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Stress and reproduction in the mare including studies on the effect of ACTH on steroid hormone levels and oestrous behaviour

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Summary

The hypothalamo-pituitary-adrenal (HPA) axis interacts with the hypothalamo-pituitary-gonadal (HPG) axis at several sites and there are both species and stress related differences in how a particular stressor affects the gonadal axis. In the pregnant mare, negative effects of stress on reproduction are often measured in terms of effect on plasma progesterone levels or abortion rate. In cycling mares, plasma levels of sex hormones, oestrous cycle duration and ovulation rates are examples of end-points measured in response to stress. Disease and low nutrition levels have been shown to cause lowered progesterone concentrations and, in the case of inadequate nutrients, abortion in pregnant mares. In non-pregnant mares, poor body condition has been shown to delay the onset of the ovulatory season and lower conception rates. Transportation, on the other hand, does not seem to adversely affect reproductive success in either pregnant or cycling mares. Feral horses have shown significantly higher foetal loss if they were removed for adoption or gathered and then re-released into the wild.

The mare's adrenal gland is capable of producing progesterone and androgens. Such extra-ovarian hormone production may be a reason why some ovariectomised mares or mares with inactive ovaries show symptoms of oestrus. In a recent study, we found that 100 % (5/5) of ovariectomised mares showed from two to 78 days of behavioural oestrus during a three month period. In another study, we investigated whether mares believed by their owners to suffer from oestrous related behavioural problems had an altered hormonal response to adrenocorticotrophic hormone (ACTH), but found they did not differ from control mares. All mares in the study showed significant increases in progesterone, androstenedione and testosterone as a result of the ACTH treatment. In other species, oestradiol has been shown to have a stimulatory effect on adrenal hormone production. However, ovariectomy in the mare was found to have no clear effect on the cortisol response to ACTH. However, either time from ovariectomy or season seemed to effect basal cortisol levels, with ovariectomised mares having lower basal cortisol concentrations in November as compared to June. Subsequent oestradiol treatment in November had no effect on basal cortisol levels. Ovariectomised mares showed a lower response in androstenedione production after ACTH stimulation, indicating androstenedione of gonadal origin in intact mares.

Introduction

Stress activates the adrenal gland and often, the activity of the hypothalamo-pituitary-adrenal (HPA) axis is used as a measurement of stress. Where reproduction is concerned, there is substantial evidence that there is a close interaction between the HPA axis and the hypothalamo-pituitary-gonadal (HPG) axis (for review: Rivier and Rivest, 1991; Handa et al., 1994; Tilbrook et al., 2002). Alexander et al. (1996) extensively studied the HPA axis in the horse by a special technique sampling pituitary venous blood and showed how stressors such

as vigorous exercise and hypoglycaemia affected the HPA axis, but they did not study subsequent effects on the HPG axis. In an earlier study, Asa and Ginther (1982) showed that treatment with a synthetic corticosteroid (dexamethasone) in pony mares prevented the preovulatory rise in plasma luteinising hormone (LH) and suppressed oestrous behaviour and follicular growth. However, the exact mechanisms of HPA-HPG interactions in the horse remain elusive and most data are derived from other species, such as rats, sheep and non-human primates.

Not all stressors are accompanied with an increase in cortisol; for example, the stress of nutritional deprivation and dehydration (Rushen, 1991; Houpt et al., 2000). Also, plasma corticosteroid levels may not be well correlated with the severity of the stressor, as shown in studies in rats using different shock intensities (Friedman et al., 1967). In addition, sexual arousal, which appears to be stressful in that it causes an increase in cortisol levels, also leads to a stimulation of LH (Irvine et al., 1983). Instead, a broader definition of stress is “the inability of an animal to cope with its environment . . . , often reflected in a failure to achieve genetic potential” as suggested by Dobson et al. (2000), and not simply increased cortisol levels. Thus, nutritional compromise may be considered a stressful event, even though it is not associated with elevated concentrations of cortisol.

This paper will review some aspects of how stress can affect reproduction in the pregnant and non-pregnant mare, including feral horses. This review is by no means complete, but provides some examples of factors that may or may not influence reproduction in the mare. Also, the paper includes a summary of some own recent studies on the effect of adrenocorticotrophic hormone (ACTH) on oestrous behaviour and adrenal hormone production in the mare.

Effect of stress in the pregnant mare

Pain and disease

A South African study investigated the effect of disease on plasma progestagen levels in 80 pregnant Thoroughbred brood mares (van Niekerk and Morgenthal, 1982). They showed that plasma progestagen levels fell in mares with colic, laminitis and navicular disease. It was concluded that the stress of severe pain could cause a decline in progestagen levels in the pregnant mare. Infectious disease, such as babesiosis, with anaemia and hyperthermia, also resulted in drops in plasma progestagen concentrations in two mares. Weaning of a foal seemed to signify a stressful event, with acute falls in progestagen levels in some mares. That the fall in progestagen was probably associated with a rise in cortisol was demonstrated by the administration of prednisolone to two mares, which caused a drop in progestagen concentrations. This study did not reveal if any of the mares aborted as a result of the lowered progestagen levels. Some of these conditions (e.g. colic and laminitis) are also associated with Gram-negative bacteria and the production of endotoxins, which cause an increase in prostaglandin concentrations that in turn have a negative effect on progesterone levels (Fredriksson et al., 1985; Daels et al., 1991). In another study, pregnant mares (gestation length 17 – 336 days) presented with abdominal disorders (uterine torsion, surgical- or medical colic) were blood sampled at admission and every 12 hours thereafter for cortisol, progestagen and oestrone sulphate content (Santschi et al., 1991). The mares that required surgical correction and subsequently aborted had the greatest mean cortisol concentrations. Progestagen concentrations declined in six of the mares of which five aborted and one delivered a compromised foal that later died. Oestrone sulphate levels fell after admission in all mares and thereafter rebounded, which the authors suggest may indicate fetal stress. However, no clear trend in the three hormones measured could be observed and thus were not considered useful in evaluating foetal viability in a clinical setting.

Nutrition

Keeping mares on a low level of nutrition after conception may cause resorption of the conceptus (van Niekerk, 1965). Also, the same author has shown that removing the concentrate ration from two pregnant mares carrying twins caused a drop in plasma progestagen concentrations (van Niekerk et al., 1983). This nutritional stress caused one mare to lose one of two foetuses and the other, both foetuses. In fact, the effect of reduction of food intake in pregnant mares has been utilised as a method to reduce twin pregnancies. Merkt et al. (1982) showed that by removing all concentrates and alfalfa (leaving mares on a diet of only grass hay) resulted in 60 % of mares, diagnosed as carrying twins between weeks three and seven of gestation, giving birth to single live foals. In 20 % of the mares both embryos were resorbed. Feral mares in poor physical condition have been found to be less often pregnant than mares in good body condition (Plotka et al., 1988). In an unmanaged population of wild ponies, a lower foaling rate was attributed to the nutritional stress of being pregnant and lactating together with an intake of poor-quality forage (Keiper and Houpt, 1984). Another study of domestic horses showed that subjecting pregnant mares to water restriction (up to 42 % of *ad libitum* water intake) caused a lowered feed intake, dehydration and a loss of body weight. However, no increase in cortisol levels was observed and none of the mares aborted (Houpt et al., 2000). The mechanisms behind abortions caused by decreased feed intake may be due to the metabolic changes seen during fasting. Diminished glucose availability and increased free fatty acid and arachnidonic acid concentrations have been observed during fasting and seem to cause an augmented prostaglandin synthesis. This increase in prostaglandin can induce labour in the mare during the second half of gestation (for review: Fowden et al., 1994).

Transportation

There is conflicting evidence as to what consequence transportation may have on abortion or early embryo loss. One study showed no effect of transportation for nine hours on early embryonic death during the third or fifth week of gestation (Baccus et al., 1990a). The transported mares in the study did, however, have increased levels of cortisol and progesterone in plasma, indicative of adrenal gland stimulation. After transportation, there was a decrease in plasma progesterone concentrations, which was suggested to be due to suppression of LH by feedback from the increased adrenal hormones. Nonetheless, progesterone values in the transported mares never declined below what is considered necessary for maintenance of pregnancy (Shideler et al., 1982). Another study reported an abortion rate of 50 % at all stages of gestation in mares transported to an abattoir (Osborne, 1975). The transportation in this study involved rough handling, food and water deprivation as well as long distances. In contrast, in seven pregnant mares in the final third period of gestation that developed salmonellosis subsequent to long distance transportation, no abortions occurred despite the stress of disease and transport (however, two mares died post transportation) (McClintock and Begg, 1990). Mares seem to be most susceptible to fetal loss induced by *Salmonella* endotoxin during the first 50-60 days of gestation, which may explain why abortions did not occur in these mares (Daels et al., 1987).

Gather-and-removal stress of feral horses

A study investigating the effects of gathering and removal of pregnant feral mares, showed that mares removed for adoption, as well as mares that were gathered and then re-released into the wild, had significantly lower reproductive rates than ungathered mares as measured by foetal loss (Ashley and Holcombe, 2001).

Exercise deprivation/confinement

Pregnant mares kept in straight stalls without regular exercise were not found to be stressed as assessed by cortisol concentrations, response to ACTH treatment and behavioural changes (Haupt et al., 2001). However, as stated in the introduction, not all stressors are accompanied with an increase in plasma cortisol levels. The mares did experience ventral oedema and oedema in the limbs as a result of the lack of exercise.

Effect of stress in the non-pregnant mare

The majority of studies concerning stress and reproduction in the horse have focused on the pregnant animal and there are few studies on how stress may affect non-pregnant mares.

Nutrition

Studies have shown that mares entering the breeding season in a thin condition have a longer interval to the first ovulation of the ovulatory season, a longer first oestrus and require more cycles per conception than mares in fat condition (condition score > 5, body fat > 15 %) (Henneke et al., 1984; Kubiak et al., 1987). In another study, mares in poor body condition and experiencing cold temperatures showed a delay in the onset of ovulatory cycles in the spring (Oxender, 1977).

Transportation and exogenous corticosteroid treatment

Twelve hours of transportation during the preovulatory stage of oestrus increased cortisol levels in mares, but did not affect ovulation rate or the number of conceptions after artificial insemination (Baccus et al., 1990b). Neither the mares' oestrous behaviour nor oestrous cycle duration were affected. Plasma concentrations of oestrogen and LH remained similar between transported and non-transported groups. In another study, when exogenous corticosteroid treatment was given (no transportation), the preovulatory LH peak was prevented and both oestrous behaviour and follicular growth were suppressed (Asa and Ginther, 1982). The divergent results may be due to the effect of endogenous corticosteroids as opposed to exogenous treatment (concentration and duration).

Adrenal gland hormone production in the mare

The adrenal gland is capable of secreting other hormones than cortisol, such as the sex steroids. Adrenal production of progesterone upon adrenal stimulation has been described in a wide variety of species, such as deer, swine and sheep (Jopson et al., 1990; Tsuma et al., 1998; van Lier et al., 1998). Also, androgens are secreted when the adrenal gland is stimulated, as shown in humans and Rhesus monkeys (Baird et al., 1969; Resko, 1971). Some research on the equine adrenal gland's capability to produce sex steroid hormones has been published. In one *in vitro* study on adrenal cortex cells, it was concluded that the mare's adrenal gland secretes more androstenedione than testosterone (Silberzahn et al., 1984). Progesterone and oestradiol were not measured in this study. Watson and Hinrichs (1996) demonstrated that treating ovariectomised mares with ACTH led to an increase in the plasma levels of cortisol and testosterone, but not in oestradiol or progesterone levels. A pilot study performed by the authors of this review demonstrated an increase in both testosterone and progesterone when intact mares were treated with ACTH (Dalin et al., 2002b). Interestingly, the most nervous mare in the study showed the highest adrenal testosterone levels, whereas the most calm mare, the lowest.

Oestrous behaviour of non-ovarian origin

Ovariectomised mares and mares with inactive ovaries, have been shown to display oestrous signs (Asa et al., 1980; Hooper et al., 1993; Dalin et al., 2002a). Such unexpected oestrous behaviour has been suggested to be caused by sex steroids of adrenal origin, since dexamethasone treatment, which inhibits the adrenal gland's endogenous hormone production, diminishes the behaviour (Asa et al., 1980). The effect of dexamethasone may also in part be due to corticosteroid affinity for the progesterone receptor, which too would have an inhibiting effect on oestrous behaviour (Asa and Ginther, 1982).

Recent research

Nymphomania

Some owners claim that their mare shows abnormal and/or exaggerated oestrous behaviour for no apparent reason (i.e. no ovarian tumours, urinary infection, back pain etc.). The term 'nymphomania' is often used for such mares, both by the owners and sometimes, also by veterinarians. However, there is very limited research concerning such cases. There is thus a need to determine if nymphomania in the mare really exists and, if so, what the causes of such behaviour are. There have been suggestions that an aberrant production of adrenal hormones may be a cause of nymphomaniac behaviour in mares (Roberts and Beaver, 1987).

Mares with suspected behavioural abnormalities

In 2003, we studied seven cases where the owner reported that their mare suffered from oestrous related behavioural problems (Hedberg et al., unpublished). The mares were all given a thorough clinical examination, including back palpation, flexion tests of all limbs, blood- and urine samples to rule out infections, and thorough gynaecological examinations. None of the mares showed any abnormalities during the clinical examinations. All mares were then followed for at least two complete oestrous cycles and their behaviour recorded. During two oestrous phases (follicle size > 30 mm and oedema in the uterus), mares were given an intravenous injection of saline (control cycle) or ACTH (0.5 ml Synachten) (treatment cycle) and blood samples taken every half hour for three hours and thereafter hourly for a total of 24 hours. Five control mares were similarly treated. The blood samples were analysed for cortisol, progesterone, testosterone and androstenedione content. All mares showed an increase in all hormones as a result of the ACTH injection. There were no differences between control horses and mares reported to have oestrous related behavioural problems. This was perhaps not surprising, since only three of the seven 'problem' mares displayed aberrant/exaggerated behaviours related to the oestrous cycle during the subjective behavioural evaluation. It was concluded that the mare's adrenal gland is capable of secreting testosterone and androstenedione, as previous studies have shown, but also progesterone, which is in contrast to Watson and Hinrichs (1996), but agrees with studies performed in other species (Jopson et al., 1990; Tsuma et al., 1998; van Lier et al., 1998).

Ovariectomised mares

In 2004, five mares used in the above study were ovariectomised (Hedberg et al., unpublished). They were thereafter teased with a stallion daily from July until October. In July and November, the mares were, one at a time, let loose in a paddock for ten minutes with the stallion, to allow more tactile contact and more natural behaviour. This 'paddock teasing' was performed weekly on three occasions in July and once in November. During conventional teasing, all mares showed from two to 78 days of oestrus during July-September. During the 'paddock teasing', three mares showed full oestrus (as defined in Asa et al., 1980) on at least two occasions in July, and one mare, on the occasion in November.

The mares were also treated with ACTH, as described for the intact mares, to see what effect ovariectomy would have on the adrenal gland response upon stimulation. In other species, such as the rat and ewe, oestradiol seem to have a stimulatory effect on the HPA axis (Kitay, 1963; Coyne and Kitay, 1968; Viau and Meaney, 1991; van Lier, 2003). The response to ACTH was compared to that of the previous year, when the mares were intact. Ovariectomy in the mare led to significantly decreased cortisol levels in the time-period four to nine hours after ACTH treatment, but there was no clear effect on the cortisol response in direct connection with the ACTH treatment. Oestradiol was measured in samples up to five hours after ACTH treatment, but no increase was found, in accordance with results from Watson and Hinrichs (1996).

The mares were thereafter treated with oestradiol (2.5 mg/day i.m.) prior to a second ACTH treatment, to further evaluate a possible stimulatory effect of oestradiol on the adrenal gland. However, before treatment with oestradiol was begun, control samples over 24 hours were taken to evaluate the effect of season/time from ovariectomy on basal cortisol levels, since the experiment was to be performed in November. This revealed overall lower cortisol levels as compared to samples taken in June. There was no difference in basal cortisol levels with oestradiol treatment and these were also lower than the cortisol concentrations in June. Hence, oestradiol treatment did not seem to effect basal cortisol levels, but either season or time from ovariectomy, seemed to have an effect. ACTH treatment was unfortunately only performed in the oestradiol treated animals, and resulted in a lower cortisol response, but a greater response in progesterone. However, if this was due to an effect of season, time after ovariectomy or the oestradiol treatment is not known. Intact mares showed a greater response in androstenedione production after ACTH stimulation, perhaps due to androstenedione of gonadal origin. Testosterone analyses have not yet been performed.

In conclusion, the mare adrenal gland can produce androgens and progesterone, but not oestradiol. Intact gonads are not required for the mare to display oestrous symptoms. Further studies concerning adrenal hormone production in the mare are needed.

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