EFFECTS OF ADMINISTRATION OF ESCHERICHIA COLI LIPOPOLYSACCHARIDES AND FLUNIXIN MEGLUMINE ON SEMEN QUALITY IN THE STALLION

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Abstract

Four clinically normal stallions were injected with endotoxin (LPS) of Escherichia coli, serotype 055:B5, in a dose of 0.3 µg/kg b.w. Twelve stallions were treated with flunixin meglumine (1.1 mg/kg b.w., IV) 5, 30 and 60 min after the administration of LPS. The administration of LPS had a negative influence on the quality of stallion semen. There have been noted a statistically significant decrease in the gel-free semen volume, and in the motility and concentration of spermatozoa. As regards each of the morphological defects of the spermatozoa, the percentage of those with cytoplasmic droplets, tail loops, loose heads and small "dwarf" heads was clearly higher. A positive influence of the administration of flunixin meglumine was noticed concerning all the measured parameters, especially a smaller percentage of the morphological defects of spermatozoa.

Key words: stallion, endotoxin, flunixin meglumine, semen quality.

It was shown that the administration of endotoxin of Escherichia coli induces a negative influence on the quality of stallion semen, especially on the motility and morphology of spermatozoa (6). In rams (20) and boars (21, 22) exposed to the action of the Salmonella typhimurium endotoxin, there is a number of semen abnormalities, including especially those concerning morphology of spermatozoa.

Non-steroidal antiinflammatory drugs (NSAIDs) are used in the treatment of many equine diseases (3, 12). Flunixin meglumine is a therapeutic agent commonly used in equine practice for its analgesic, antiinflammatory and antipyretic properties (10, 11, 15). Apart from many other effects characteristic for NSAIDs, in case of flunixin meglumine, its abilities to prevent adverse effects of endotoxin on a number of body systems in horses and other animals are mentioned (2, 7-9, 13, 14, 16-19). Flunixin meglumine administration also can be used to prevent abortion in cases of endotoxaemia in the mare (4). Studies on boars (23) showed that flunixin meglumine decreased the levels of 15-ketodihydro-PGF2α and modulated endotoxin-induced negative effects on the testicular function.

However, there is no such research concerning stallions. That is why the purpose of this study is to determine the effects of endotoxin and non-steroidal antiinflammatory drug injection on the macro- and microscopic characteristics of the stallion semen.

Material and Methods

Sixteen 16 clinically healthy stallions of Polish Primitive breed, aged 4-13 years and weighing 220-420 kg, were used in the study. The experiments were carried out during the mating season (April-July). The stallions were divided into 4 equal groups. Group E received endotoxin (LPS) of E. coli, serotype 055:B5, (Sigma), group E+FM1 – LPS and flunixin meglumine (FM, Finadine vet. Scanvet, Denmark) 5 min later, group E+FM2 – LPS and FM 30 min later and group E+FM3 – LPS and FM 60 min later. The LPS, dissolved in apyrogenic physiological saline solution, was injected intravenously in a single dose of 0.3 µg/kg b.w. FM was administered as a single intravenous injection in a dose of 1.1 mg/kg b.w.

Semen was collected with an artificial vagina (Missouri type AV, Nasco, Fort Atkinson, USA) twice a week during 4 weeks and 24 and 72 h before (time A) and 24 and 72 h and twice a week during 9 weeks after the drug administration (time B).

The gel-free semen volume (GFSV) and total motility of spermatozoa (TSM) were recorded and the concentration of spermatozoa (SC) was counted in a haemocytometer. Semen morphology (eosin-nigrosin smears) were studied according to the previous work (1). The percentage of abnormal forms of spermatozoa was determined, especially the spermatozoa 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, spiraling of the tail and tail looped around the head (defects 4-8), loose heads (form 9), and small „dwarf” heads (form 15).

The data were analysed statistically using the Statistica PL (StatSoft, Poland) program with ANOVA variance analysis. The mean values were compared using Fisher test. The differences were statistically significant at P<0.05.

Results

In all the stallions, there were changes in the gel-free semen volume, motility, and concentration of spermatozoa (Figs. 1-3) In relation to the initial time (time A) the stallions treated only with the endotoxin (group E) demonstrated a statistically significant decrease in the gel-free semen volume from 50.4 ml to 41.4 ml (Δ - 17.8%) In the remaining groups of stallions, there were no essential changes.

Greater changes were related to the motility of spermatozoa. In three groups of stallions, there was a statistically significant decrease in the motility of spermatozoa: in group E from 79.5% to 60.5% (Δ - 24.0%), in group E+FM1 from 82.4% to 75.5% (Δ - 8.4%), and in group E+FM3 from 81.3% to 75.3% (Δ - 7.4%), whereas in group E+FM1 the changes were statistically insignificant.

The concentration of spermatozoa in the stallions of E and E+FM3 groups decreased from 199.8x10^6 /ml to 167.8x10^6 /ml (Δ - 16.0%) and from 178.2x10^6 /ml to 148.4x10^6 /ml (Δ - 16.7%), respectively. In comparison, concentration of spermatozoa in semen of groups E+FM1 and E+FM2 decreased insignificantly.

Figs. 4-6 show the percentages of spermatozoa with a cytoplasmic droplet (defects 1-3), with a tail loop (defects 4-8) and with loose head (form 9). Percentage of defects 1-3 increased in group E from 1.9% to 7.1% (Δ - 273.6%), in group E+FM1 from 4.5% to 5.3% (Δ - 17.7%), in group E+FM2 from 3.3% to 6.1% (Δ - 84.8%), and in group E+FM3 from 2.3% to 6.2% (Δ - 169.5%). When analysing the changes related to each defect, the stallions demonstrated a particularly high rise of spermatozoa with a cytoplasmic droplet in distal position (Fig. 4a). The increase in form 1 was noted in group E, from 1.2% (at the time A) to 4.6 % (at the time B), Δ - 283.3%). At the same time an increase in form 1 was noted in group E+FM1 from 2.1% to 4.5 % (Δ - 114.3%), in group E+FM2 from 2.7% to 5.0 % (Δ - 85.2%) and in group E+FM3 from 2.3% to 5.1 % (Δ - 121.7%).

Percentage of spermatozoa with tail loops (defects 4-8) was higher in all groups of stallions. In relation to time A a statistically significant increase in spermatozoa with these defects was noted in group E, from 3.3% to 8.6% (Δ - 160.6%), in group E+FM1, from 4.5% to 5.9% (Δ - 31.1%), in group E+FM2, from 4.2% to 7.1% (Δ - 69.0%), and in group E+FM3, from 4.7% to 6.4% (Δ - 36.1%). Among these defects, the largest increase in spermatozoa with a single tail loop was observed (Fig. 5a). The increase in form 4 was noted in group E, from 2.0% (at the time A) to 4.8 %, Δ - 140.0% (at the time B). At the same time an increase in spermatozoa with this form in group E+FM1 ranged from 2.9% to 4.8% (Δ - 65.5%), in group E+FM2 from 2.7% to 5.1% (Δ - 89.0%), and in group E+FM3 from 3.0% to 4.8% (Δ - 60.0%).

In the stallions from groups E and E+FM1-2, there were changes in the percentage of loose heads (form 9). The growth, in comparison to time A occurred in group E, from 0.90% to 1.52% (Δ - 69.0%), and in group E+FM2, from 0.33% to 0.50% (Δ - 51.5%). At the same time in group E+FM1 the percentage of loose heads decreased from 0.95% to 0.57% (Δ -40.0%)
Fig. 2. Motility of spermatozoa (mean±SD).
* - significant differences to the time A, a:b:c - significant differences between groups, at P<0.05.

Fig. 3. Concentration of spermatozoa (mean±SD).
* - significant differences to the time A, a:b - significant differences between groups, at P<0.05.

Fig. 4. Spermatozoa with a cytoplasmic droplet (mean±SD).
* - significant differences to the time A, a:b:c - significant differences between groups, at P<0.05.
**Fig. 4a.** Spermatozoa with cytoplasmic droplet in distal position (mean±SD).

*- significant differences to the time A, a:b - significant differences between groups, at P<0.05

**Fig. 5.** Spermatozoa with tail loops (mean±SD).

*- significant differences to the time A, a:b - significant differences between groups, at P<0.05.

**Fig. 5a.** Spermatozoa with single tail loops (mean±SD).

*- significant differences to the time A, a:a – non significant differences between groups, at P<0.05
Fig. 6. Spermatozoa with loose head (mean±SD).

*- significant differences to the time A, a:b - significant differences between groups, at P<0.05

Fig. 7. Spermatozoa with small "dwarf" head (mean±SD).

*- significant differences to the time A, a:b - significant differences between groups, at P<0.05

After administration of endotoxin (group E), and endotoxin+flunixin meglumine (group E+FM3), there was an increase in the percentage of spermatozoa with small "dwarf" heads (form 15) (Fig. 7). This growth, in comparison to time A, varied from 0.68% to 1.66% (Δ-144.1%) in group E, and from 0.80% to 1.26% (Δ- 57.5%) in group E+FM3.

**Discussion**

In stallions from group E, a statistically significant decrease in the gel-free semen volume, and in the motility and concentration of spermatozoa occurred. As regards each of the morphological defects of the spermatozoa, the percentage of those with cytoplasmic droplets, tail loops, loose heads and small "dwarf" heads was clearly higher after administration of LPS (time B). As for spermatozoa with cytoplasmic droplets in distal position and with a single loop of the tail a statistically significant increase was observed also in this time.

In the previous work (6) it was showed that during 9 weeks after administration of E. coli LPS there was a significant decrease in the motility (Δ-9.4%) and also an increase in the percentage of spermatozoa with a cytoplasmic droplet in distal position (Δ-386.6%), with a single tail loop (Δ-114.7%) and loose heads (Δ-105.8%). During this time there was also an increase in the percentage of spermatozoa with “dwarf” head (Δ-242.2%).

Flunixin meglumine had a specially positive effect on most semen characteristics which had been changed under the influence of endotoxin. The effects of
FM were especially visible for motility and concentration of spermatozoa. A significantly smaller decrease in those values in the following weeks of the experiment was observed in stallions of group E+FM1. As regards morphological changes, a positive influence of FM was noticed in all measured parameters. The use of this drug limited a decrease in the percentage of normal spermatozoa as well as increase in the percentage of primary and secondary changes. Especially a smaller percentage of the morphological defects of spermatozoa in stallions which received FM injected 5 min after the infusion of endotoxin was noted.

According to studies by Wallgren et al. (23) it is clear that applying the FM to boars 10 min. before administration of S. typhimurium endotoxin influenced the level of 15-ketodihydro-PGF2α in the blood serum, but the inhibition of cyclooxygenase, at the drug dose of 1.1 mg/kg, was not complete. These studies also showed that FM reduced the endotoxin-induced infiltration of PMN into the testicular interstitium and morphological changes of Leydig cells.

Smaller changes in the quality of stallions' semen after endotoxin and flunixin meglumine administration can be attributed first of all to antiinflammatory and antipyretic properties of the drug and as for the latter, to the limitation of the increase in body temperature and, undoubtedly, changes in intrasctral temperature in stallions. It has been confirmed by a research in which a rise in the scrotal skin temperature occurred after endotoxin administration in the stallions (5).

References