \pm 0.08$  versus  $3.2 \pm 0.25$  observations/foal/d  $\pm$  SEM;  $p < 0.0001$ ), observations of aggression ( $2.4 \pm 0.27$  versus  $7.6 \pm 0.52$  observations/foal/d  $\pm$  SEM;  $p < 0.0001$ ), and distance travelled ( $1275 \pm 150$  versus  $4679 \pm 497$  m/d  $\pm$  SEM;  $p < 0.0001$ ). Prior to treatment, foals lay  $34.91 \pm 12.4$  min/bout in  $8 \pm 3.4$  bouts/d. During nutritional separation, control foals lay down more than TS foals ( $p < 0.0031$ ). There was no treatment effect on lying duration ( $p > 0.75$ ) or number of lying bouts ( $p > 0.17$ ) upon physical separation, but lying time was significantly less in all foals ( $p < 0.01$ ). Prior to treatment, mares lay an average of  $14.16 \pm 11.21$  min/bout in  $2 \pm 1.7$  bouts/d. Mares displayed no difference in lying duration or number of lying bouts between treatments ( $p > 0.99$ ) or upon physical separation ( $p > 0.37$ ). Two-stage mares vocalized less frequently than control mares upon physical separation ( $7.6 \pm 0.6$  versus  $12.6 \pm 0.9$  vocalizations/mare/d  $\pm$  SEM;  $p < 0.0001$ ). Fecal cortisol concentrations increased in both mares and foals upon physical separation ( $146 \pm 13.5$  versus  $193 \pm 21.9$  ng/ml  $\pm$  SEM;  $p < 0.0032$ ). Although some minor reductions in stress response were achieved by use of a two-stage approach to weaning, these results more clearly show the effect on mares and foals to physical separation. Large variation in both behavioural and physiological responses to stress may indicate a role of personality and attachment theory in effective approaches to weaning.

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### 1. Introduction

Under natural conditions, weaning is a gradual process where young mammals' milk consumption is reduced as their intake

of solid foods is increased (Martin 1984). In horses in the non-domestic environment, this cessation of nursing typically occurs at around 10 months of age, shortly before the arrival of a subsequent foal (Barber and Crowell-Davis 1994). Weaning is also usually associated with reduced physical contact between the dam and her offspring but in many cases their bond will extend beyond the young's nutritional independence and even beyond the birth of subsequent offspring (Newberry and Swanson 2008), with the foal

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often remaining in its natal band (Carson and Wood-Gush 1983) until it reaches sexual maturity (Goodwin 1999).

The gradual and mare-mediated “natural” weaning process is drastically different from the typical “artificial” weaning methods used by breeders in the equine industry. Domesticated foals are weaned younger than their undomesticated counterparts, with the average age at weaning between 5.2 and 6.8 months (Parker et al., 2008). The most common weaning method is total and abrupt (Gooding and Merkies, 2008), meaning that the foal is separated from its dam with no visual or auditory contact (Waran et al., 2008), and the pair will not be reunited.

At the time of weaning, foals are exposed to a wide variety of stressors including maternal deprivation (Nicol, 1999), social disruption (Henry et al., 2012), nutritional changes (Nicol et al., 2005), physical environment changes (Henry et al., 2012), and changes in management (McCall et al., 1985). These cumulate in a variety of measurable stress responses which can be both behavioural (e.g. locomotion, vocalizations, development of stereotypies, and aggression; Henry et al., 2012; Haley et al., 2009; McCall et al., 1985) and physiological (e.g. increased heart rate, Moons et al., 2005; increased cortisol, Henry et al., 2012; McCall et al., 1987; decreased body weight, Waran et al., 2008; decreased immune response, Malinowski et al., 1990).

As an alternative to abrupt methods, gradual weaning, either by short-term separations, or removing dams from a group of mares and foals one at a time (Waran et al., 2008) has been suggested. However it appears that mares and foals do not become habituated to the temporary separations (Moons et al., 2005), and removing mares from a group one at a time was found to be ineffective in preventing significant weight loss (measured by average daily gain; Rogers et al., 2004).

Fence line weaning is another gradual weaning method, where mothers and their offspring retain auditory and visual contact but are separated by a fence (Waran et al., 2008). Fence line weaning has had success in reducing the behavioural indices of stress in foals (when compared to isolation-weaned foals; McCall et al., 1985), and in beef calves this technique reduced both behavioural indices of distress (inappetance and vocalizations) and minimized the loss in weight gain typically associated with separation from the dam (Price et al., 2003).

Two-stage weaning takes fence line weaning one step further by dividing the weaning process into two “stages”: a nutritional separation followed by a physical separation (Haley et al., 2001). During the nutritional separation stage, all forms of mother-offspring contact are allowed except nursing, which is prevented by means of a physical barrier (e.g. a nose flap in calves; see Haley et al., 2005). After a period of nutritional separation, animals are then physically separated in a manner similar to other weaning techniques (Haley et al., 2005). This method has shown success in reducing behavioural and physiological measures of stress in both beef calves (Haley et al., 2005; Loberg et al., 2008) and lambs (Schichowski et al., 2008). Preliminary work by Haley et al. (2009) also found promising results in horses, where two-stage weaning reduced acute behavioural stress for both mares and foals.

The purpose of this study was to build upon previous work done by Haley et al. (2009) by applying a two-stage weaning technique to horses, and combining live behavioural measurements with automated behavioural recording devices and physiological parameters to analyze stress responses. It was hypothesized that two-stage weaning would reduce the stress response in mares and foals as evidenced by fewer signs of behavioural (e.g. vocalizations, locomotion) and physiological (e.g. heart rate, concentrations of fecal cortisol metabolites) stress than experienced by the control group weaned in the typical abrupt manner.

## 2. Materials and method

### 2.1. Experimental herds and management

All experimental procedures were approved by the Animal Care Committee at the University of Guelph (Animal Utilization Protocol No. 11R051) in accordance with the Canadian Council of Animal Care for the ethical treatment of animals used in research.

Seventeen mare-foal pairs were assigned to either two-stage weaning (TS) or control (CON) group, balanced for foal age and sex. Experimental procedures took place in October at two different farm locations in Ontario, Canada with similar management. Seven mare-foal pairs at location A were of warmblood breeding, and ten mare-foal pairs at location B were ponies (Table 1). Parity of the mares ranged from 0 to 10 with an average of three prior foals. All animals were housed outdoors in a single group at each location with ad libitum access to hay and water for at least four months before the start of this study. Animals at location A also had access to grass.

### 2.2. Procedures and timeline

For each location, the same timeline was followed. Baseline data was collected on each animal for the first four days (BL: d1–4). On the morning of d5, treatments were applied. Two-stage mares were fitted with an udder cover to prevent the foals from nursing (Fig. 1A). Control mares were fitted with a sham device that left the udder exposed (Fig. 1B). The covers were worn continuously for four days while data continued to be collected (Nutritional Separation period, NS: d5–8). On the morning of d9, the covers were removed from all the mares and the mare-foal pairs were physically separated. At location A, the mares were moved approximately 300 m away but still had auditory and partial visual contact with their foals. At location B, the mares were moved a greater distance away and had no visual or auditory contact with the foals after separation. At both locations, mares and foals remained as discrete groups after physical separation (i.e. no other horses were introduced). Data continued to be collected for four days after separation (Physical Separation period, PS: d9–12).

### 2.3. Behavioural data collection

#### 2.3.1. Live observations

Behaviour was observed by live observation by two trained independent observers. A Cohen's kappa test on trial observations noted consistency between the observers ( $\kappa = 0.89$  (95% CI, 0.82–0.96),  $p < 0.0001$ ). Observations occurred for eight hours per day from 0900 h to 1300 h and 1400 h to 1800 h. During this time, behaviour of all mares or all foals was sampled every ten minutes. The sampling was staggered every five minutes so the observer was recording either mare behaviour or foal behaviour. The behaviours sampled were: walking, running (any forward motion faster than a walk), standing idle, eating, defecating, vocalizing, aggressing, drinking and nursing. Aggressing was defined as the initiation or threat of a kick (orient hind quarters toward another individual with or without hind leg raised) or bite (ears laid back, neck stretched out, head in line with neck with or without mouth open) toward another individual if the individual the threat was aimed toward responded (e.g. moved away). Due to the small udder size of mares and the distance of the observers, it was difficult to determine when a foal actually had a teat in its mouth, therefore ‘nursing’ was defined as a foal's muzzle being in contact with the inguinal area of a mare. Instantaneous sampling at the five-minute mark was recorded for walking, running, standing idle, and eating, while defecations, drinking and aggressive acts were sampled using 1–0 sampling for the duration of each 5-min interval. The instigator

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