EFFECT OF DEXAMETHASONE ON THE CHANGES OF SEMEN QUALITY INDUCED BY ENDOTOXIN IN STALLION

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Received for publication December 10, 2007

Abstract

The effect of steroidal anti-inflammatory drug - dexamethasone on the changes of semen quality in stallions after endotoxin administration was studied. Three clinically healthy stallions were injected intravenously with endotoxin (LPS) from Escherichia coli 055:B5 at the dose of 0.3 µg/kg b.w.; while four stallions were treated with dexamethasone (0.069 mg/kg b.w., i.m.) 10 min before the administration of LPS. The administration of the endotoxin elicited a significant (P<0.05) decrease in the gel-free semen volume (GFSV), total spermatozoon motility (TSM), and spermatozoon concentration (SC). As regards to each of the morphological defects of the spermatozoa, the percentage of those with cytoplasmic droplets – droplet in distal (form 1), proximal and atypical position (defects 1-3); tail loops – single loop (form 4), double loop, loop of the end part of the tail, spiralling of the tail, and tail looped around the head (defects 4-8); two (or more) heads (form 13), and small (“dwarf”) heads (form 15) were clearly higher in endotoxin treated stallions. The administration of dexamethasone (DEX) had a different effect on endotoxin-induced changes of the semen’s quality. There were noted smaller changes in TSM, SC, defects 1-3, form 1, and form 13. Dexamethasone had no influence on GFSV. A negative influence of the administration of LPS+dexamethasone, in relation to the stallions treated only with LPS, was especially noticed in forms 4 and 15. This data suggest that the dexamethasone does not prevent and to a certain degree enhances an adverse influence of endotoxin on the semen quality in stallions.

Key words: stallion, endotoxin, dexamethasone, semen quality.

The effect of endotoxin on the reproductive functions of males of domestic animals has been partly elucidated. In rams (24, 28) and boars (27) exposed to S. typhimurium endotoxin, there was a number of clinical, endocrinological and seminological changes, including the morphology of spermatozoa. Studies in boars showed that endotoxin induced infiltration of polymorphonuclear neutrophils (PMN) into the testicular interstitium and morphological changes of Leydig cells (29, 30). In stallions, the administration of Escherichia coli endotoxin had a negative influence on the levels of blood serum and seminal plasma testosterone, quality of semen, especially on the sperm motility and morphology of spermatozoa, and on the concentration of seminal plasma albumin and activity of aspartate aminotransferase (5, 7, 9).

Steroidal anti-inflammatory drugs are widely used in equine practice. Dexamethasone is a long-acting, synthetic analogue of hydrocortisone (cortisol) widely used in various equine disorders, including allergic, cutaneous, circulatory, intestinal, and musculoskeletal diseases (15, 16). Apart from many other effects characteristic for steroidal anti-inflammatory drugs, dexamethasone has an ability to prevent adverse effects of endotoxin on a number of body systems in horses and other animals (11, 12, 21, 22, 25). In lipopolysaccharide-challenged swine (19), dexamethasone decreased blood plasma tumour necrosis factor (by approximately 60%), and interleukin-6, but did not alter interleukin-10 levels. Co-incubation with 100 µM dexamethasone (this concentration greatly exceeds the therapeutic dose of the drug for horses) suppresses the production of TNFα by lipopolysaccharide-stimulated equine peritoneal macrophages (18). Dexamethasone also significantly inhibited TNF production by equine mammary exudate macrophages when the agent was added one hour before lipopolysaccharide administration (17). Dexamethasone treatment had differential effects on LPS-induced testicular inflammation and steroidogenesis inhibition in adult rats (13). There are no such studies concerning stallions.

In the previous paper (7), it was shown that non-steroidal anti-inflammatory drug - flunixin meglumine had a positive effect on most semen characteristics, which had been changed under the influence of endotoxin. The aim of this study was to determine the effects of dexamethasone on the changes of semen quality in stallions induced by endotoxin administration.
Material and Methods

Animals. Seven clinically healthy stallions (Polish Primitive Horses) were investigated during the mating season (April-July). The stallions were divided into two groups: E (three stallions aged 8-12 years, and weighing 370-400 kg) and E+DEX (four stallions aged 4-14 years and weighing 280-400 kg).

Treatment with endotoxin and dexamethason. Lipopolysaccharide (LPS) from Escherichia coli (serotype 055:B5; Sigma Chemical Co.) dissolved in 500 mL of apyrogenic physiological saline solution was infused (i.v.) at the dose of 0.3 µg/kg b.w. The experimental stallions (group E+DEX) received intramuscularly a single dose (0.069 mg/kg b.w.) of dexamethasone in the form of preparation Dexafort (Intervet, Holland). The presence of bi-esters of dexamethasone causes Dexafort to act immediately and at least for 8 d after its administration. Dexafort contains 2 mg of dexamethasone phenylpropionate and 1 mg of dexamethasone sodium phosphate. Dexafort was injected 10 min before the infusion of endotoxin.

Collection and examination of semen. Semen was sampled by means of an artificial vagina (Missouri model) twice a week during four weeks and 72 and 24 h before treatment (mean marked as time 0), and 24 and 72 h and twice a week during 9 weeks after the LPS injection. The gel-free semen volume and motility of spermatozoon were recorded and the concentration of spermatozoon was counted in a haemocytometer. Semen morphology (eosin-nigrosin staining) was studied according to Bielański et al. (2). The percentage of abnormal forms of spermatozoon, especially the spermatozoon with cytoplasmic droplet in distal (form 1), proximal and atypical position (defects 1-3), with loops of the tail: single loop (form 4), double loop, loop of the end part of the tail, spiralling of the tail, and tail looped around the head (defects 4-8), two (or more) heads (form 13), and small ("dwarf") heads (form 15), was determined.

Statistical analysis. The data were analysed statistically using the Statistica StatSoft programme, with an analysis of variance (ANOVA). Differences between mean values were analysed with the Tukey test. Results are expressed as means ± SD and significance was defined as P<0.05.

Results

The present work claims that the administration of endotoxin had a negative influence on the quality of stallion semen. The changes in the gel-free semen volume, motility, and concentration of spermatozoon were observed (Figs 1-3). In group E, there was a statistically significant decrease in the gel-free semen volume up to 21.5 ml (ΔGFSV - 126.0%) at 6 week after the administration of LPS. In the group E+DEX, there was significantly increased GFSV, up to 19.7 ml, ΔGFSV - 116.2%. Greater changes were related to the motility of spermatozoon. In relation to the initial time (time 0) and the experimental group, the stallions receiving endotoxin demonstrated a statistically significant decrease in the motility of spermatozoon: in group E at weeks 1 and 3 and between 5-8 (with the maximal decrease at week 6, up to 53.1%, ΔTSM - 47.6%), and in group E+DEX at week 3, up to 66.1% (ΔTSM - 24.8%). In relation to the initial value, the concentration of spermatozoon was significantly lower in the stallions from both groups. After endotoxin administration, the concentration of spermatozoon decreased between weeks 6-8 (maximum at week 6, up to 95.0 x 10^6/ml, ΔSC - 148.2%) in the E group, and at week 2 and between weeks 5-8 (maximum at week 5, up to 103 x 10^6/ml, ΔSC -53.1%) in the E+DEX group.

Figs 4-5 show the percentage of spermatozoon with a cytoplasmic droplet (defects 1-3), and with a tail loop (defects 4-8). The percentage of spermatozoon with a cytoplasmic droplet (defects 1-3) was higher in the experimental stallions’ semen. In the groups E and E+DEX, there was a significant increase between weeks 2-9 (maximum at week 3, up to 9.2%, ΔDefects 1-3 - 311.0%), and between weeks 3-9 (maximum at week 6, up to 6.8, ΔDefects 1-3 - 134.4%, respectively). When analysing the changes related to each defect, the experimental stallions demonstrated a particularly high rise of spermatozoon with a cytoplasmic droplet in distal position (Fig. 4a); in the group E, it increased between weeks 2-9 (maximum at week 5, up to 6.5% (ΔForm 1 - 377.9%), and in the group E+DEX between weeks 3-8 (maximum at week 6, up to 5.8%, ΔForm 1 - 129.3%). The percentage of spermatozoon with tail loops (defects 4-8) was also higher in both groups of experimental stallions. In the groups E and E+DEX, the number of these defects was significantly increased between hour 72 and week 9 (maximum at week 3, up to 10.6%, ΔDefects 4-8 - 221.2%), and at weeks 1-4, and between weeks 6-9 (maximum at week 8, up to 7.6%, ΔDefects 4-8 - 245.4%, respectively. Among these defects, the experimental stallions demonstrated the largest increase in the percentage of spermatozoon with a single tail loop (Fig. 5a). The increase of spermatozoon with this defect was noted in group E, between hour 24 and week 9 (maximum at week 3, up to 5.4% (ΔForm 4 - 157.1%), and in group E+DEX between hour 72 and week 9 (maximum at week 8, up to 5.3% (ΔForm 4 - 211.7%).

After the administration of LPS and LPS+DEX, there was an increase in the percentage of spermatozoon with two (or more) heads (form 13), and small ("dwarf") heads (form 15). In the group E, there was an increase in form 13 of spermatozoon at week 8, up to 1.2%, ΔForm13 -300.0%, and form 15; at weeks 6-7, with maximum increase at week 6, up to 2.7%, ΔForm15 -350.0%. In the group E+DEX, the percentage of form 13 spermatozoon increased at week 5, up to 1.6%, ΔForm13 -142.4%, and of form 15 spermatozoon at week 5, up to 2.1%, ΔForm15 -600.0%. (Figs 6-7).
**Fig. 1.** Gel-free semen volume of stallions after DEX and LPS administration (mean ± SD). E - group of stallions with LPS, E+DEX group of stallions with LPS+DEX, * - significant difference to the time 0, a:a – no significant difference between groups, at P<0.05

**Fig. 2.** Total motility of spermatozoa in the semen of stallions after DEX and LPS administration (mean ± SD). E - group of stallions with LPS, E+DEX group of stallions with LPS+DEX, * - significant difference to the time 0, a:b - significant difference between groups, at P<0.05
**Fig. 3.** Concentration of spermatozoa in the semen of stallions after DEX and LPS administration (mean ±SD). Symbols are explained in the footnotes to Fig. 2.

**Fig. 4.** Spermatozoa with a cytoplasmic droplet (distal, proximal, and atypical position) in the semen of stallions after DEX and LPS administration (mean ±SD). Symbols are explained in the footnotes to Fig. 1.
Fig. 4a. Spermatozoa with cytoplasmic droplet in distal position in the semen of stallions after DEX and LPS administration (mean ±SD). Symbols are explained in the footnotes to Fig. 1.

Fig. 5. Spermatozoa with tail loops (single loop, double loop, loop of the end part of the tail, spiralling of the tail, and tail looped around the head) in the semen of stallions after DEX and LPS administration (mean ±SD). Symbols are explained in the footnotes to Fig. 2.
Fig. 5a. Spermatozoa with single tail loops in the semen of stallions after DEX and LPS administration (mean ±SD). Symbols are explained in the footnotes to Fig. 1.

Fig. 6. Spermatozoa with two (or more) heads in the semen of stallions after DEX and LPS administration (mean ±SD). Symbols are explained in the footnotes to Fig. 2.
Fig. 7. Spermatozoa with small ("dwarf") heads in the semen of stallions after DEX and LPS administration (mean ±SD). Symbols are explained in the footnotes to Fig. 1.

Discussion

In the present work, it was demonstrated that the administration of endotoxin had a negative influence on the quality of stallion semen. A statistically significant decrease in the gel-free semen volume, motility, and concentration of spermatozoa after LPS administration was noted. As regards each of the morphological defects of the spermatozoa, the percentage of those with cytoplasmic droplets, tail loops, loose heads, two (or more) headed, and small "dwarf" heads was clearly higher. The stallions (5, 7) receiving endotoxin demonstrated a decrease in sperm concentration and increase in the percentage of spermatozoa with secondary changes, i.e. with a cytoplasmic droplet and with tail loops and loose heads. Among the primary defects, spermatozoa with "dwarf" and "gigantic" heads were prevailing.

The obtained results are close to the observations of other authors in males of different animal species. In rams (28), treated with single doses (3.0 µg/kg b.w.), of S. typhimurium LPS as well as in the case of repeated endotoxin administration (0.3 µg/kg b.w., 10 times, at 12 h intervals) and in a dose of 0.6 µg/kg b.w. (five times, at 24 h intervals), the semen volume decreased in week 5, and the motility of spermatozoa decreased in weeks 2 and 4-6, without any negative influence on the overall number of spermatozoa in the semen. In weeks 1 and 3-6, according to the LPS dose applied, there was a statistically significant increase in the percentage of spermatozoa with abnormal heads. In these animals, apart from primary changes, there was an increase in the percentage of spermatozoa with cytoplasmic droplets in proximal position (week 2-4). The administration of endotoxin S. typhimurium to boars (27) had no any significant influence on the semen volume, motility, and total number of spermatozoa, but the percentage of spermatozoa with morphological changes was higher. Particularly marked increase in spermatozoa with cytoplasmic droplets took place in weeks 2-3 after injection of 2.0 µg LPS/kg b.w., and with coiled tail in week 4 after injection of 2.5 µg LPS/kg b.w. Other defects such as nuclear pouch formation and abnormally shaped sperm heads occurred in weeks 3-4 after the administration of the endotoxin.

Earlier reports also showed that steroidogenesis and spermatogenesis are affected during bacterial LPS-induced acute inflammation. Studies in boars showed that endotoxin induced infiltration of polymorphonuclear neutrophils into the testicular interstitium and morphological changes of Leydig cells (29). In adult rats treated with LPS, it was found that the damage to the seminiferous epithelium during inflammation is more likely to be due to direct effects of the inflammation, rather than disturbances in androgen production (20). Oxidative stress is a major causal factor in altered steroidogenesis and spermatogenesis, and perhaps the infertility during endotoxin-induced acute inflammation (23).

Dexamethasone belongs to a group of drugs with anti-inflammatory and antipyretic properties. The effects of corticosteroids were assessed in endotoxaemia, especially during endotoxin shock, in horses (10, 12, 25). The role of DEX in testicular steroidogenesis or its influence on sperm production and seminal characteristics is partially known (1, 8, 14). However, in vivo evidence suggests that glucocorticoids (DEX) may decrease testosterone production indirectly via the hypothalamic-pituitary-gonadal axis (26). Dexamethasone, so as IL-1β, inhibits LH-stimulated testosterone release from mouse testicular cells (3). In the present study, DEX treatment prevented an endotoxin-induced decrease in the concentration of...
spermatozoa and had no clear influence on the gel-free semen volume and on percentage of form 4 spermatozoa. A negative influence of DEX administration was noticed especially, in percentage of forms 9 and 15 of spermatozoa and seminal plasma albumin concentration in the stallions treated with endotoxin. A previous paper (6) showed that injection of DEX only had a negative influence on the morphological properties of spermatozoa in the stallions. As regards each of the morphological defects of the spermatozoa, the percentage of those with loose heads and small ("dwarf") heads was clearly higher.

Flunixin meglumine (non-steroidal anti-inflammatory drug) at the dose of 1.1 mg/kg given after the injection of Escherichia coli LPS (0.3 µg/kg b.w.) had a positive influence on the seminological changes occurring during endotoxaemia in the stallions (7). This paper showed that the effects of flunixin were especially visible for the motility and concentration of spermatozoa and for morphological defects of spermatozoa in stallions, which received the compound injection 5 min after the infusion of endotoxin. In adult rats (13), it was found that co-administration of dexamethasone inhibited the systemic inflammatory response, but did not prevent the local inflammation in the testes. Dexamethasone did not prevent the lipopolysaccharide-induced early inhibition of testosterone, but reversed later inhibition of this hormone.

In conclusion, the administration of glucocorticoid - dexamethasone had a different effect on endotoxin-induced changes of semen quality in the stallions. A positive influence of DEX was noticed in certain macro- and microscopic characteristics of semen parameters. There were noted smaller changes in total motility and concentration of spermatozoa and in the percentage of spermatozoa with a cytoplasmic droplet and two (or more) heads, without influence on the gel-free semen volume. Greater, negative changes in the quality of stallions’ semen after administration of endotoxin+DEX, in relation to the group of stallions with alone LPS, were noticed in the percentage of spermatozoa with tail loops, loose head, and small ("dwarf") head. Dexamethasone in accepted therapeutic doses did not prevent and to a certain degree enhanced the adverse influence of endotoxin on the semen quality in the stallions.

Acknowledgments: This study was granted by the Polish State Committee for Scientific Research (grant No. 5P06K 040 18). The study was executed in the National Veterinary Research Institute in Pulawy, Poland.

References


