Original Article

Post-partum concentrations of serum progesterone, oestradiol and prolactin in Arabian mares demonstrating normal maternal behaviour and Arabian mares demonstrating foal rejection behaviour

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A B S T R A C T

The aim of this prospective study was to investigate possible endocrine components to foal rejection behaviour in post-partum Arabian mares. Arabian mares were divided into two groups based on their maternal behaviour: (1) mares with normal post-parturient behaviour (8 mares); and (2) mares that demonstrated foal-rejecting behaviour post-partum (15 mares). Most mares were visited and sampled twice, in the first and third days post-partum. Serum samples were used for measurement of progesterone, oestradiol and prolactin concentrations. There were no statistically significant differences in oestradiol, progesterone or prolactin concentrations between the groups. In the rejecting mares, there was a statistically significant decrease in the progesterone (mean ± standard deviation, SD, 3.14 ± 6.2 ng/dL on day 1 and 0.49 ± 0.18 ng/dL on day 3) and prolactin (mean ± SD 216.2 ± 325.4 ng/mL on day 1 and 145.2 ± 311.4 ng/mL on day 3) concentrations between days 1 and 3, while the oestradiol concentration did not change significantly. In the non-rejecting mares, progesterone concentrations decreased significantly (mean ± SD 0.8 ± 0.23 ng/dL on day 1 and 0.43 ± 0.22 on day 3) while the oestradiol and prolactin concentrations did not change significantly. The oestradiol to progesterone ratio was significantly higher in non-rejecting mares on day 1 (mean ± SD 114.8 ± 140.2 on day 1 and 143.4 ± 72.6 on day 3) and this ratio increased significantly from days 1 to 3 in the rejecting mares (mean ± SD 47.3 ± 21.1 on day 1 and 122.1 ± 123.7 on day 3).

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Introduction

Maternal foal rejection is a serious problem in equine practice. Disruption of normal post-partum behaviour prevents the establishment of mare-foal bonding, prevents the foal from suckling colostrum and, when the mare is especially aggressive, it may severely harm the foal (Crowell-Davis and Houpt, 1986; Houpt, 1987; Asbury, 1993; Houpt and Feldman, 1993; Juarbe-Díaz et al., 1998; Żurek and Danek, 2012). The rejecting mare may avoid the foal and resist its suckling, or may be aggressive towards the foal. These behaviours are characteristic of a mare's reaction towards a foal that is not her own (Houpt, 1987; Houpt and Feldman, 1993; Juarbe-Díaz et al., 1998; Żurek and Danek, 2012).

Foal rejection is more prevalent in primiparous mares than multiparous mares, and in Arabian mares more than in other

breeds, with a prevalence of 5.13% in Arabian mares and 1.93% in Paint mares (Juarbe-Díaz et al., 1998); however, the underlying causes are unclear. Juarbe-Díaz et al. (1998) did not find differences between rejecting and non-rejecting mares in breeding methods, presence of people during parturition, presence of other horses nearby, or human assistance during parturition.

Mares with health problems after parturition may be more prone to reject their foals (Żurek and Danek, 2012). The higher prevalence of foal rejection in primiparous mares may be related to excess nervousness, inexperience and/or pain during suckling. Most primiparous mares and those that are not highly aggressive may eventually learn to accept their foal. In extreme cases, the mare's behaviour does not change and eventually the foal has to be separated from the mare and raised with milk replacement or by a foster mare (Żurek and Danek, 2012).

Maternal behaviour is influenced by hormones, but the importance and influence of hormones varies and findings in one species should be cautiously interpreted when applied to other species (González-Mariscal et al., 2007; Saltzman and Maestripieri,

2011). High plasma oxytocin levels have been related to normal maternal behaviour in human beings, primates, sheep, rats and mice (Siegel, 1986; Douglas, 2010; Gordon et al., 2010; Saltzman and Maestripieri, 2011; Strathearn et al., 2012). The association between plasma oxytocin concentrations and maternal behaviour is difficult to interpret due to the fluctuating nature of its secretion, as well as the importance of intracranial local effects. Furthermore, circulating oxytocin does not enter the central nervous system and thus may not necessarily be a reliable indicator of parental behaviour (Leng et al., 2008).

In human beings, a positive relationship has been found between pre-partum oestrogen concentrations and the quality of maternal-infant bonding and affection (Saltzman and Maestripieri, 2011); however, post-partum concentrations were not directly related to maternal behaviour. A relationship between post-partum progesterone plasma concentrations and aggressive maternal behaviour towards intruders was found in rats (de Sousa et al., 2010).

Prolactin appears to play an important role in establishing maternal behaviour in mammals and in nesting birds (González-Mariscal et al., 2007; Angelier and Chastel, 2009). Reports on abnormal maternal behaviour in human beings deal mostly with post-partum depression (PPD) (Brummelte and Galea, 2010; Wylie et al., 2011) and there is evidence of hormonal involvement in this syndrome. Since aggression and lack of interest in the infant are symptoms of PPD, as well as signs of foal rejection, it is possible that there is a common aetiology to both syndromes.

The aim of the current study was to identify hormonal differences between mares that exhibit normal maternal behaviour and mares that reject their foals. In addition, the study aimed to describe the abnormal maternal behaviours and the changes in these behaviours in the first few days after parturition.

Materials and methods

Mares

Arabian mares were identified and monitored in the first 24 h and on the third day after parturition. The mares were divided into two groups based on their maternal behaviour: (1) non-rejecting mares, with normal behaviours towards their foals, including allowing and prompting the foal to suckle, calling and searching for the foal if they are separated, protecting the foal from intruders, licking the foal and absence of signs of aggression towards the foal; these mares were randomly chosen from breeding farms and from healthy mares that were housed in the Koret School of Veterinary Medicine Veterinary Teaching Hospital (KSVM-VTH) for breeding purposes; and (2) rejecting mares, which demonstrated abnormal behaviours towards their foals, including indifference to the foal, threatening the foal, attacking the foal, with or without it approaching the udder, and any other aggressive behaviour towards the foal. In order to include a mare in the 'rejecting group', the behaviours had to be severe enough to warrant human intervention and did not resolve after the first suckling sessions. These mares were identified by field veterinarians and were either managed on the farm or at the KSVM-VTH. The study was approved and sponsored by the Clinical Studies Fund of the KSVM-VTH (2011–2012) and The Hebrew University of Jerusalem, Israel, ethics committee (approval number KSVM-VTH/023_2011; date of approval 20th May 2011).

Collection of mare data including maternal behaviour

For each mare, two forms were filled by the investigators with the aid of the owner and the attending veterinarian. The first form included pre-partum information on age, parity, names of the dam and sire of the sampled mare, and the sire of the foal, history of foal rejection in the mare's family, history of foal rejection or other previous post-parturient problems in the sampled mare, breeding method, health problems and treatments during the current pregnancy. The second form included parturition and post-partum information, and was filled on both sampling days. The form included questions on final gestation length, milk dripping before parturition, description of the delivery if attended, assistance during delivery, the foal's sex, the mare's behaviour towards the foal, human intervention, final outcome with regards to foal acceptance or permanent rejection, and any other problems. For most of the mares, information regarding history of foal rejection in the family was not available.

Collection of blood samples and analysis of hormones

Blood was collected within the first 24 h and on the third day post-partum via jugular venepuncture into plain vacuum tubes. Serum was separated by centrifugation at 1000 g for 5 min within 4 h of collection. The serum samples were stored at −80 °C until they were analysed. All non-rejecting mares were sampled twice; however, not all the rejecting mares were sampled twice due to logistical difficulties. Nine out of the 15 rejecting mares were sampled twice, two were sampled only on the first day and four were sampled only on the third day post-partum.

Oestradiol-17β (E2) and progesterone (P4) concentrations were determined by solid-phase, competitive chemiluminescent enzyme immunoassay using an Immulite 2000 analyser (Siemens). For serum progesterone concentrations in mares, the sensitivity and specificity were determined at 94% and 95%, respectively, with an intra-assay coefficient of variance (CV) of 9% (Relave et al., 2007). For serum oestradiol concentrations, there was intra-assay CV < 10% in all concentration ranges ($r = 0.993$) in comparison to the standard method (Yang et al., 2004). Serum prolactin analysis was performed in duplicate in one assay using the Horse Prolactin ELISA Kit (Cusabio), which has a range of detection of 2.5–1000 ng/mL, with an intra-assay CV < 15% according to the manufacturer.

Statistical analysis

Results were analysed using SPSS-PASW (IBM). The mean, median, standard deviation (SD) and interquartile range of each hormone on each sample day were calculated for each group of mares. The oestradiol to progesterone ratio (E2:P4) was calculated as a fraction for each day. Since the groups were relatively small and were not normally distributed, the Mann–Whitney U test was used for comparisons of each hormone, ratio and sampling day between groups. For comparisons between sampling days for each hormone and ratio, only mares that were sampled twice were used and the Wilcoxon signed-rank test for paired-samples was used. Fisher's exact test was used to compare between the most common breeding methods of each group. The Levene's test of equality was used to compare the variances. The level of significance was set at $P < 0.05$.

Results

Pre-partum and post-partum general information

Twenty three mares were included in the study; eight mares were defined as non-rejecting and 15 mares were defined as foal-rejecting. Details of age, parity, behavioural category, breeding method and sex of foals are presented in Table 1. The age range was 3.5–14 years (mean ± SD 5.2 ± 2.3 years). In the non-rejecting group, 5/8 (62.5%) mares were primiparous and none of the three multiparous mares had a history of foal rejection. In the foal-rejecting group, 7/15 (47.7%) mares were primiparous and 5/8 (62.5%) multiparous mares had rejected their previous foals. Most mares (21/23, 91.3%) did not have any medical problems before, during or after the parturition; one rejecting mare had a retained placenta and partial uterine prolapse that were treated in the field, while another rejecting mare had an uncomplicated retained placenta. In the foal-rejecting group there were nine fillies and six colts, while in the non-rejecting group there were five colts and three fillies (Table 1). There was no significant difference in the sex of foals between groups. There was a significant difference in the most common breeding method of each group ($P = 0.027$); most of the non-rejecting mares were inseminated with fresh semen (7/8) and the most common breeding method in the rejecting mares was natural cover (5/15).

Post-partum behaviour

Table 2 summarises the behaviours of the rejecting mares. One mare's behaviour data was not complete; thus, it was not included in the table. Most of the rejecting mares tried to move away from the foal (12/14, 86%), bite (9/14, 64%), kick (11/14, 79%) and/or make threatening gestures, such as ear pinning (9/14, 64%), when the foal approached the udder. Most of the rejecting mares did not resist hand milking (10/14, 71%) and were not aggressive towards people (8/14, 57%). The mares did not seem to have an unusually oedematous or painful udder. Restraint (holding, tying and/or