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Behavioural and physiological measures of welfare of pregnant mares fitted with a novel urine collection device

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Abstract

An alternative collection procedure for pregnant mare urine (PMU) has been developed in Australia, which allows mares to be loose housed either in indoor stables or outdoors in paddocks, rather than in tether stalls as is common practice in Canada and North Dakota. The present study examined the welfare risks to mares of collecting urine using this alternative procedure. The study involved 24 pregnant mares at 3–5 months of gestation. The mares were allocated to two treatments: 12 mares were fitted with the pregnant mare urine collection device and 12 mares acted as controls. All mares were housed in two large paddocks during the day, in two groups of 12. During the night, all mares were housed in six small enclosures in groups of four, while the PMU treatment mares wore the collection device.

Mares wearing the PMU collection device showed little or no behavioural change relative to the control mares. While brief bouts of forward lifting of the hind leg in the region of the PMU collection device were observed in some mares on day 1, this disappeared by day 2. Observations on the time budgets of behaviour in weeks 3 and 6–7 indicated similar patterns of behaviour in the two treatments. The only difference in behaviour was in lying behaviour. PMU collection mares spent less ($P < 0.01$) time lying during the night, with fewer horses observed lying down in the PMU collection treatment (4/11 versus 10/12). It is unclear what implication this may have on horse welfare since there was no evidence of a rebound in this behaviour when the device was removed. Based on heart rate and cortisol concentrations measured on days 1 and 2 of study, there was no evidence of acute stress associated with fitting the device. Limited measurements on salivary cortisol

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concentrations at weeks 6–7 of the study provided no evidence of an increase in basal cortisol concentrations or an increase in the sensitivity of the adrenal cortex to ACTH in the PMU collection mares. Therefore, it is concluded that wearing the PMU collection device at night for five nights per week for a total of 6–7 weeks does not pose a serious challenge to the welfare of pregnant mares.

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1. Introduction

There is a worldwide market for pregnant mare urine (PMU) to produce oestrogenic products used in human hormone replacement therapy. The segment of the horse industry presently involved in collecting PMU is concentrated in Canada and the USA (North Dakota), where more than 35,000 mares are housed on about 450 farms. Horses are housed in tether stalls for 5–6 months of the year, during which time some horses may have very limited opportunities for exercise outside the stall. In these PMU collection situations, horses wear a loose fitting, light-weight pouch, held in position by a system of pulleys. As a consequence of the restriction of movement of the mares, animal protection groups have voiced concern about the welfare implications of the housing system for this PMU collection procedure. An alternative collection procedure has been developed in Australia by Thorgard Pty. Ltd., which allows mares to be loose housed either in indoor stables or outdoors in paddocks. The present experiment examined the welfare risks to mares of collecting urine using this alternative procedure. The control for this examination was mares in which the collection device was not fitted but which were housed and handled in similar loose conditions. The welfare implications of the treatments were examined by measuring the behaviour and stress physiology of the horses in both the short and long term. The company intends to collect urine only during the evening and night and to limit collection to 5 days a week out of welfare considerations for the mares. This collection protocol was followed during the duration of the trial.

To study the short-term effects on the welfare of mares in the present study, cortisol concentrations, heart rate and behaviour before and after fitting the device were measured. After 6–7 weeks of treatment, basal plasma cortisol concentrations, adrenal responsiveness to ACTH and general behavioural activity were measured. Salivary cortisol is predictive of plasma cortisol in many species (Fell et al., 1985; Mendl et al., 1991, 1992) and this sampling procedure, which is generally non-traumatic, was used in this study to measure both basal salivary cortisol concentrations and salivary cortisol concentrations in response to a challenge with ACTH. The responsiveness of the adrenal cortex to ACTH administration may indicate a state of chronic stress when the secretion of cortisol is increased in response to an injection with ACTH (Terlouw et al., 1997). A long-term inability to perform a behaviour that is highly motivated (such as lying down, grooming and exploration) may lead to a stereotypy at the time of the restriction or a rebound in behaviour when the restriction is removed (Mueller et al., 1999). Specific mild and repeated localised stimulation that is disturbing may lead to excessive rubbing and in turn injury, and thus, both behaviour and

injuries were also monitored in the present study. Pressure and uneven weight distribution may result in localised pain of skin and muscle and even affect the spinal column, and therefore, a chiropractic examination was undertaken on the study horses.

2. Materials and methods

The study was conducted in March and April (autumn) at a horse stud in Victoria, Australia. A total of 24 mares of several breeds were used, although most were thoroughbreds and Australian Stock horses. They were sourced from three different breeders and ranged in age from 4 to 23 years old. All mares were weighed and confirmed pregnant, and were 3–5 months in gestation at the start of the study.

The mares were allocated to two treatments: 12 mares were fitted with the PMU collection device (PMU collection treatment) and 12 mares acted as control mares (control treatment). The PMU collection device consisted of a harness, a perineal silicon rubber pad and a urine collection bag as illustrated in Fig. 1. The harness, which stabilises and supports the other components, consists of: a breastplate; a stabiliser that passes over the wither; a sheet portion, which houses the straps and pads that support the urine reservoir; a fitted rump section, which supports the perineal pad. The perineal silicon rubber pad sits against the perineal body between the anus and vulva, and funnels urine into the collection bag. It is designed to prevent faecal contamination of the urine. The urine collection bag or reservoir is flexible and is connected to the perineal pad via tubing. Potential pressure points were protected as much as possible with the use of sheepskin for the breastplate; the use of close cell foam pads over the weight bearing areas of the back, which also raised the weight bearing straps up off the tips of the dorsal spinous processes of the vertebrae; the use of soft silicon rubber for the perineal fitting; making use of a fitted rump sheet; and preventing the mares from moving around too much, by keeping them confined to small paddocks.

The PMU collection mares wore the device for 15–17 h a day, 5 days of the week, for 6–7 weeks, while the control mares did not wear the device. The mares were housed at night in six small enclosures in groups of four consisting of two PMU collection mares and two control mares, and housed during the day in two large paddocks in groups of 12. Allocation



Fig. 1. The PMU collection device used in this study.

to treatments was random within the group of four mares allocated to the same small night enclosure.

Animals were gathered up twice daily at 08:00 and 16:00 h. At 16:00 h, the PMU collection mares were led into an indoor enclosure to fit the PMU collection device, and at 08:00 h, the mares were again led to the indoor enclosure to remove the PMU collection device. Control animals were treated in a similar manner and taken to the indoor enclosure, without the device being fitted or removed. The horses were housed in small enclosures of similar dimensions (average dimensions were 29 m × 15 m, with a range in area of 380–555 m²) in groups of four (two PMU collection and two control mares per group) during the night and urine was collected over night from the horses wearing the device. During the day, the collection devices were not worn and horses were turned out in two large paddocks of approximately 2 ha each. Routine husbandry (such as feeding, watering, veterinary inspection and supervision) was similar for all animals.

The allocation to six enclosures was as far as possible according to the source of the mares. Because the horses were housed in small enclosures during the night, it was important that the social hierarchy was established within each group. The horses were housed in their allocated groups 1 week before the start of the experiment. The groups were introduced to the PMU collection device in a staggered manner, with the first three groups during the first 3 days of the first week, and the second three groups during the first 3 days of the second week. The first three groups were allocated to the same day paddock and the second three groups were allocated to the other day paddock.

3. Standard protocol

At 16:00 h, the horses were moved from the group paddocks to their night enclosures. Good quality meadow hay with a 60% ryegrass and 40% clover mix was placed in the night enclosures to facilitate movement of horses from the day paddocks. After all horses had entered the night enclosures, they were taken in pairs consisting of one control mare and one PMU collection mare by two people into an indoor fitting area in the same order every day. They were either fitted with a device (PMU collection treatment) or received brief handling (brief stroking on neck and rump, control treatment). Once all horses were treated and returned to their night enclosures, they received a mixed feed consisting of whole oats, mixed cereal and lucern chaff (12% crude protein). A feed balance supplement formulated for pregnant mares was added as well as 60 g of canola oil.

At 08:00 h, the horses were led in pairs to the fitting area in the same order as at 16:00 h. After the urine collection bag was emptied, the device was removed from the PMU collection mares by two people in a standard manner. The control horses received brief handling and the pairs were returned to their night enclosures. Once all horses were returned to their night enclosures, the horses were moved to their day paddocks. Removal of all the devices from the PMU collection mares generally took about 1 h per day. Grass hay was provided ad libitum in the day paddocks.

4. Measurements

4.1. Veterinary inspection

One week prior to the start of the study, all horses underwent a visual inspection to determine pre-existing injuries and scars and an internal examination to confirm pregnancy. The veterinary inspection was repeated in weeks 6–7 to determine injuries and scars that may have been caused by the PMU collection treatment. The inspection was conducted by an independent veterinarian.

4.2. Chiropractic examination

The chiropractic examination was conducted by a qualified veterinarian and equine chiropractor. The examination included assessment of the physical effects of the device on the horse's skin, muscles and the spinal column. The horses were examined several days prior to commencement of the study, and again in week 7 within 5 days after the conclusion of the study. The examination included the following.

4.2.1. Gait/spinal restriction

Limb gait and spinal motion restriction were visually appraised and scored via an ordinal scale as described below. The mares were first walked 30 m in a straight line, and then directly back to the examiner. This procedure was repeated at a trot. Lameness was graded according to the American Association of Equine Practitioners lameness grading system (Stashak, 2002). Short stride was scored according to the estimated degree of stride restriction. Score 1 being mild; score 2, moderate; score 3, moderate to severe; score 4, severe. Shoulder, pelvic and lower back motion were graded in a similar fashion.

4.2.2. Skin soreness and muscle tenderness

The tenderness of the muscle was also scored via subjective analysis based on the reaction of the horse to gentle to firm prodding of the muscle concerned. Again an ordinal scale of measurement was applied. The prodding was done with the fingers and thumbs in a blunt fashion in such a way as would not be uncomfortable to normal human skin or muscle. Signs of tenderness exhibited by the horse include: the horse flinching or moving away to avoid contact; guarding of the area examined, for example via tight muscle contraction; throwing up its head or swishing its tail; muscle tightening or tensing with contact and skin twitching or flickering (Jeffcott, 1981; Martin and Klide, 1999; Harman, 2001). Signs of skin swelling or oedema and abrasion/ulceration were also noted.

4.2.3. Chiropractic (vertebral motion) analysis

Chiropractic analysis was performed on a regional basis. Regional motion restriction was subjectively assessed and scored on an ordinal scale as for the skin and muscle assessments. The subjective assessment was based on the degree of vertebral restriction and number of vertebrae restricted in each particular region. Vertebral motion was assessed inducing motion of the vertebral motion segments. A vertebral motion segment consists of two adjacent vertebrae and their interposed soft tissue structures (Hausler, 1999). The

motion segments were tested for mobility in lateral flexion and rotation. The method of testing involved inducing motion, via the examiner's hands, along the planes of the surfaces of the dorsal articular, or zygapophyseal, joints of the vertebrae. The sacro-iliac motion was tested by inducing motion along the plane of the sacro-iliac joint of either side. Motion was induced by imparting slow, rhythmic, gentle thrusts, through the joint planes. Restrictions of vertebral motion was scored as follows: score 0 being unrestricted regional mobility; score 1 mildly restricted regional mobility; score 2 moderately restricted regional mobility; score 3 moderate to severe regional mobility restriction; score 4 severely restricted regional mobility. The examination was focussed on detecting restriction greater than score 1. As a result, a restriction of score 1 is likely to be underestimated. At each examination, an attempt was made to accustom the horse to touch by repeated rubbing, starting with gentle rubbing and increasing to firm rubbing of the tissue.

Three to five days prior to the start of treatment, heart rate and behaviour similar to behaviour observations during 'day 1' were measured for 1 h between 15:00 and 16:00 h in all horses while they were housed in individual indoor enclosures. During this period a single saliva sample was collected from each horse for subsequent assay for cortisol concentrations. These data were used as covariates in the analysis of the treatment effects on heart rate and salivary cortisol. The body weights of the mares were also recorded before and after the study.

The PMU collection device was fitted for the first time on day 1 of week 1 of the experiment. Mares in groups 1, 2 and 3 commenced in the first week of study over three consecutive days (group 1 on Monday, group 2 on Tuesday and group 3 on Wednesday), while mares in groups 4, 5 and 6 started 1 week later on three consecutive days (Monday, Tuesday and Wednesday, respectively).

4.3. Behavioural observations

4.3.1. Day 1

All mares were housed in individual indoor stables after the first treatment imposition (PMU collection and control treatment). The mares were housed in the individual stables only for the first night to minimise injury that may occur to the PMU collection mare in the possible event of a flight response when wearing the PMU collection device for the first time in an open area.

Observations were made by two observers who observed two horses each by direct observation for 10 s every minute during the first hour. Observers each observed a control and a PMU mare with mares within treatment randomly allocated to observer. During each 10-s observation bout, mares were recorded as either being relaxed or alert, and as walking or standing still (whichever was the predominant behaviour during the 10-s bout). The definition of 'relaxed' was a mare that stood with the neck more or less level with the withers, and ears relaxed or pointed in one direction. A mare was considered 'alert' when the neck was raised to above the level of the withers and the ears were pointed in several directions in quick succession (Hutson and Haskell, 1999). All other behaviours, such as pawing, tail swishing, yawning, head shaking, rubbing against objects, lifting the hind leg forward towards the stomach, social interactions, vocalisations, urinating and defecating, were recorded when observed during the 10-s bouts.

4.3.2. Day 2

Video observations were conducted during the second night, while the animals were housed in their night enclosures in groups of four for the first time. Time-lapse video recorders and low-light video cameras fitted with auto-iris lenses were used. The paddocks were lit with 500 W floodlights covered with red heat-resistant theatre gel, to assist night-time video recording. Behaviours recorded from the video records were standing, eating, drinking, urinating, defecating and walking, rubbing against objects, lifting the hind leg forward at the device or stomach, lying down and locomotion. The behaviours were recorded every 10 min using the scan sample technique (Lehner, 1979; Mitloehner et al., 2001).

4.3.3. Weeks 3 and 6

Video observations, similar to those on day 2, were made during one night from 16:00 to 08:00 h at 3 and 6 weeks. Videos were analysed as for those recorded on day 2.

On another day during each of weeks 3 and 6, direct observations were made on all mares from 08:00 to 16:00 h after the removal of the devices from the PMU collection mares. Observations were made every 15 min using the scan sample technique. Observations were made on general activity and time budgets (eating, standing, walking, drinking and lying) and latency to urinate after the device was removed from the PMU collection mares. Observations commenced as soon as the horses were released in the day paddocks and were completed when horses were taken back to their night enclosures.

4.4. Physiological measurements

4.4.1. Day 1

Saliva samples were collected at 15 and 60 min after the treatment was first imposed. Saliva samples were taken with a Salivette (cotton wool swab, Sarstedt, Nuembrecht, Germany), held in the mouth of the horse by the observer for about 1 min. Each observer collected the saliva samples from both horses under observation within 3 min of entry to the stable, to minimise effects of saliva sampling on cortisol concentrations. No behaviour observations were made for 5 min during and directly after saliva collection. The saliva samples were placed on ice and then transported to the laboratory, where the saliva was extracted from the cotton wool using a centrifuge and stored at about -20°C until assayed. After thawing, saliva samples were centrifuged at 12,000 rpm in a microcentrifuge to sediment any particulate matter. The samples were analysed by a commercial RIA kit for cortisol (Orion Diagnostica, Turku, Finland) according to the protocol for salivary samples. The within- and between-assay coefficients of variation for cortisol concentrations of 3.4 and 13.3 nmol/L were 11 and 7%, and 17 and 10%, respectively.

Heart rate measurements were also taken during this first hour after the treatments were imposed on the first day. Polar heart rate monitors (Polar HorseTrainer S610TM) were used and were held in place by a girth strap. The heart rate monitors were attached 15 min before imposition of treatment to acclimatise the mares to wearing the heart rate monitors. Heart rate was measured every 5 s and the average heart rate over the test period was displayed and recorded.

4.4.2. Weeks 3 and 6

After the horses had been in treatment for 3 and 6 weeks, heart rate was measured during imposition of treatment. Heart rate monitors were attached to both control and PMU collection mares prior to the horses entering the indoor enclosure for treatment imposition. The heart rate monitors commenced recording when the horses were taken from the night enclosures to the indoor enclosure and were terminated when the horses returned to the night enclosure, which took on average about 4 min per pair.

4.4.3. End of study

Because of the staggered start of the experiment, horses were in treatment for 6–7 weeks at the end of the study. Several measurements were conducted during the last week of the study and the days following the completion of the study. Immediately prior to treatment imposition on the last day of the study, a saliva sample for basal cortisol concentrations was collected from each mare while in the indoor enclosure. Following this sample, each mare was injected with 100 IU ACTH im (0.25 mg/mL, Synacthen, Ciba-Geigy, Australia). A second saliva sample was taken 1 h after the ACTH injection while the mares were in the night enclosure. Saliva samples were collected by two observers and the sampling of the horses in each enclosure was completed within 3 min to minimise effects on cortisol concentrations by the collection procedure.

4.5. Gait analysis

A 3 m wide and 25 m long laneway was constructed with tape on a freshly raked sand surface. The horses were led through this lane way three times at both a walk and a trot, and each passage was recorded on video. The two PMU collection mares from each day enclosure were taken to the test area in pairs with one of the two horses wearing the device with the collection bag filled with 8 L of water (the average volume of urine collected each day). The other horse in the pair was observed while not wearing the device. After all horses were initially tested, the test was reversed. That is, the horses that were initially tested with 8 L of water in the PMU collection device were tested without the device and vice versa. Only horses in the PMU collection treatment were used for this measurement to compare the ease of movement with and without the device. Comparisons were made for each horse with and without the device for the following parameters: stride length (first full stride, direct measurement of hoof prints in the raked sand), time taken over 20 m and number of strides over 20 m (from video observations). The first 5 m of the laneway was used to ensure that the horse was moving in a straight line and had settled into a rhythm and was not included in the measurements. The other horse of the pair was held at the end of the laneway to encourage movement.

5. Statistical analysis

5.1. Measurements taken immediately after imposing treatments

Measurements that were taken immediately after imposing the two treatments were analysed using a paired comparison analysis of variance, with a pair taken to be the two

mares simultaneously observed by one observer. The majority of measurements were transformed, prior to analysis, to reduce the skewness of the data. Results are presented as backtransformed means. Angular transformations were used for most behaviour observations. A \log_{10} transformation was applied prior to analysis of the heart rate data, as well as for analysis of latency to urinate after removal of the PMU collection device.

Due to the highly discrete nature of the data for the number of kicks, these data were analysed using an exact Wilcoxon signed rank test. For the cortisol concentrations data, there were some missing values at 60 min due to insufficient saliva collected (four samples), and thus, these data were analysed using restricted maximum likelihood analysis (Payne, 2000) with random effects for enclosures and observer within enclosure, and fixed effects for group or enclosures and whether a PMU collection device was fitted.

5.2. *Subsequent measurements*

Subsequent data were analysed as a block design with two replicates for two treatments (PMU collection and control) within each block. The experimental unit was the individual mare. A number of the measurements were analysed after the data were transformed, and for these measurements backtransformed means are presented. For the chiropractic measurements, the same chiropractic measurement taken prior to the start of the experiment was used as a covariate. Other data collected prior to the study were not used as covariates, as these covariates had no significant effects on the variables studied.

6. Results

Two mares aborted during the trial, and both mares were in the PMU collection treatment. The mares were able to abort unassisted while wearing the collection device. The first mare aborted with twins 14 days after commencing the study due to placental insufficiency to carry twins. The foetuses were examined post-mortem at the Victorian Institute of Animal Science, Attwood, Australia. There was no evidence of infectious causes and the cause was considered unrelated to the device. This mare was removed from the group for 5 days after which she returned, without wearing the device. Data were only collected and utilised from this mare until the day before the abortion because of some general concern for her health. The second mare aborted 22 days after commencing the study. This mare was noticed showing signs of a uterine infection 7 days after the PMU collection device was first fitted, and a veterinarian was called. The foetus was confirmed dead by ultrasound 2 days later and the mare aborted 13 days after this diagnosis. The foetus was sent to the Victorian Institute of Animal Science, for post-mortem examination. The cause of death was most likely an ascending bacterial placentitis. The time of death of the foetus was estimated to be from 6 days prior to the mare commencing the study to possibly 10 days after the mare commenced the study, according to the age of the foetus and the last serving date of the mare. It is likely that the infection started before commencement of the study considering the likely interval between the start of the infection and the death of the foetus. Although it cannot be totally excluded, in the professional opinion of the independent attending veterinarian, the PMU collection device was not responsible for the mare aborting, or the death of and mummification of the foetus. The mare was retained in the

group while being treated. Five days after abortion, this mare was returned to treatment and data collected from this mare were included in the results. There were no signs of further infection after the mare recommenced wearing the collection device.

Measurements taken after treatment imposition for the first time are presented in Table 1. Behaviour observations during the first 15 min indicate that PMU collection treatment mares and control treatment mares behaved in a similar manner. There was no significant ($P > 0.05$) difference between treatments in the percentage of time the mares were relaxed and standing still. The mares were observed to mainly stand still and showed several other behaviours very infrequently, in fact too infrequent to be analysed statistically. Behaviours that were observed infrequently included head shaking, tail swishing, social interaction (nose to nose), vocalisation, urinating, defecating, pawing the ground and rubbing the head.

Measurements from 20 to 60 min after treatment imposition also showed few treatment differences (Table 1). There was no significant ($P > 0.05$) difference between treatments in the percentage of time the mares were relaxed and the mares in both treatments spent most of their time standing still. However, over the 60 min after treatment imposition, PMU collection mares were observed to significantly ($P < 0.05$) lift a hind leg more towards their stomach area, where the collection bag was fitted, than the control treatment mares. Out of the 12 horses fitted with the device, 7 horses lifted a hind leg towards the bag attached underneath the stomach. However, only three horses showed this behaviour frequently, with lifting a hind leg observed in either 11 or 12 bouts over a total of 55 observation bouts. This behaviour was too infrequent to analyse the treatment effects on raw means values but was analysed with an exact Wilcoxon signed rank test.

6.1. Observations during the night

The frequency of behaviour of mares in the two treatments during the first night in the night enclosure (day 2) is presented in Table 2. There were no significant ($P > 0.05$) differences between treatments in frequency of walking, standing, eating and lying down.

Table 1
Measurements conducted on the two treatments of mares after the first imposition of treatment on day 1

Measurement	Control treatment	PMU collection treatment	S.E.D.	<i>P</i> -value
Relaxed (%)				
0–15 min	66	64	9.45	0.882
20–60 min	60	74	6.47	0.184
Standing (%)				
0–15 min	96	93	4.98	0.518
20–60 min	94	93	3.60	0.891
Heart rate (beats/min)				
0–15 min	40.5	40.9	0.39	0.558
20–60 min	40.7	38.8	1.29	0.207
Cortisol (nmol/L)				
15 min	10.2	8.5	0.095	0.769
60 min	8.2	6.2	0.11	0.224

Behaviour reported as percentage of observation bouts in which the behaviour was recorded.

The average time spent in main activities over three observation periods was calculated from the video observations. The results of the analysis are presented in Table 2 and indicate that the mares spent most of the night eating and standing. There were no significant ($P > 0.05$) treatment effects on time standing still and time spent eating/grazing. There was a trend ($P < 0.1$) for control mares to walk more than the PMU collection mares (3.8% versus 2.9%). A significant difference ($P < 0.05$) was found in the time spent lying down, with control mares spending 2.8% of the observation time lying down, while PMU collection mares spent only 0.5% of the time lying down. Several mares were not observed to lie down during any of the three observation nights: two control mares and seven PMU collection mares. Other behaviours were observed very infrequently and no behaviours indicative of possible irritation with the device, such as kicking at the device or rubbing, were observed during these periods.

6.2. Observations during the day

There were no significant ($P > 0.05$) differences in walking, standing, eating or lying down between treatments during the day and most time was spent eating or grazing (Table 2). The remainder of the time was spent standing still and walking. Urinating, defecating, drinking and rolling were observed, but they occurred infrequently.

There were no differences ($P > 0.05$) between treatments in latency to urinate after the collection device was removed and the horses were moved to the day paddocks in weeks 3 and 6. While only two observation periods were used, the latency to urinate may have been affected by the weather. The weather was warmer during the first observation, which may have lead to increased water intake, and the latency to urinate was on average 18.7 min for control mares and 13.4 min for PMU collection mares ($P > 0.05$). On the days of the second observations, the weather was wet and much colder. Two horses in one paddock and four horses in the other paddock were not observed to urinate at all and in the statistical analysis the maximum time value (480 min) was used for these horses that did not urinate

Table 2

Behaviour of mares in the two treatments during the second night, the average during the second night, and nights in week 3 and week 6 and the average during the day in week 3 and week 6.

Measurement	Control treatment	PMU collection treatment	S.E.D.	<i>P</i> -value
Walking (second night)	3.9	3.3	0.008	0.476
Standing (second night)	48.3	47.3	0.036	0.794
Eating (second night)	39.7	42.8	0.033	0.361
Lying (second night)	1.8	1.6	0.010	0.806
Walking (night, average)	3.8	2.9	0.517	0.103
Standing (night, average)	50.0	53.3	2.29	0.163
Eating (night, average)	40.2	40.7	2.12	0.824
Lying (night, average)	2.8	0.5	0.63	0.002
Walking (day, average)	6.4	5.8	0.017	0.73
Standing (day, average)	8.9	6.9	0.014	0.178
Eating (day, average)	81.9	83.5	0.023	0.505
Lying (day, average)	0.8	1.0	0.008	0.809

Behaviour reported as percentage of observation periods in which the behaviour was recorded.

during the observation period. Average time to urinate was 67.4 min versus 59.6 min for control and treatment horses, respectively ($P > 0.05$).

7. Physiological measurements

7.1. Heart rate

During the first fitting on day 1 the average heart rate over 15 min was not significantly ($P > 0.05$) different between the two treatments. There were also no significant treatment effects on average heart rate from 20 to 60 min ($P > 0.05$, Table 1). Heart rate measured while mares were taken to the fitting area and during fitting with the collection device in weeks 3 and 6 is presented in Table 3. Heart rates were significantly lower ($P < 0.05$) in the PMU collection treatment than in the control treatment in both weeks.

7.2. Cortisol and ACTH challenge test

Salivary cortisol concentrations measured after 15 min and 1 h of imposing the treatments on the first day were not significantly ($P > 0.05$) different between the two treatments (Table 1). There was also no significant ($P > 0.05$) difference either in basal cortisol concentrations in weeks 6 or 7 or in response to ACTH (Table 3).

7.3. Gait analysis

The results of the gait analysis are presented in Table 4. There were no significant ($P > 0.05$) differences in stride length of first stride, number of strides and time taken over 20 m in walk and trot for the PMU collection mares with and without the collection device. However, there was a trend ($P < 0.1$) for mares with the PMU collection device to take more time to trot 20 m.

7.4. Body weight

Mares were weighed before and after the study. The average body weight was 514 kg before the start of the study and 559 kg immediately after the completion of the study.

Table 3

Heart rate measurements (average beats per min) taken at weeks 3 and 6 during treatment imposition and cortisol concentrations (nmol/L) in saliva before and 60 min after an ACTH injection

Measurement	Control treatment	PMU collection treatment	S.E.D.	<i>P</i> -value
Heart rate				
Week 3	50.9	44.4	0.0811	0.020
Week 6	47.9	40.2	0.1125	0.0064
Basal cortisol (pre-ACTH)	5.9	5.9	0.59	0.929
Cortisol 60 min after ACTH	34.9	35.6	2.58	0.789

Table 4

Gait characteristics (mean values) in walk and trot of PMU treatment mares with and without the collection device over 25 m

Gait characteristic	Without PMU collection device	With PMU collection device	S.E.D.	<i>P</i> -value
Number of strides in walk	10.4	10.8	0.21	0.160
Stride length first stride in walk (mm)	2154	2143	41.4	0.806
Time taken in walk (s)	12.6	12.6	0.42	0.903
Number of strides in trot	7.0	7.6	0.35	0.192
Stride length first stride in trot (mm)	2779	2587	132.2	0.219
Time taken in trot (s)	5.1	5.9	0.33	0.091

Weight gain over the study period was 48.6 kg for the control mares and 43.7 for the PMU mares ($P > 0.05$).

7.5. Chiropractic assessment

The mean scores of the chiropractic assessment are presented in Table 5. There were no significant differences between treatments.

7.5.1. Gait/spinal restriction

Apart from pelvic and lower back restriction, minimal lameness and short leg stride of more than mild nature, were observed. There was no indication of any change in gait or spinal restriction from pre- or post-study.

7.5.2. Skin soreness

No abrasion, ulceration, swelling or other indicators of skin soreness were noted in areas associated with the device pre or post-study. In a few cases, focal areas of swelling and soreness were noted. These did not appear to be associated with the device and were attributed to bites from other mares.

7.5.3. Muscle soreness

Assessment of both the control and treatment groups did not reveal any muscle soreness that could be attributed to the device. Mild lower neck muscle tension and reactivity were

Table 5

Mean scores of vertebral motion in lower back and hip region of mares in both treatments at the conclusion of the study

Measurement	Control treatment	PMU collection treatment	S.E.D.	<i>P</i> -value
Lower back and pelvic restrictions at gait (visual appraisal)	3.68	3.22	0.421	0.297
Lumbar motion restriction (palpable)	2.83	2.83	0.322	1.000
Sacroiliac and lumbosacral motion restriction (palpable)	3.7	3.74	0.303	0.878

the main features of the pre-study examination, but this was less apparent at the post-study examination. Other muscle soreness that was noted at the pre-study examination was less apparent at the post-study examination of the mares from both treatments.

7.5.4. Chiropractic motion analysis

Of the body regions associated with the device, only the lower back and pelvic regions exhibited more than mild motion restriction. However, there were no significant differences ($P > 0.05$) between treatments in the observed restrictions (Table 5).

8. Discussion

The measurements taken after the first imposition of the treatment and during the second night indicate that the horses had no serious problems adapting to wearing the collection device. The horses in the study were generally familiar with wearing rugs and saddles, and so the PMU collection equipment was probably not a highly novel experience. The significant increase in forward lifting of the hind leg in some horses fitted with the PMU collection device indicates some discomfort with this device on initial fitting. However, it should be noted that at no stage was aggressive kicking in the region of the device observed. Furthermore, only 3 of the 12 horses frequently showed this behaviour on day 1, and the behaviour was not observed during subsequent observations. Although scan sampling may not be sensitive to behaviours of short duration, adverse behaviours were also not observed during non-observation times. Rubbing, indicative of irritation, was not observed during initial fitting, or during later observations. This was also confirmed by the veterinary inspection, which found no sign of rubbing in the ano-genital area or any other part of the body of the PMU collection mares. General activity levels of the two treatments were very similar, indicating that no suppression of activity took place. The fact that general activity and the alertness were not increased in PMU collection mares suggests that there was no escape or avoidance behaviour in response to wearing the device. Heart rates and cortisol concentrations also indicate that the initial fitting of the PMU collection device did not increase the acute stress response above that associated with introduction to the fitting area and handling. These data suggest therefore that the PMU collection device, when fitted for the first time, was not painful, aversive, or physically restrictive for the mares.

Fitting in weeks 3 and 6 resulted in lower heart rates in horses in the PMU collection treatment. These horses received more handling in the indoor enclosure than horses in the control treatment. Although visual contact with humans was similar, PMU collection mares received more tactile contact during fitting of the collection device. Although the control horses received some attention from the handlers at this time, they were mainly left standing without close human contact. This additional human contact for the PMU collection mares, presumably habituating any fear responses to humans, may explain the lower heart rate in horses fitted with the device. It has been shown that frequent handling reduces the subsequent heart rate of horses in the presence of humans compared to horses that received minimal previous handling (McCann et al., 1988).

The time budgets of horses in the two treatments at night was very similar, with the exception of lying behaviour. Horses wearing the device laid down significantly less. Seven

of the eleven PMU collection mares were not observed to lie down at all during the three sets of night observations in comparison to only 2 of 12 control horses. However, it should be recognised that the control treatment mares were only observed to lie down for relatively short times at night. Furthermore, while the literature reports average total lying times of 2 h in a 24-h period (with most or all lying down occurring at night, [Waring, 1983](#); [Shaw et al., 1988](#)), which was not observed in the present study, lying times reported in the literature are highly variable with some horses not observed to lie down. There are reports that horses that do not lie down over very long periods may buckle at the knees and fall down while resting standing up ([Houpt et al., 2001](#)). This was not observed in the horses in the present study. Horses do not need to lie down for sleep, as they can sleep standing using a unique structure, the stay apparatus ([Adams, 1966](#)). Lying down during sleep is actually less energy efficient than sleeping while standing ([Winchester, 1943](#)). Nevertheless, horses in natural environments as well as stabled horses normally choose to lie down for several periods during the day. It is thought that the main function is to take pressure off the feet and joints, and may play a function in REM sleep (see [Waring, 1983](#)). It is possible that the night enclosures were restrictive because of their size, and did not encourage lying down. With close confinement in the small night enclosures, it was possible that horses that had trouble getting up quickly may have preferred not to lie down. Especially lower ranked horses wearing the device may have reduced their lying time, as [Fader and Sembraus \(2004\)](#) found that lower ranked horses tended to lay down for shorter periods than higher ranked horses when confined together in a relative small enclosure. Wearing the PMU collection device may slow the response time in getting up, and in turn discourage lying down. In addition, very short bouts of lying down may have been missed due to the method of observation (scan sampling every 10 min). Because the horses only wore the devices for five nights of the week, it is also possible that the horses did lie down during the two nights without the device. Furthermore, mares were only observed during three nights in total, so it is possible that the mares that were not observed lying down may have done so on other occasions when they were not observed.

During the day observation periods, when the horses were not wearing the PMU collection devices, the time budgets of mares in both treatments were very similar. The absence of a rebound effect in lying behaviour adds to the argument that the motivation of PMU collection mares to lie down when wearing the device was not high. Deprivation of a behaviour that is highly motivated may result in an increase in the behaviour once the animal is capable of performing the behaviour ([Mueller et al., 1999](#)). This rebound effect may result from an increase in motivation during deprivation and hence has been considered by some authors as potentially indicative of suffering ([Metz and Wierenga, 1984](#)). Most time in the day paddock was spent eating and only four horses were observed lying down, two control treatment and two PMU collection treatment horses. Nevertheless, the fact that wearing a urine collection device reduced lying behaviour is of potential concern and requires further investigation.

The measurements on basal salivary cortisol taken at the end of the study provide no evidence of a substantial activation of the hypothalamic–pituitary–adrenal axis. The ACTH challenge test taken after the horses had been wearing the PMU collection device for 6–7 weeks also provide no evidence of chronic stress in these mares. A chronic activation of the HPA axis can lead to an increased sensitivity of the adrenal cortex to ACTH ([Terlouw et al.,](#)

1997). This lack of treatment effects cannot be explained in terms of human contact confounding treatment effects. Saliva samples from a group of four horses in the enclosure were collected by two technicians and were generally taken within a 3-min period. This collection interval is likely to be insufficient for cortisol concentrations to have been affected by the handling associated with collection (Broom and Johnson, 1993).

While there was a tendency for horses to trot slower while wearing the PMU collection device, the overall gait analysis indicates that the device did not inhibit movement of the mares. The chiropractic examination indicated no effects of the device on muscle soreness and spinal restrictions and further supports the contention that the device did not inhibit movement.

Visual analysis and statistical analysis of the results for the observation of gait and spinal restriction, and manual examination of the skin, muscles and regional vertebral motion, of the mares of the control group and those that were fitted with the device, did not reveal any significant effects of the device. The absence of any detectable skin or muscular damage, as a result of the device, is quite surprising in that one would expect that at least one or two of the treatment mares would have shown some chafing or similar, from the harness. The anticipation of pressure points and methods of protecting them appear, from the results of this short-term study, to be very adequate.

The results of this study indicate that the device appears to cause no obvious welfare problems under the studied management condition, over a 6–7 week period. However, in a commercial situation the device will be worn for a 6–7 month period and as the mares progress with their pregnancy welfare problems cannot be excluded and need to be evaluated in a separate study.

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