Contents lists available at ScienceDirect

## Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

### Original Research

## Physiological Stress Responses of Mares to Gynecologic Examination in Veterinary Medicine

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#### A R T I C L E I N F O

Article history: Received 7 March 2016 Received in revised form 14 April 2016 Accepted 15 April 2016 Available online 30 April 2016

Keywords: Horse Rectal palpation Stress Cortisol

#### ABSTRACT

Based on salivary cortisol, heart rate, and heart rate variability (HRV), the stress response of horse mares (n = 21) to transrectal palpation and ultrasonographic examination of the genital tract was analyzed. Mares differed in experience with regard to the examination and were either pluriparous (n = 13) or in their first breeding season (n = 8). They also differed with regard to examination frequency and were either examined every 6 hours (n = 10) or at 24- to 48-hour intervals (n = 11). All mares were followed for three examinations and 13 mares for four examinations. We hypothesized that gynecologic examination causes a stress response, which decreases with repeated examinations. Heart rate increased (P < .01) during the veterinary procedure but neither changed from examination 1 to 4 nor differed between experienced and inexperienced mares. The increase in heart rate was higher in mares examined every 6 hours compared with every 24 to 48 hours. During gynecologic examinations, HRV did not change while cortisol concentration increased. The cortisol response was more pronounced in mares examined at 6-hour intervals (from 1.5  $\pm$  0.7 to 2.4  $\pm$  1.3 ng/mL) than in mares examined every 24 to 48 hours (from 1.5  $\pm$  1.1 to 1.9  $\pm$  1.2 ng/mL; time  $\times$  examination frequency, P < .01). No differences existed between experienced and less experienced mares and between examinations 1 and 4. In conclusion, transrectal examination of mares was not perceived as a major stressor, but, the response was influenced by examination frequency.

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#### 1. Introduction

Veterinary interventions are primarily judged by their outcome and by the ratio of costs to potential benefits. However, in case several diagnostic or therapeutic options exist, the least stressful approach for the animal should be chosen. The gynecologic examination of female horses and cattle includes transrectal palpation and usually also ultrasonography of the genital organs. Except in extremely uncooperative mares, this examination is performed without sedation of the animal. Several studies, however,

\* Corresponding author at: Natascha Ille, Centre for Artificial Insemination and Embryo Transfer, Department for Small Animals and Horses, Vetmeduni Vienna, 1210 Vienna, Austria. indicate that rectal palpation may be perceived as stressful in cattle [1–4]. Therefore, gynecologic examinations in large animals as part of research projects or veterinary student teaching in many countries are classified as animal experimentation requiring approval by an ethics committee or competent authority. Horses are flight animals and potentially dangerous or stressful situations can trigger a violent flight response. Potentially stressful challenges in horse mares during veterinary examinations therefore should not only be minimized or at best avoided for animal welfare reasons but may also endanger safety of the examining veterinarian and the horse itself.

The stress response of horse mares to transrectal gynecologic examination has been analyzed in two preliminary studies. In a study from our group based on fecal cortisol metabolite concentrations, rectal examination of







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<sup>0737-0806/</sup>\$ – see front matter © 2016 Elsevier Inc. All rights reserved. http://dx.doi.org/10.1016/j.jevs.2016.04.010

the ovaries and uterus four times per day (for artificial insemination with cryopreserved semen) evoked a measurable cortisol release, whereas no such response was detected in mares examined only once daily (for artificial insemination with fresh or cooled semen; [5]). In more recent studies, however, we have demonstrated that fecal cortisol metabolites are elevated only in response to either marked or prolonged stressors and are relatively insensitive to assess the effect of less pronounced transient stressors [6]. Based on physiological stress parameters, the response of horse mares to transrectal ultrasonographic pregnancy examination has recently been analyzed with inconclusive results. Interactions between the stage of pregnancy and lactation were suggested [7], whereas experience of the mare and frequency and duration of the examination were not taken into account.

Stressful stimuli initiate a hypothalamo-pituitaryadrenocortical, an adrenomedullary, and a sympathetic nervous system response. Short-term stress, as may occur in animals during veterinary procedures, increases cortisol release [8]. Nonprotein-bound cortisol rapidly diffuses into saliva and, as in humans [9], salivary cortisol concentrations mirror changes of free cortisol in blood of horses [10]. Acute stress may also elicit a shift of the autonomous nervous system toward sympathetic dominance as indicated by the release of epinephrine, an increase in heart rate, and a decrease in heart rate variability (HRV). Heart rate variability, that is, short-term fluctuations of the cardiac beatto-beat (RR) interval, reflects the oscillatory antagonistic influence of the sympathetic and parasympathetic branch of the autonomous nervous system on the sinus node of the heart. A decrease in HRV due to high sympathetic or low parasympathetic activity is interpreted as part of a stress response [11].

Based on salivary cortisol concentrations, heart rate, and HRV, we have analyzed the stress response of horse mares to transrectal palpation and ultrasonographic examination of the genital tract. Mares were either pluriparous broodmares well accustomed to gynecologic examinations or maiden mares in their first breeding season and thus differed with regard to experience. They also differed with regard to the frequency of examinations and were either examined four times per day for insemination with cryopreserved semen or at 24- to 48-hour intervals for insemination with cooled semen. We hypothesized that gynecologic examination does evoke a stress response in mares, which increases with frequent examinations but decreases once mares learn that the examination is not harmful.

#### 2. Material and Methods

#### 2.1. Animals

A total of 21 mares presented to the Centre for Artificial Insemination and Embryo Transfer at Vetmeduni Vienna were included into the study. Mares were aged 9.7  $\pm$  4.7 (mean  $\pm$  standard deviation [SD], range 3–19) years and from the following breeds: Warmblood sport horse n = 15, Quarter Horse n = 3, Standardbred n = 2, and Friesian n = 1. All mares included into the study were in estrus as

characterized by the presence of a follicle with at least 35 mm in diameter and uterine edema on ultrasound examination. Mares were either examined four times daily (6 AM, 12 PM, 6 PM, and 12 AM) for insemination with cryopreserved semen within 6 hours after ovulation (n = 10)or every 24 to 48 hours between 8 AM and 11 AM for preovulatory insemination with cooled shipped semen (n = 11). Eight mares were in their first breeding season and classified as inexperienced with regard to gynecologic examination, and 13 mares were at least in their second breeding season and classified as experienced. Four mares were lactating and 17 without a foal. All mares were followed for at least three examinations and 13 mares also for a fourth examination. Individual mares were examined five and six times, but because of the low number of animals, these examinations were not included in the analysis.

The mares remained at the Centre for Artificial Insemination and Embryo Transfer throughout the study. They were housed in individual loose boxes on straw or wood chippings depending on the preference of the owners. Mares had access to an outdoor paddock for 1 to 2 hours per day and were fed oats and concentrates three times daily and hay twice daily. Water was available at all times.

#### 2.2. Experimental Procedures

The study was approved by the Ethics and Animal Welfare Committee of Vetmeduni Vienna (protocol number ETK-08/03/2015). During examinations, horses were placed in stocks and restrained by a door at the back and a belt around the mare's neck in the front. In lactating mares, foals remained with their dams during examination and were held in front of the mare by one person. The mares did not receive any medications and were not sedated for transrectal examinations.

Examinations started with removal of feces from the rectum and transrectal palpation of the uterus and both ovaries followed by ultrasonographic examination of the ovaries and uterus in the same way. All examinations were performed by veterinarians with ample experience and training in equine reproduction, and examinations lasted 185.0  $\pm$  5.6 (mean  $\pm$  SD; range 120–300) seconds. Depending on the findings, mares were either scheduled for a follow-up examination or inseminated. Examinations that were directly followed by insemination of the mare were not included in the study.

#### 2.3. Salivary Cortisol

Saliva samples for cortisol determination were taken in the stable 30 and 10 minutes before gynecologic examinations, directly after examinations in the examination stocks and 15, 30, 60, and 120 minutes after examinations again in the stable. Saliva was collected as described [6,12] with cotton rolls (Salivette; Sarstedt, Nümbrecht-Rommelsdorf, Germany) placed onto the tongue of the horses with the help of a surgical arterial clamp for 1 minute until the cotton was well soaked with saliva. The cotton roll was then returned to the Salivette polypropylene tube and centrifuged at 1,000 g for 10 minutes. At least 1-mL saliva per sample was obtained and frozen at  $-20^{\circ}$ C until analysis. Download English Version:

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