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Increased cortisol release and transport stress do not influence semen quality and testosterone release in pony stallions

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ABSTRACT

The use of breeding stallions for equestrian competitions requires that fertility is not negatively affected by competition or transport to the competition site. In this study, effects of cortisol release induced by road transport (600 km), adrenocorticotropic hormone (ACTH) administration (3 × 0.5 mg synthetic ACTH) and placebo treatment on semen quality and testosterone release were investigated in Shetland stallions (N = 13) using a crossover design. Saliva for cortisol and blood for testosterone analysis were collected for 10 weeks after treatments. Semen was collected daily for 5 days directly after treatments and twice weekly for another 9 weeks. Total sperm count, sperm morphology, motility, and membrane integrity were analyzed. We hypothesized that elevated cortisol decreases testosterone concentration and semen quality. Cortisol concentrations increased in response to transport and ACTH ($P < 0.001$) but not control treatments (peak concentration, transport: 7.6 ± 2.4, ACTH: 13.7 ± 1.5, control: 3.8 ± 0.9 ng/mL). No treatment effects on testosterone existed. Total sperm count decreased with daily semen collections in week 1 ($P < 0.01$) but did not differ between the treatments. The percentage of motile, progressively motile, membrane-intact, and morphologically defective spermatozoa did not change over time from Days 2 to 6, and there existed no differences between the treatments. In conclusion, road transport evoked a stress response which was mimicked by ACTH treatment. Both treatments had no effect on testosterone release and semen quality. Testicular function in stallions is apparently well protected against transiently elevated cortisol concentrations, and stallions can be transported over longer distances without negatively affecting their fertility.

1. Introduction

Sport horse stallions are often used for breeding and equestrian competitions in parallel. This requires that the stallions' fertility is not negatively affected by competition and training. Stallion owners and riders are often concerned that either sport or breeding activity could compromise the stallion's performance for the other purpose. In sexually experienced stallions, regular semen collection is not perceived as stressful [1]. Also, semen quality was not impaired in sport horse stallions entered in competitions either occasionally or on a regular basis [2]. However, equestrian sports require regular transport of the horses to the competition site. With an increase in

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international equestrian events by nearly 30% from 2009 to 2012 [3], both the number and the distance of horse transports have grown considerably. On the basis of analysis of cortisol release, road transport is far more stressful for horses [4–6] than equestrian training and competitions [7–10].

Species differences exist in the response to stress and the neuroendocrine regulation of reproduction. Glucocorticoid hormones are important mediators of the stress response. In some species, glucocorticoids suppress reproductive function at the hypothalamic, pituitary, ovarian, and also uterine level [11]. Thus, in male rats, boars, and humans, glucocorticoids inhibit the expression of proteins involved in testosterone biosynthesis [12–14].

Although stressful events may contribute to low reproductive efficiency in several species, effects of transport stress on semen quality in the horse have not been studied so far. It was thus the aim of the present study to investigate the influence of road transport and elevated cortisol concentrations on steroidogenesis and semen quality in the horse. We hypothesized that an increase in cortisol concentrations caused by 12 hours of road transport will lead to a temporary decrease in testosterone concentrations and a transient impairment of semen quality. Because the effects of transport are mediated *via* cortisol, they can be mimicked by repeated injection of adrenocorticotropic hormone (ACTH).

2. Materials and methods

2.1. Animals

A total of 13 fertile Shetland stallions were used in this study. All stallions were well accustomed to semen collection and transport. From the stallions, ejaculates are collected and examined on a regular basis (two to three times per week) either for breeding or teaching purposes throughout the year. At the start of the experiment, the animals were aged between 5 and 22 years (10.9 ± 1.9 years) and weighed between 118 and 199 kg (157 ± 8 kg). Stallions were kept in two groups in spacious stables. One group ($n = 5$) had access to an outdoor paddock from 7:00 AM to 6:00 PM, and the other group ($n = 8$) had access to a paddock at all times. The stallions were fed hay twice daily, and water was freely available.

2.2. Experimental procedures

Experiments were carried out from March to October. All stallions were transported over a distance of 600 km (treatment T; transport time approximately 12 hours) and received treatments of synthetic ACTH (Synacthen, 0.25 mg/mL; Defiante Farmaceutica, Funchal, Portugal) on 1 day (three treatments of 0.5 mg at 4-hour intervals; treatment A) or received 0.9% NaCl as the control (treatment C). All stallions received all three treatments in a triple crossover design and thus served as their own controls. The interval between the treatments was always 10 weeks.

The study was approved by the competent authority for animal experimentation (Austrian Federal Ministry for Science and Research, license number BMWF-68.205/0230-II/3b/2012).

2.3. Transport

Always four to five pony stallions were transported together in a standard horse trailer (floor size 3.4×1.7 m). They were not tied and allowed to move loosely in the trailer. Transport was started immediately after loading and followed two-lane national roads in Lower Austria State, Austria. The region is predominantly flat and the transport neither led through bigger cities nor through hilly or undulating terrain. Every 2nd hour, the transport was stopped for collection of saliva samples. The stallions received hay on the trailer and water every 4 hours. Immediately after the end of the transport, they were unloaded and returned to their stable or paddock.

2.4. Sample collection and hormone analysis

Saliva for cortisol determination was collected at 60-minute intervals starting 2 hours before experimental treatments. Further saliva samples were taken at 2-hour intervals until 16 hours after treatment. During the subsequent 5 days, saliva samples were taken three times per day (6:00 AM, 12:00 PM, 6:00 PM) and once a week during the following 9 weeks. Saliva was collected with cotton-based swabs (Salivette; Sarstedt, Nümbrecht-Rommelsdorf, Germany) as described [9]. The Salivette was placed onto the tongue of the horses with the help of a surgical arterial clamp for at least 1 minute until it was well soaked with saliva. The cotton roll was then returned to the Salivette polypropylene tube and stored at 4 °C until centrifugation at the end of the transport or centrifuged within 10 minutes at $1000 \times g$ for 10 minutes. At least 1-mL saliva per sample was obtained and frozen at −20 °C until analysis. The sampling procedure was well tolerated by all stallions and conducted by a single person without restraining the animal.

Cortisol was determined by direct enzyme immunoassay without extraction as described [9]. The antibody was raised in rabbits against cortisol 3-CMO-BSA in the authors' laboratory, and cortisol obtained from Steraloids (Wilton, NH, USA) was used for preparation of the standard curve. Because the antiserum cross-reacts with cortisone and several corticosterone metabolites, values obtained have to be interpreted as cortisol immunoreactivity. The intra-assay coefficient of variation determined from duplicates of a control saliva in each assay was 4.5%, the interassay variation was 11.7% ($n = 66$), and the minimal detectable concentration defined as two standard deviations from zero binding was 0.05 ng/mL.

For testosterone analysis, blood was collected from one jugular vein into polystyrene tubes containing lithium heparin (Vacuette; Becton Dickinson, Schwechat, Austria). On the experimental days, blood was taken one hour before start of individual treatment (5:00 AM) and again in the late afternoon (6:00 PM). Further samples were collected daily for 5 days and afterward once weekly for 9 weeks. Blood samples were centrifuged immediately after

collection for 10 minutes at 3000 ×*g*; the supernatant was aspirated and frozen at −20 °C until testosterone analysis.

Testosterone concentrations were determined by direct enzyme immunoassay without extraction (Testosterone ELISA; Demeditec Diagnostics, Kiel, Germany). Wells were coated with a mouse monoclonal antitestosterone antibody. The assay was validated for equine plasma in our laboratory. Recovery of testosterone standard added to plasma was 100%, and increasing dilutions of plasma samples resulted in changes in optical density parallel to the standard curve. All samples were assayed in duplicates. The intra-assay coefficient of variation determined from duplicates of a control saliva in each assay was 7.4%, the interassay variation was 8.4% (n = 9), and the minimal detectable concentration was 0.01 ng/mL.

2.5. Semen collection and semen analysis

Semen was collected with an artificial vagina (Hannover model; Minitube, Tiefenbach, Germany) on a dummy daily for 5 days after the individual experimental treatments (treatments T, A, C) and twice a week for the following 9 weeks. For semen collections, stallions were exposed to a teaser mare until erection and readiness to mount, followed by mounting of the dummy. Immediately after collection, the gel fraction of the ejaculate was removed. Semen was filtered through sterile gauze, and volume and color were determined. Sperm concentration was measured by NucleoCounter (ChemoMetec, Allerød, Denmark). Total sperm count was calculated from volume and sperm concentration. The pH was determined with test strips (Merck, Darmstadt, Germany). Sperm morphology was evaluated by an experienced laboratory assistant as previously described [15]. Spermatozoa were evaluated in a wet mount preparation as unstained samples fixed in buffered formal saline (1:4 ratio). At least 200 spermatozoa were checked for morphologic aberrations (abnormalities of acrosome, head, neck, midpiece, and tail) under a phase-contrast microscope with oil immersion (magnification: ×1000). The percentage of motile (total motility), progressively motile spermatozoa, and membrane-intact spermatozoa was evaluated with a computer-assisted sperm analyzer (SpermVision, Minitube) as described [16,17]. Thirty frames per field were evaluated. To select cells from debris, the camera recognizes the position of the sperm heads in successive frames. At least seven fields per sample with approximately 100 cells per field were evaluated. Spermatozoa with average orientation change less than 8 μm were considered immotile. Spermatozoa with curvilinear velocity 10 μm/s or greater, distance straight line 6 μm or greater, and radius 15 μm or greater were considered progressively motile. For assessment of sperm membrane integrity, 100 μL of semen was mixed with 2 μL of SYBR-14/propidium iodide and incubated for 10 minutes at room temperature in darkness. One droplet was placed onto a glass slide, covered with a glass coverslip, and evaluated by fluorescence microscopy at magnification × 400 (Olympus AX70; Olympus, Vienna, Austria; U-MWB filter block, BP420–480 excitation filter, BA515 suppressor filter, dichromatic mirror: DM500). At least 15 fields were evaluated, and the average value was calculated by the computer-assisted sperm analyzer system. Results are given as percent of membrane intact cells. For further analysis, the mean of the two weekly samples in weeks 2 to 9 was calculated.

2.6. Statistical analysis

Statistical analysis was performed with the SPSS statistics program (version 20.0; IBM-SPSS, Armonk, NY, USA). Data were analyzed for normal distribution by the Kolmogorov–Smirnov test, and for nonnormally distributed parameters, data were log-transformed before further analysis. Changes in testosterone and salivary cortisol concentrations and semen parameters were determined by ANOVA using a general linear model for repeated measures with time and treatment as within subject factors, thus taking into account the successive treatments in the same animals. A P value less than 0.05 was considered significant. All data given are the mean ± standard error of the mean.

3. Results

Cortisol concentrations in saliva of stallions increased significantly during road transport and in response to ACTH injections ($P < 0.001$ over time) but not after control treatment (Fig. 1). Mean salivary cortisol concentrations on the day of treatments did also differ between transport and ACTH treatment (overall effect $P < 0.001$, individual *post hoc* comparisons T vs A vs C at least $P < 0.05$; interactions treatment × time $P < 0.001$). Peak cortisol concentration toward the end of transport was 7.6 ± 2.4 ng/mL, and peak cortisol concentration in saliva after injection of ACTH was 13.7 ± 1.5 ng/mL. Near baseline cortisol values were reached again at two hours after the end of transport and unloading and at four hours after the last ACTH administration, respectively. After the treatment day, i.e., during the subsequent 10 weeks, cortisol concentrations were low and at no time differed between the treatments.

Testosterone concentrations in plasma changed slightly but significantly from Days 1 to 6 ($P < 0.05$; Fig. 2A). No

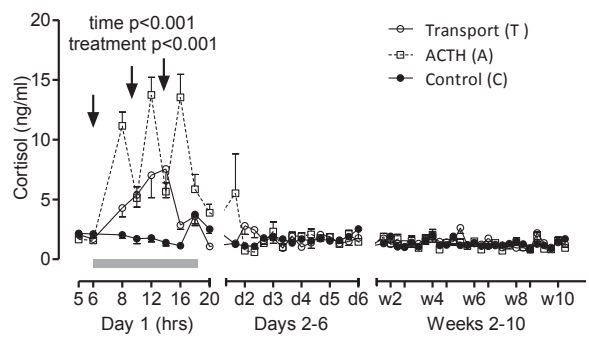

Fig. 1. Cortisol concentration (mean ± standard error of the mean) in saliva of Shetland stallions (N = 13) transported for 12 hours (gray bar = transport time), treated with synthetic ACTH three times at four-hour intervals (arrows), or left untreated as controls on the day of transport or treatment (Day 1) and until 10 weeks thereafter. For Day 1, significant changes over time ($P < 0.001$) and between the treatments (overall effect $P < 0.001$, T vs A vs C at least $P < 0.05$; interactions time × treatment $P < 0.001$). No significant differences between the groups from Days 2 to 6 and weeks 2 to 10 after transport or treatment, respectively.

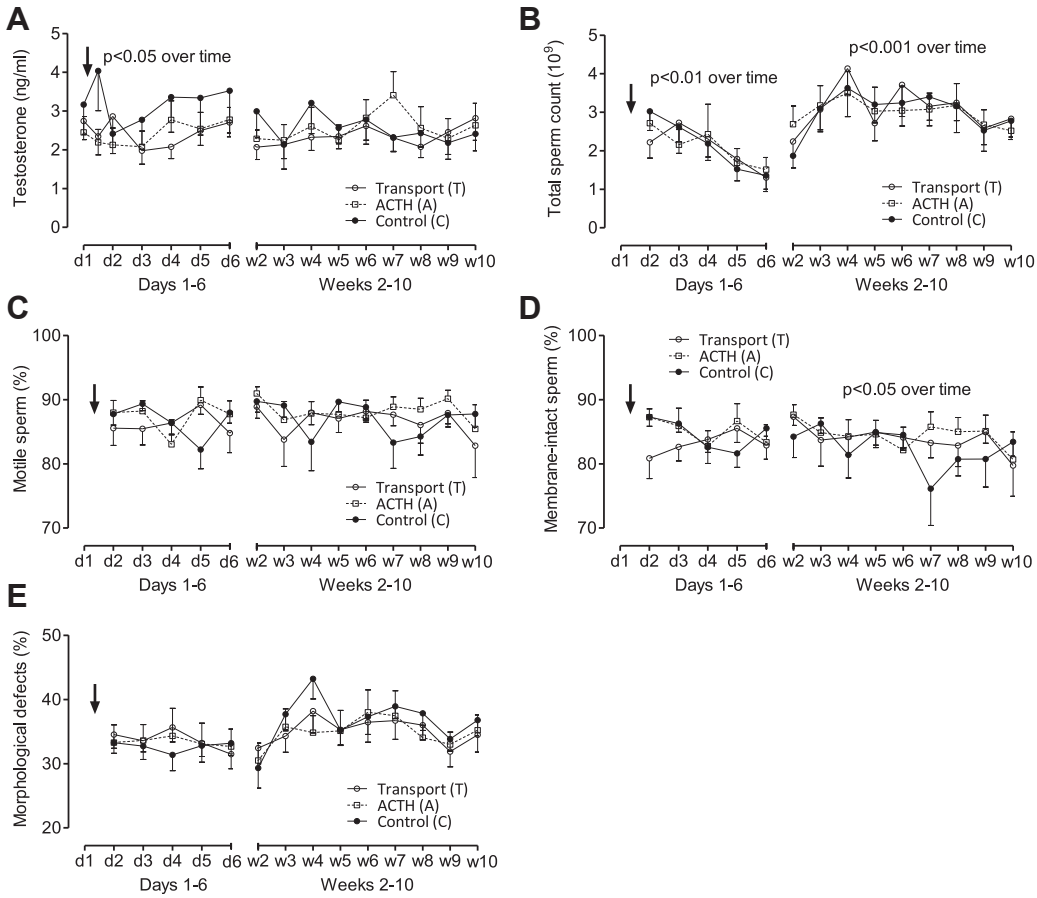

Fig. 2. (A) Testosterone concentration in plasma and (B) total sperm count, (C) percentage of motile spermatozoa, (D) membrane-intact spermatozoa, and (E) percentage of morphologically defective spermatozoa in semen of Shetland stallions (N = 13) transported for 12 hours, treated with synthetic ACTH three times at 4-hour intervals or left untreated as controls, day of experiment = Day 1 (arrow). Values are mean ± standard error of the mean. Significant differences over time during Days 2 to 6 and weeks 2 to 9 after treatment are indicated in the figures, no significant differences between the treatments.

significant differences existed between the groups. When pre and posttreatment testosterone concentrations on Day 1 were compared individually for each group, significant changes did not exist, neither for transport ($P = 0.154$) nor for ACTH ($P = 0.416$) and control treatments ($P = 0.300$). From week 2 to week 10, neither significant changes over time nor differences between the groups could be found (Fig. 2A).

Total sperm count decreased continuously with daily semen collections in week 1 after treatments ($P < 0.01$) but did not differ between the treatments T, A, and C. When semen was collected twice weekly thereafter, total sperm count calculated as the mean of the two weekly semen collections increased during weeks 2 and 3 but again did not differ between the treatments (Fig. 2B). The percentage of motile, progressively motile, membrane-intact, and morphologically defective spermatozoa did not change significantly over time from Days 2 to 6 after treatments, and there existed no significant differences between the groups (Fig. 2C–E). During weeks 2 to 10, the percentage of membrane-intact spermatozoa tended to decrease with time ($P < 0.05$) but did not differ between the treatments.

4. Discussion

In the present study, road transport elicited an immediate and marked increase in salivary cortisol concentrations in Shetland stallions. On the basis of salivary cortisol, heart rate, and heart rate variability, it has been reported previously that transport is perceived as a stressful challenge not only when horses were transported for the first time but also when they were accustomed to regular transportation [4–6]. Cortisol concentrations in the stallions increased with the onset of transport, remained elevated throughout transport time, and decreased to baseline values within 2 hours after unloading. Transport is thus not only a stressor to which most sport horses are regularly exposed but, in equine stress studies, provides a well controllable stress model. It also evokes a more pronounced stress response than equestrian training or competitions [7,8,10]. Stallions were allowed to move freely on the trailer, but transport-induced cortisol release did not differ from previous studies in which Warmblood horses were transported either facing the direction of transport [5,6] or perpendicular to the transport direction [4].

Stress effects on testicular function have been suggested to be mediated *via* cortisol [11–14]. Thus, in our study, as a second treatment, we stimulated cortisol concentrations in stallions by administration of synthetic ACTH. Salivary cortisol concentrations reached a maximum at 2 hours after ACTH injections but decreased rapidly thereafter, which is in agreement with previous studies [18–21]. To achieve elevated cortisol concentrations for the same duration as in the transported stallions, three ACTH treatments at 4-hour intervals had to be given. This treatment schedule resulted in peak cortisol concentrations approximately twice as high as during road transport, whereas cortisol nadirs in between ACTH administration did not fall below the concentrations measured during transport. With three ACTH treatments at 4-hour intervals, we could thus mimic an elevated cortisol concentration for up to 12 hours.

Although cortisol concentrations were clearly elevated in response to both road transport and ACTH treatments, both treatments had no effects on plasma testosterone concentrations and semen parameters. The lack of medium or long-term changes in semen quality and testosterone concentrations after both transport and ACTH treatment indicates that testicular function in stallions is apparently well protected against increased cortisol release. In mature stallions, this is probably achieved by conversion of active cortisol to inactive cortisone [22] *via* oxidative 11β-HSD enzyme activity which is present both in Leydig cells and epididymal cells [23]. The horse is a prey species that responds to stressful situations with increased cortisol release [24,25], enabling a "flight or fight" reaction. Protecting testicular functions from negative effects of such a stress response will avoid reductions in fertility in a stressful environment. In the wild, such mechanisms will ensure survival of the species. The negative results with regard to endocrine and germinative testicular function in the stallions of this study are in agreement with results on road transport and reproductive functions in horse mares. Transport over 700 km which is similar to the situation in the present study induced a marked cortisol release but this was not associated with estrous cycle irregularities, reduced conception rates in mares transported close to ovulation [26], or an increase in embryonic mortality when mares were transported in early pregnancy [27]. Thus, not only on the male, but also on the female side, equine reproductive function is more stress resistant than in other species such as cattle and sheep [28–32].

In boars [33–35], bulls [36], and male rabbits [37], ACTH treatment as in the stallions of the present study induced an immediate cortisol release. However, in these other species, ACTH also caused a rapid increase in plasma testosterone concentrations lasting for 1 to 2 hours [33–36], followed by a depression in testosterone concentrations [35–38]. Although the transient increase occurred independently from LH and might be due to a cortisol-associated increase in testicular blood flow, the second phase with decreased testosterone concentrations is apparently due to an inhibition of LH release [36–38]. This immediate stimulatory effect of transport or ACTH on testosterone release is not in contrast to our results in stallions. The aim of our study was to detect potential effects of transport or ACTH-induced cortisol release on testosterone and on semen quality lasting at least until the day after transport or ACTH treatment. If changes of shorter duration were present, they were not detected with the sampling protocol in our study.

In the present study, semen was collected once daily for 5 days after transport and ACTH treatment. As expected from previous studies in horses [39–41], daily total sperm count decreased during this time because of depletion of sperm from the epididymis and vas deferens. As expected [39], with twice daily semen collection thereafter, total sperm count increased again. Sperm motility, membrane integrity, and percentage of morphologically normal spermatozoa changed over time with repeated semen collections but were neither affected by road transport nor by ACTH treatment. Spermatogenesis in the stallion takes 55 to 57 days and is followed by maturation and storage in the epididymis for approximately 9 days [42]. In the present study, semen was collected for 10 weeks after each experimental treatment; thus, spermatozoa collected during week 10 had undergone spermatogenesis completely after the respective treatment.

In agreement with the findings of the present study, moderate regular exercise did not affect equine sperm motility [2,43]. A negative influence of repeated strenuous treadmill exercise on semen quality could be attributed to increased scrotal temperature due to the treadmill setup and not to increased cortisol release [44]. Three months of overtraining caused a decrease in plasma testosterone concentrations and total sperm count in human athletes [45], and prolonged psychosocial stress has been associated with decreased sperm concentration and motility in previously fertile men [46]. Comparable studies on the effects of long-term stress on fertility do not exist in horses. However, as shown in the present study, transient stress in horses had no effect on long-term testosterone concentrations in plasma and semen quality.

4.1. Conclusions

Road transport evokes a marked stress response in stallions which could be mimicked by ACTH treatment. Increased cortisol release had no effect on testosterone concentrations in plasma and on semen quality when assessment was started 1 day after treatment. Equine semen quality and testosterone secretion are thus resistant to short-term stress, and breeding stallions can be transported without negatively affecting their fertility.

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Competing Interests

The authors do not report any conflict of interest.

References

[1] Pasing S, von Lewinski M, Wulf M, Erber R, Aurich C. Influence of semen collection on salivary cortisol release, heart rate, and heart rate variability in stallions. Theriogenology 2013;80:256–61.
[2] Lange J, Matheja S, Klug E, Aurich C, Aurich JE. Influence of training and competition on the endocrine regulation of testicular function and on semen parameters in stallions. Reprod Domest Anim 1997; 32:297–302.
[3] German Equestrian Federation. Jahresbericht 2013 (Annual report 2013). Available at: http://www.pferd-aktuell.de/shop/index.php/cat/c106_Verband.html#21004. Accessed November 19, 2014.
[4] Schmidt A, Biau S, Möstl E, Becker-Birck M, Morillon B, Aurich J, et al. Changes in cortisol release and heart rate variability in sport horses during long-distance road transport. Domest Anim Endocrinol 2010;38:179–89.
[5] Schmidt A, Hödl S, Möstl E, Aurich J, Müller J, Aurich C. Cortisol release, heart rate, and heart rate variability in transport-naive horses during repeated road transport. Domest Anim Endocrinol 2010;39:205–13.
[6] Schmidt A, Möstl E, Wehnert C, Aurich J, Müller J, Aurich C. Cortisol release and heart rate variability in horses during road transport. Horm Behav 2010;57:209–15.
[7] Becker-Birck M, Schmidt A, Lasarzik J, Aurich J, Möstl E, Aurich C. Cortisol release and heart rate variability in sport horses participating in equestrian competitions. J Vet Behav 2013;8:87–94.
[8] Ille N, von Lewinski M, Erber R, Wulf M, Aurich J, Möstl E, et al. Effects of the level of experience of horses and their riders on cortisol release, heart rate and heart-rate variability during a jumping course. Anim Welf 2013;22:457–65.
[9] Schmidt A, Aurich J, Möstl E, Müller J, Aurich C. Changes in cortisol release and heart rate and heart rate variability during the initial training of 3-year-old sport horses. Horm Behav 2010;58:628–36.
[10] von Lewinski M, Biau S, Erber R, Ille N, Aurich J, Faure JM, et al. Cortisol release, heart rate and heart rate variability in the horse and its rider: different responses to training and performance. Vet J 2013;197:229–32.
[11] Kalantaridou SN, Makrigiannakis A, Zoumakis E, Chrousos GP. Stress and the female reproductive system. J Reprod Immunol 2004;62: 61–8.
[12] Claus R, Wagner A, Lambert T. Characterization of 11beta-hydroxysteroid dehydrogenase activity in testicular tissue of control and GnRH-immunized boars as a possible regulator of spermatogenesis. Exp Clin Endocrinol Diabetes 2005;113:262–7.
[13] Nacharaju VL, Muneyyirci-Delale O, Khan N. Presence of 11 beta-hydroxysteroid dehydrogenase in human semen: evidence of correlation with semen characteristics. Steroids 1997;62:311–4.
[14] Rengarajan S, Balasubramanian K. Corticosterone has direct inhibitory effect on the expression of peptide hormone receptors, 11beta-HSD and glucose oxidation in cultured adult rat Leydig cells. Mol Cell Endocrinol 2007;279:52–62.
[15] Heckenbichler S, Deichsel K, Peters P, Aurich C. Quality and fertility of cooled-shipped stallion semen at the time of insemination. Theriogenology 2011;75:849–56.
[16] Aurich C, Seeber P, Müller-Schlösser F. Comparison of different extenders with defined protein composition for storage of stallion spermatozoa at 5 degrees C. Reprod Domest Anim 2007;42:445–8.
[17] Schäfer-Somi S, Aurich C. Use of a new computer-assisted sperm analyzer for the assessment of motility and viability of dog spermatozoa and evaluation of four different semen extenders for predilution. Anim Reprod Sci 2007;102:1–13.
[18] Hedberg Y, Dalin AM, Forsberg M, Lundeheim N, Hoffmann B, Ludwig C, et al. Effect of ACTH (tetracosactide) on steroid hormone levels in the mare. Part A: effect in intact normal mares and mares with possible estrous related behavioral abnormalities. Anim Reprod Sci 2007;100:73–91.
[19] Hedberg Y, Dalin AM, Forsberg M, Lundeheim N, Sandh G, Hoffmann B, et al. Effect of ACTH (tetracosactide) on steroid hormone levels in the mare. Part B: effect in ovariectomized mares (including estrous behavior). Anim Reprod Sci 2007;100:92–106.
[20] Watson ED, Hinrichs K. Adrenal production of sex steroids in the mare. Theriogenology 1989;32:913–9.
[21] Wiest JJ, Thompson Jr DL, McNeill-Wiest DR, Garza Jr F, Mitchell PS. Effect of administration of adrenocorticotropic hormone on plasma concentrations of testosterone, luteinizing hormone, follicle stimulating hormone and cortisol in stallions. J Equine Vet Sci 1988;8:168–70.
[22] Draper N, Stewart PM. 11beta-Hydroxysteroid dehydrogenase and the pre-receptor regulation of corticosteroid hormone action. J Endocrinol 2005;186:251–71.
[23] Herrera-Luna CV, Budik S, Helmreich M, Walter I, Aurich C. Expression of 11beta-hydroxysteroid dehydrogenase type 1 and glucocorticoid receptors in reproductive tissue of male horses at different stages of sexual maturity. Reprod Domest Anim 2013;48: 231–9.
[24] Forkman B, Boissy A, Meunier-Salaun MC, Canali E, Jones RB. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol Behav 2007;92:340–74.
[25] Hada T, Onaka T, Takahashi T, Hiraga A, Yagi K. Effects of novelty stress on neuroendocrine activities and running performance in thoroughbred horses. J Neuroendocrinol 2003;15:638–48.
[26] Baucus KL, Squires EL, Ralston SL, McKinnon AO, Nett TM. Effect of transportation on the estrous cycle and concentrations of hormones in mares. J Anim Sci 1990;68:419–26.
[27] Baucus KL, Ralston SL, Nockels CF, McKinnon AO, Squires EL. Effects of transportation on early embryonic death in mares. J Anim Sci 1990;68:345–51.
[28] Dobson H. Effect of transport stress on luteinizing hormone released by GnRH in dairy cows. Acta Endocrinol 1987;115:63–6.
[29] Dobson H, Smith RF. What is stress, and how does it affect reproduction? Anim Reprod Sci 2000;60–61:743–52.
[30] Hughes PE. Factors affecting the natural attainment of puberty in the gilt. In: Cole DJA, Foxcroft GR, editors. Control of pig reproduction. Butterworth-Heinemann; Oxford, UK; 1982. p. 117–38.
[31] Lamond DR. Synchronization of estrus and ovulation in beef heifers. Proceedings Australian Society of Animal Production 1962. p. 72–8.
[32] Maziero RRD, Martins AC, Mollo MR, Martin I, Bastos MR, Ferreira JCP, et al. Ovarian function in cows submitted to acute stress during proestrus. Livest Sci 2011;138:105–8.
[33] Hahmeier W, Fenske M, Pitzel L, Holtz W, Konig A. Corticotropin- and lysine-vasopressin induced changes of plasma corticosteroids and testosterone in the adult male pig. Acta Endocrinol (Copenh) 1980;95:518–22.
[34] Juniewicz PE, Johnson BH. Influence of adrenal steroids upon testosterone secretion by the boar testis. Biol Reprod 1981;25: 725–33.
[35] Liptrap RM, Raeside JI. Increase in plasma testosterone concentrations after injection of adrenocorticotrophin into the boar. J Endocrinol 1975;66:123–31.
[36] Johnson BH, Welsh TH, Juniewicz PE. Suppression of luteinizing hormone and testosterone secretion in bulls following adrenocorticotropin hormone treatment. Biol Reprod 1982;26:305–10.
[37] Pitzel L, Kendoff A, Osterloh B, König A. The stimulatory effect of corticotrophin on testicular testosterone release in male rabbits. Exp Clin Endocrinol 1984;83:297–302.
[38] Pitzel L, Hartig A, Fenske M, Holtz W, König A. The effect of long-acting ACTH on plasma corticosteroids, testosterone, and LH levels in the male pig. Exp Clin Endocrinol 1984;83:14–20.
[39] Amann RP, Thompson Jr DL, Squires EL, Pickett BW. Effects of age and frequency of ejaculation on sperm production and extragonadal sperm reserves in stallions. J Reprod Fertil Suppl 1979;27: 1–6.
[40] Clay CM, Squires EL, Amann RP, Pickett BW. Influences of season and artificial photoperiod on stallions: testicular size, seminal characteristics and sexual behavior. J Anim Sci 1987;64:517–25.
[41] Magistrini M, Chanteloube P, Palmer E. Influence of season and frequency of ejaculation on production of stallion semen for freezing. J Reprod Fertil Suppl 1987;35:127–33.
[42] Franca LR, Avelar GF, Almeida FF. Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology 2005;63:300–18.
[43] Dinger JE, Noiles EE, Hoagland TA. Effect of controlled exercise on semen characteristics in two-year-old stallions. Theriogenology 1986;25:525–35.
[44] Janett F, Burkhardt C, Burger D, Imboden I, Hassig M, Thun R. Influence of repeated treadmill exercise on quality and freezability of stallion semen. Theriogenology 2006;65:1737–49.
[45] Roberts AC, McClure RD, Weiner RI, Brooks GA. Overtraining affects male reproductive status. Fertil Steril 1993;60:686–92.
[46] Gollenberg AL, Liu F, Brazil C, Drobnis EZ, Guzick D, Overstreet JW, et al. Semen quality in fertile men in relation to psychosocial stress. Fertil Steril 2010;93:1104–11.