EFFECT OF *ESCHERICHIA COLI* ENDOTOXIN ON THE LEVELS OF TESTOSTERONE AND ESTRADIOL-17β IN BLOOD SERUM AND SEMINAL PLASMA AND ON THE SEMEN CHARACTERISTICS IN THE STALLION

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The purpose of this study was to evaluate the effects of administration (i.v.) of *Escherichia coli* endotoxin (055:B5), in the dose of 0.3 μg/kg b.w., on the level of testosterone (T) and estradiol-17β (E2) in blood serum and seminal plasma and on the semen characteristics in stallions. A statistically significant decrease in T concentration in blood serum has been demonstrated at 6 h and 8-24 h (maximum at 6 h, ΔT - 0.47 ng/ml) and an increase at 48-72 h (maximum at 48 h, to 2.20 ng/ml, ΔT - 1.44 ng/ml) after an intravenous administration of *E.coli* LPS. In stallions, a decrease of T concentration in the seminal plasma has also been noticed at 24 h to 0.08 ng/ml (ΔT - 0.15 ng/ml) after endotoxin administration. A statistically significant increase was noticed in estradiol-17β concentration in the blood serum at 3 h to 140.9 pg/ml (ΔE2 - 39.1 pg/ml) and a decrease at 6 h and week 1 (maximum at 6 h, to 61.2 pg/ml, ΔE2 - 40.6 pg/ml). In endotoxin-treated stallions there was a significant increase of E2 concentration in the seminal plasma at 72 h - week 2 (maximum at 72 h to 156.4 pg/ml, ΔE2 - 75.9 pg/ml). Administration of endotoxin has also a negative influence on the quality of stallion semen. In the 9th week after LPS administration, a significant decrease in the motility (to the mean value of 74.9%, Δ - 9.4%) was demonstrated and also an increase in the percentage of spermatozoa with a cytoplasmic droplet in distal position to the mean value of 4.7% (Δ - 386.6%), with a single tail loop to the mean value of 4.5% (Δ - 114.7%), and with loose heads to the mean value of 1.5% (Δ - 105.8%). During this time there was also an increase in the percentage of spermatozoa with “dwarf” head to the mean value of 1.5% (Δ - 242.2%) and “gigantic” head to the mean value of 0.4% (Δ - 117.1%).

Key words: stallion, *Escherichia coli*, endotoxin, testosterone, estradiol-17β, semen.

Endotoxin, a lipopolysaccharide (LPS) is the main component of the outer membrane of the cell wall of Gram-negative bacteria. This toxic heteropolimer, released during bacterial cell lysis, plays a great role in the interaction of the bacteria with immune system cells of higher organisms. This interaction results in the synthesis and release of several mediators, also inflammatory cytokines - tumour necrosis factor (TNF), interferon (IFN), interleukin-1 (IL-1), and interleukin-6 (IL-6) (8, 11, 21).

*Escherichia coli* endotoxin results in multisystemic organ failure. The effect of endotoxin action on the male reproductive functions has been partly discovered.
Bosmann et al. (4) demonstrated a decrease in the testosterone concentration in blood serum in rats after LPS administration. Christeff et al. (7) found also that endotoxaemia in these animals is characterized by both a decrease in androgens (T) and an increase in estrogens level (E₁ and E₂) in blood. In rams (25) and boars (26, 27) exposed to the action of the *Salmonella typhimurium* endotoxin, there are a number of clinical changes, both endocrinological and seminological, including specially those concerning morphology of spermatozoa. Morphological changes indicate a negative influence on epididymis as well as on seminiferous epithelium.

Endotoxin-treated stallions display many alterations in clinical sings (10) and changes in seminal plasma biochemical values (9). Horses are specially sensitive to endotoxin action. Endotoxaemia is often recognized in veterinary practice in connection with many pathologic conditions of the gastrointestinal tract (23). The investigation of the effects of endotoxin on reproduction in mature stallions, with special emphasis on endocrinological changes as well as andrological changes has also great practical importance.

Thus the aim of the present study is to estimate the effect of endotoxin administration on the levels of testosterone and estradiol-17β in blood serum and seminal plasma and on the semen quality in stallions.

**Material and Methods**

**Animals.** Ten clinically healthy Polish Primitive Horse stallions were used in the study that was carried out in the reproductive season (April - July). The horses were divided into two groups: CONTROL (3 stallions aged 8-12 years and weighing 370-400 kg and ENDO (7 stallions aged 4-14 years and weighing 280-400 kg).

**Endotoxin administration.** Freeze-dried lipopolysaccharide from *E. coli* serotype 055:B5 (Sigma Chemical Co, St. Louis, USA) was used. This endotoxin was administered intravenously to experimental stallions after dissolution in 500 mL of apyrogenic physiological saline solution. The stallions ENDO were infused with LPS in a dose of 0.3 µg/kg b.w. The controls were infused with saline solution as the experimental group.

**Blood sampling.** The whole blood was taken from the jugular vein with the use of catheter (Secalon® Kathy 1, Viggo, Swindon, U.K.). Blood samples were collected twice a week during 4 weeks and at 72, 48, 24 h (mean of all marked as time A) and at 1, 0 h before, and at 1, 2, 3, 4, 5, 6, 7, 8, 24, 48, 72 h and twice a week during 9 weeks after the LPS administration. Serum samples were obtained and stored at -20°C until assayed.

**Collection of semen.** Semen was collected with an artificial vagina (Missouri type AV, Nasco, Fort Atkinson, USA). Semen was sampled, twice a week during 4 weeks and at 24, 72 h before (mean of all marked as time A) and at 24, 72 h and twice a week during 9 weeks after the LPS administration (mean of all marked as time B). Seminal plasma samples were obtained through centrifugation of gel-free semen volume at 1500g for 15 min and stored at -20°C until assayed.

**Examination of semen.** The total and gel-free semen volume and motility of spermatozoa were recorded and the concentration of spermatozoa was counted in a haemocytometer. Semen morphology (eosin-nigrosin smears) was studied according to previous work (1). The percentage of abnormal forms of spermatozoa was assigned, especially with: distal cytoplasmic droplet, single loops of the tail, loose heads, „dwarf”, and „gigantic” heads.
**Hormonal analysis.** The levels of testosterone and estradiol-17β were measured with the use of RIA kit (Spectria, Orion Diagnostica, Espoo, Finland).

**Statistical analysis.** 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**

**Effect of LPS administration on the level of testosterone in blood serum and seminal plasma.** After the endotoxin administration there was a small increase in the T concentration in the first hour and then a statistically significant decrease at 6 h and 8-24 h, after which there was an increase at 48-72 h. At 6 h there was a decrease in the T concentration up to 0.29 ng/ml, ΔT - 0.47 ng/ml, and at 48 h T concentration in the blood serum increased up to 2.20 ng/ml, ΔT - 1.44 ng/ml (Fig. 1). Concentration of testosterone in seminal plasma was subject to a statistically significant change after endotoxin injection. In group ENDO after LPS administration a significantly decrease in its concentration was noticed at 24 h up to 0.08 ng/ml, ΔT - 0.15 ng/ml (Fig. 2).

![Fig. 1. Level of testosterone in blood serum (mean ± SD).](image1)

* - significant difference to the time A, a:b - significant difference between groups, at P<0.05; wk - week

![Fig. 2. Level of testosterone in seminal plasma (mean ± SD).](image2)

* - significant difference to the time A, a:b - significant difference between groups, at P<0.05; wk - week
Effect of LPS administration on the level of estradiol-17β in blood serum and seminal plasma. Estradiol-17β concentration was also subject to statistically significant changes in endotoxin-treated stallions. In group ENDO a significant increase in the hormone concentration was noticed at 3 h to 140.9 pg/ml, $\Delta E_2 = 39.1$ pg/ml and a decrease at 6 h up to 61.2 pg/ml, $\Delta E_2 = 40.6$ pg/ml and at week 1 up to 76.7 pg/ml, $\Delta E_2 = 25.1$ pg/ml (Fig. 3). After an intravenous injection of endotoxin $E_2$ concentration in the stallions’ seminal plasma was also changed. In group ENDO an increase in the $E_2$ concentration was observed at 72 h - week 2, with maximum at 72 h up to 156.4 pg/ml, $\Delta E_2 = 75.9$ pg/ml (Fig. 4).

Fig. 3. Level of estradiol-17β in blood serum (mean ± SD). *-significant difference to the time A, a:b - significant difference between groups, at P<0.05; wk - week

Fig. 4. Level of estradiol-17β in seminal plasma (mean ± SD). *-significant difference to the time A, a:b - significant difference between groups, at P<0.05; wk - week
Effect of LPS administration on the semen characteristics. In the ENDO stallions, there were no changes in the gel-free semen volume, and concentration of spermatozoa. Greater changes were related to the motility of spermatozoa. At the 9th week the stallions receiving endotoxin demonstrated a significant decrease in the motility to the mean value of 74.9%, Δ -9.4% as compared to time A and control group (Fig. 5).

Fig. 5. Motility of spermatozoa (mean ± SD).
* - significant differences and Δ(%) to the time A, a:b significant differences between groups, at P<0.05

ENDO stallions demonstrated also a particularly high increase of spermatozoa with a cytoplasmic droplet in distal position, with a single tail loop, and loose heads. The percentage in spermatozoa with a cytoplasmic droplet in distal position increased significantly to the mean value of 4.7%, Δ - 386.6% (Fig.6), of those with a single tail loop increased to the mean value of 4.5%, Δ -114.7% (Fig. 7), and of those with loose heads increased to the mean value of 1.5%, Δ - 105.8% (Fig. 8).

Fig. 6. Spermatozoa with distal cytoplasmic droplet (mean ± SD).
* - significant differences and Δ(%) to the time A, a:b significant differences between groups, at P<0.05
Fig. 7. Spermatozoa with single loop of the tail (mean±SD).
*- significant differences and Δ (%) to the time A, a:b significant differences between groups, at P<0.05

Fig. 8. Loose heads of spermatozoa (mean ± SD).
*- significant differences and Δ (%) to the time A, a:b significant differences between groups, at P<0.05

Fig. 9. Spermatozoa with dwarf-head (mean ± SD).
*- significant differences and Δ (%) to the time A, a:b significant differences between groups, at P<0.05
In the ENDO stallions there was an increase in the percentage of spermatozoa with “dwarf” head. This growth reached 1.5%, Δ-242.2% (Fig. 9). In this time percentage of spermatozoa with “gigantic” head also increased significantly to 0.4%, Δ-117.1% in the 9th week after LPS administration (Fig. 10).

Fig. 10. Spermatozoa with gigantic-head (mean ± SD).

*- significant differences and Δ (%) to the time A, a:b significant differences between groups, at P<0.05

Discussion

Administration of endotoxin resulted in a statistically significant decrease in the T concentration at 6 h and at 8-24 h (maximum at 6 h) and again an increase at 48-72 h. In case of E2 a statistically significant increase of this hormone took place at 3 h and a decrease at 6 h and week 1 after the administration of the LPS.

Endotoxaemia resulting in elevated cytokine release is associated with a male hypogonadism and decreased serum androgen concentrations. TNF-α antagonizes the influence of LH gonadotrophins on steroidogenesis through limiting the use of cholesterol substrates by Leydig cells mitochondria (17). IL-1β is a potential inhibitor of testosterone biosynthesis in Leydig cells in rats and its unfavorable action occurs as an inhibiting influence on the expression of mRNA cytochrome P-450scc (16). Bosmann et al. (4) showed that in mice subjected to an influence of sub-lethal doses of LPS a depression of serum testosterone takes place as a result of the decrease, first of steroidogenic acute regulator (StAR) protein levels and then protein and transcript levels of steroidogenic enzymes in Leydig cells. On the basis of tests carried out on male laboratory animals it has been accepted that after administration of LPS there occurs a decrease of T content in the blood serum as a result of androgen synthesis impairment in Leydig cells. Bosmann et al. (4) demonstrated also a decrease in the testosterone concentration in blood serum in rats after LPS administration (by 90% at 2 h), which persisted for 9 d. Hales et al. (13) showed a significant decrease in serum testosterone within 2 h after bacterial lipopolysaccharide injection in mice. In this study a decrease in the level of steroidogenic acute regulatory (StAR) protein in Leydig cells
was demonstrated but no changes were observed in the level of mRNA for P450 scc. According to other research (7) steroid response to endotoxin administration in rats is characterized by both a decrease in androgens (T) and an increase of the estrogens levels (E₁ and E₂) in blood as a result of the intensification of estrogen conversion. In rats, foot shocks, endotoxaemia, and turpentine lower basal plasma LH and T levels and blunt the T response to hCG (22). Wallgren et al. (25) state that in rams a decrease in testosterone concentration in blood serum occurs for about 12 – 24 h, while in boars an increase in the concentration of this hormone takes place especially at 1-2 h after endotoxin S. typhimurium administration with a later tendency for a decrease in its concentration. The initial increase of testosterone might have resulted from a leakage of this hormone from interstitium and lymph into the blood (26, 27).

In rams after infection with T. congolense, an impairment of testosterone production in Leydig cells in response to hCG was also demonstrated, which resulted in a decrease in the testicular testosterone concentrations (19). A decrease in T concentration and a rapid increase in estrogens’ (E₁ and E₂) level was noticed in men in septic shock (6). The research by Munabi et al. (18) proves a decrease in sperm counts and impaired sperm motility in men with varicocele. An increase in the temperature affects testes and stimulates of important testicular steroidogenic enzymes, which causes an increase in E₂ synthesis. As Blanchard et al. (3) demonstrated in stallions with thermally-induced testicular degeneration, a decrease in the T concentration in blood plasma occurs at 16, 24, 30, 38 h and 44 h as well as E₂ at 24 h and 26 h after the onset of scrotal insulation.

After an intravenous administration of E. coli endotoxin there was a decrease in seminal plasma T concentration at 24 h. As far as E₂ is concerned, a non significant decrease in the level of this hormone at 24 h there was an increase in its concentration at 72 h - week 2 after LPS administration. These results can not be compared with the research results of other authors as there are no similar publications concerning stallions and other animals.

Testosterone in blood correlates with T content in stallions’ testicles (14) and with T concentrations in semen - for example the levels of T in stallions blood plasma and in seminal plasma in season are similarly the lowest in December and the highest in May (5). From the research on humans (29) it appears that T concentration in seminal plasma is lower in men with abnormal sperm characteristics than with normozoospermia. Laudat et al. (15) state that there is an important dependence between seminal T concentrations and the percentage of spermatozoa with a residual cytoplasmic droplet. The changes in T concentration in seminal plasma can thus be related, to some extent, to concentration changes of this hormone in testicles and blood. However, in order to confirm this there has to be some more analysis done. Similar dependencies relating to E₂ are not that clear, though.

Infusion of the 0.3 µg of LPS/kg b.w. caused also a decreased motility of stallion spermatozoa. No statistically significant changes were related to volume of semen and concentration of spermatozoa. Even more disadvantageous deviations from the standard values concerned morphology of spermatozoa. The stallions receiving the endotoxin demonstrated an increase in the percentage of spermatozoa with secondary changes, i.e. those with a cytoplasmic droplet, tail loops and loose heads. Among the primary defects, spermatozoa with “dwarf” and „gigantic” heads were prevailing.

Administration of endotoxin caused a number of changes, both macro- and microscopic ones, in the males of different animal species. In rams (25) the changes
involved semen volume, and motility of spermatozoa, without any bad influence on the overall number of spermatozoa in semen. According to the LPS dose applied, there also 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. Administration of endotoxin *S. typhimurium* to boars (26) did not have any significant influence on the semen volume, motility and total number of spermatozoa, but the percentage of spermatozoa with morphological changes was higher. Particularly clear increase was observed in spermatozoa with cytoplasmic droplets, with coiled tail, nuclear pouch formation and abnormally shaped sperm heads.

Many changes in the quality of stallion semen were noticed during the EAV experimental infection (20). One group of stallions demonstrated the changes in the morphology of spermatozoa, without any significant changes in the values of other indices of semen quality. In other group of stallions the changes involved the motility of spermatozoa, and concentration of spermatozoa in the gel-free semen. Particularly distinct was the increase in the percentage of spermatozoa with distal cytoplasmic droplets. The fever and scrotal edema associated with EVA is most likely the cause of the observed decline in semen quality following infection. The authors claim that unfavorable action of fever and scrotal edema can be limited by cold environmental temperatures (in November) or intensified by ambient temperatures and humidity (in June).

The seminal changes after endotoxin administration indicate a disturbance located in the epididymis as well a short-term mild degeneration in the seminiferous epithelium and are similar to those seen after heat stress and scrotal insulation (2, 3, 12, 24, 28). Endotoxin infusion caused an increase in stallion scrotal skin temperature (10). It should be pointed out that the main reason of these changes is fever with scrotal thermoregulatory disturbances occurring during endotoxaemia.

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References


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