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The influence of lameness-caused stress, pain and inflammation on health and reproduction in Holstein-Friesian bulls

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ABSTRACT

In this study, a group of 11 Holstein-Friesian bulls used for semen production has been monitored. Due to inadequate living space, some of bulls (experimental group, n = 5) with higher body measures developed ulcera and blisters on claws and distal extremities. At the beginning of the trial these animals were treated by surgical removal of ulcera, claw correction and supportive therapy. The other bulls (control group, n = 6) only had claw correction. All 11 bulls were monitored daily for next 90 days following surgery. Blood samples were collected weekly for haemogram and clinical biochemistry. After short interval of recovery, bulls were returned in semen collection and spermiograms were performed regularly. According to laboratory data, bulls after surgery and during supportive therapy (n = 5) had shown changes specific for reversible renal damage and endotoxaemia (altered total proteins, creatinin, urea, lymphopenia) for more than 30 days, although they appeared clinically healthy. All bulls that were cured for ulcera and blisters, were returned back to semen collection and gave ejaculat of acceptable quality. There was no noticeable difference in spermiograms of two investigated groups of bulls.

Key words: bull, lameness, stress, endotoxemia, spermiogram

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Introduction

The bull exerts major influences on herd fertility and production whether it is bred with many females, using assisted reproductive technologies, or with relatively few via natural service. Although general physical condition and fertility are monitored strictly and regularly in all bulls involved in semen production, there are some other, equally important factors, which in some circumstances could compromise health, fertility and reproductive competence of bulls. For example, influence of lameness-caused stress, pain and inflammation on health and reproduction traits in Holstein-Friesian cows is already known and well understood (COLLICK et al., 1989; KOOPS et al., 1995; DOBSON and SMITH, 2000; DOBSON et al., 2000). Lameness is one of the main reasons for involuntary culling in the majority of dairy herds. Apart from increasing replacement rates, lameness reduces food intake, depresses yield and impacts adversely on reproductive performance. In the UK the average cost of a lameness case is close to £200. With an estimated 15% of the national herd affected at any one time it is easy to see how costs soon mount up (ESSLEMONT et al., 2000; ESSLEMONT and KOSSAIBATI, 2002; YOUNGQUIST and THRELFALL, 2007). Although lameness is primarily a management issue, it is influenced also by body shape, general constitution and production, or putting it all together, by breed and production type of the animal (CERGOLJ and SAMARDŽIJA, 2006; YOUNGQUIST and THRELFALL, 2007).

Holstein-Friesian bulls are predominant in semen production in dairy industry. They are regularly kept and managed with maximal care and in optimal environment. Unfortunately, their size and body frame increases due to selection. Increase of body mass and size of the animals makes boxes and stanchions too small and could lead to stress and environment-linked diseases. In the same time their body weight and body frame makes them sensitive to lameness and related problems. The aim of this study is to investigate influence of lameness on health and fertility in Holstein-Friesian bulls.

Materials and methods

Study was performed on a group of 11 Holstein-Friesian bulls. All animals were kept in boxes and stanchions of Unit for semen production, that was originally built for Simmental bulls. Although boxes still meet professional standards, some of the largest bulls developed ulcera and blisters on claws and distal extremities due to inadequate living space. Bulls in experimental group (n = 5) were chosen according to pathological findings on orthopedic examination, while control group bulls were chosen according to healthy status also based on orthopedic examination. At the beginning of the trial, experimental group bulls were treated by surgical removal of ulcera, claw correction and supportive therapy (antibiotics and NSAIDs as painkillers, if needed). The other bulls (control group, n = 6) only had claw correction. All 11 bulls were monitored daily

for next 90 days following surgery. Blood samples were collected weekly and samples for the first three weeks were analysed. Haemogram and biochemical profile of animals were proceeded and analysed immediately. After short interval of recovery, bulls were used for semen collection and spermiological and andrological tests were performed after each ejaculation. Data were summarized on descriptive statistical level and analysed for clinical significance.

Results

Since we analysed different data (clinical findings, spermiograms, haemograms and clinical biochemistry) it was decided to describe the findings chronologically. Data from spermiograms and heaemograms, combined with clinical biochemistry, for the first three weeks are given in Table 1 and 2 and in Figs. 1. and 2. due to its clinical significance. According to clinical findings, recovery interval depended on healing process and varied from 72 h (claw correction only) till 30 days (surgical removal of blisters and ulcera followed with supportive therapy). In 2 bulls surgery was repeated after 70 days since ulcera reoccurred. According to clinical and laboratory findings only repeated surgery bulls needed and received NSAID therapy to remove pain. They received flunixin-meglumine 2.5 mg/kg IV/24 h during 3 consecutive days. According to laboratory data bulls after surgery and during supportive therapy (n = 5) all had altered haemogram and biochemical parameters specific for reversible renal damage and endotoxaemia (altered total proteins, creatinin, urea, lymphopenia) for more than 30 days although they clinically appeared healthy. In comparison to them, control group had normal findings.

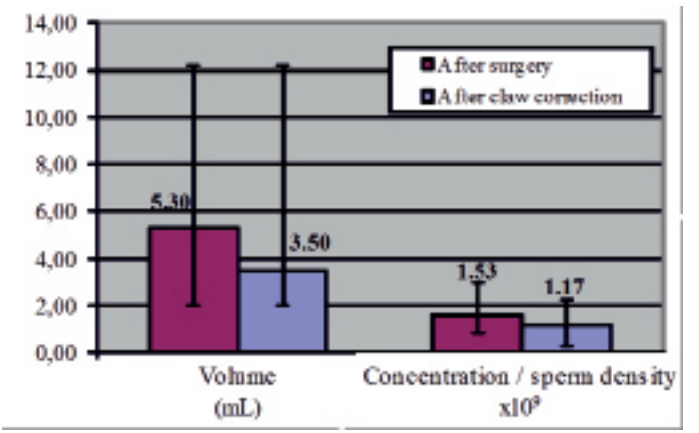

Fig. 1. Comparison of experimental group (surgery) with control group (claw correction) in spermiogram (volume and concentration/sperm density) - median, max. and min. values during trial

Table 1. Haemograms of bulls after surgery/claw correction (median values - above, min. and max. - below)

	RBC $\times 10^{12}$	Hb (g/L)	PCV u %	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC $\times 10^{9}$	Segm. Neutr.%	Lymph. %	Eos. %	Mono. %	Platelet $\times 10^{9}$
One week after surgery/claw correction												
Surgery	7.8	133.0	49.0	56.0	17.0	308.0	8.0	62.0	30.0	8.0	2.0	370.5
min.	5.4	92.0	47.0	42.0	13.0	298.0	5.2	50.0	9.0	2.0	1.0	313.0
max.	8.7	147.0	56.0	62.0	18.0	317.0	11.9	81.0	34.0	14.0	2.0	778.0
Claw correction	7.0	116.5	38.5	55.5	17.0	308.5	6.3	57.5	38.0	6.0	9.0	343.0
min.	6.1	103.0	34.0	53.0	16.0	300.0	5.7	37.0	21.0	2.0	9.0	239.0
max.	8.1	145.0	42.0	59.0	18.0	320.0	11.9	77.0	63.0	8.0	9.0	536.0
Two weeks after surgery/claw correction												
Surgery	7.4	120.0	38.0	51.0	17.0	323.0	7.2	56.0	39.0	3.0	2.0	367.0
min.	6.4	106.0	31.0	38.0	14.0	283.0	4.7	51.0	30.0	1.0	1.0	226.0
max.	8.2	132.0	42.0	63.0	18.0	379.0	7.6	64.0	43.0	8.0	5.0	504.0
Claw correction	7.4	123.5	38.0	52.5	17.0	322.5	6.9	44.5	46.0	5.0	2.0	477.0
min.	5.8	98.0	31.0	50.0	16.0	305.0	4.9	32.0	27.0	3.0	1.0	431.0
max.	7.7	131.0	42.0	55.0	18.0	329.0	11.8	72.0	63.0	9.0	3.0	552.0
Three weeks after surgery/claw correction												
Surgery	7.8	122.0	40.0	52.0	16.0	312.0	6.6	56.0	41.0	2. 0	1.0	603.0
min.	7.0	100.0	32.0	40.0	13.0	302.0	4.3	43.0	35.0	1.0	1.0	435.0
max.	8.1	136.0	44.0	57.0	17.0	317.0	10.5	62.0	46.0	5.0	6.0	1.201.0
Claw correction	8.0	123.5	40.0	50.5	16.0	309.5	7.3	51.5	47.0	3.50	1.0	457.5
min.	6.3	99.0	32.0	49.0	15.0	305.0	5.9	41.0	40.0	1.0	1.0	286.0
max.	8.4	133.0	42.0	53.0	17.0	320.0	8.5	55.0	58.0	5.0	1.0	754.0

Table 2. Clinical biochemistry results after surgery/claw correction (median values - above, min. and max. - below)

	Urea (mmol/L)	Creat. (μmol/L)	Phos. (mmol/L)	Ca (mmol/L)	TP (g/L)	Alb. (g/L)	Glob. (g/L)	ALT (U/L)	AST (U/L)	ALKP (U/L)	GGT (U/L)
One week after surgery/claw correction											
Surgery	4.9	149.0	1.7	2.2	88.0	36.0	53.0	40.0	105.0	112.0	41.0
min.	2.8	137.0	1.3	2.2	84.0	31.0	48.0	0.0	52.0	59.0	34.0
max.	5.8	196.0	2.0	2.4	106.0	44.0	62.0	51.0	109.0	133.0	48.0
Claw correction	5.2	162.5	1.9	2.3	93.5	37.0	56.5	40.0	129.0	71.5	41.0
min.	4.0	108.0	1.5	2.0	82.0	34.0	45.0	0.0	100.0	52.0	27.0
max.	6.5	198.0	2.3	2.4	104.0	41.0	66.0	65.0	268.0	147.0	56.0
Two weeks after surgery/claw correction											
Surgery	4.4	159.0	2.0	2.2	84.0	31.0	53.0	58.0	98.0	57.0	43.0
min.	4.1	128.0	1.5	2.1	81.0	29.0	50.0	38.0	88.0	24.0	38.0
max.	4.8	206.0	2.3	2.4	91.0	38.0	58.0	68.0	120.0	126.0	47.0
Claw correction	5.4	155.0	1.9	2.2	83.5	31.0	53.5	61.5	119.0	59.0	46.5
min.	4.4	100.0	1.6	2.0	72.0	27.0	43.0	39.0	77.0	49.0	40.0
max.	7.1	191.0	2.4	2.3	92.0	33.0	60.0	87.0	479.0	123.0	49.0
Three weeks after surgery/claw correction											
Surgery	4.1	156.0	1.8	2.2	89.0	31.0	52.0	56.0	114.0	57.0	45.0
min.	3.7	147.0	1.5	2.1	78.0	28.0	47.0	39.0	88.0	49.0	42.0
max.	6.0	211.0	2.1	2.3	95.0	37.0	61.0	77.0	151.0	140.0	51.0
Claw correction	4.3	101.0	1.9	2.2	81.5	35.0	48.0	59.5	102.5	76.5	46.0
min.	3.3	70.0	1.8	2.0	76.0	28.0	43.0	37.0	80.0	23.0	33.0
max.	5.7	115.0	2.5	2.3	91.0	37.0	63.0	79.0	115.0	135.0	52.0

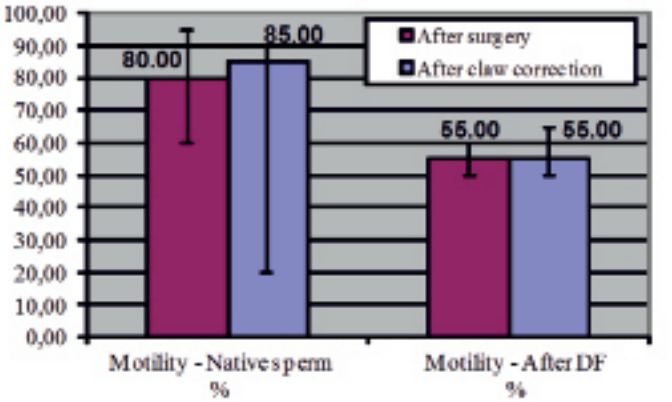

Fig. 2. Comparison of experimental group (surgery) with control group (claw correction) in spermiogram (motility - native sperm and motility - after deep freezing) - median, max. and min. values during trial

Discussion

Fertility and infertility are remarkably difficult terms to define in the context of male animals. While it is generally easy to determine whether a male is sterile, decisions over whether fertility is normal or impaired have to be made in the knowledge of the circumstances of the animal's use and the expectations that have been placed upon its performance. One of the long standing goals of breeding soundness examinations of production animals has been to be able to prognosticate upon the level of fertility, in terms of per-service conception rates or final pregnancy rates that individual sires can achieve. This is partly a negative response to the costs of maintaining male animals, but also represents a positive intention to increase selection intensities for sires. Whether it is in fact possible to predict the actual fertility level is still, after perhaps 50 years of research, still open to conjucture. While there are *in vitro* tests and even some aspects of clinical examination that provide some indication of actual fertility levels of specific sires in specific circumstances (e.g. service testing of beef bulls or in vitro production of acrosome reactions of AI bulls semen), the most valuable application of breeding soundness examinations of sires is the identification and elimination of subfertile and sterile animals. This is closely aligned with the livestock industries goals of minimization of animal health-related risk. Even so, it is difficult to develop binary pass/fail criteria for many aspects of male fertility, largerly as a result of the inherent difficulties in calculating sensitivity and specificity data for such criteria. Furthermore, since spermatogenesis is longterm process, it is extremely difficult to determine exact cause of impaired or decreased fertility in bulls. It is certain that any intercurrent illness and/or stress could

influence reproductive performance in the bull. Diseases of locomotor system can significantly affect the reproductive performance of the sires. In particular, hindlimb pain (mainly hock and foot) and back pain are generally incompatible with normal mating behaviour. Furthermore, not only does locomotor pain limit mating directly, but also the stress of prolonged, unresolved pain may cause corticosteroid-mediated impairment of spermatogenesis as described earlier by numerous authors (FOOTE et al., 1976; KOOPS et al., 1995; DANEK, 2003; LOPATE, 2012). Other systemic illnesses also can affect male animals reproductive performance. However, it should be noted that short-term pyrexia or illness does not generally have this result: prolonged pyrexia is required to cause temperature limited impairment of spermatogenesis as described by YOUNGQUIST and THRELFALL (2007). Although mild, subclinical longterm endotoxemia follows many pathological conditions in domestic mammals, it is still unknown how it influences male reproductive function in ruminants. As described earlier by DANEK (2003) mild chronic endotoxemia in mares and stallions accompanies different forms of disorders in the body, including the reproduction functions. The stallions under the influence of the endotoxin show disorders in steroidogenesis, followed by decreased concentration of testosterone and estradiol-17β in the blood as well as a decreased semen quality. The administering of non-steroid anti-inflammatory drug, Flunixin meglumine, eliminates described disorders of the reproduction functions of stallions caused by endotoxemia but only to some extent. Apart from thermoregulatory disorders of testicles a great deal of changes in the quality of the stallion's semen can be particularly observed with reference to the concentration of the sperm cells, the percentage of the mobile/alive sperm cells and those morphologically normal ones (DANEK, 2000; DANEK, 2002a; DANEK, 2003). Flunixin only to some, modulating extent, affects the changes of the concentration of testosteron and estradiol-17β in stallions' blood, which are caused by the activity of the endotoxin. The beneficial influence of flunixin meglumine on the quality of semen of stallions under LPS influence is noticed especially with reference to the concentration of the sperm cells and the percentage of the normal sperm cell (DANEK, 2002b).

Similar changes are observed in stud dogs due to endotoxemia induced by release of bacterial endotoxins, as a consequence of antibiotic therapy for Lyme disease or leptospirosis caused by amoxicillin+clavulonic acid combination. Dogs on mentioned therapy suffered from decreased steriodogenesis and decreased spermiogram 6 weeks after therapy (LOPATE, 2012). Similar data are obtained in male rats. REDDY et al. (2006) used an LPS rat model to investigate the role of oxidative stress in spermatogenesis. Intraperitoneal administration of bacterial LPS (5 mg/kg body weight) to adult male albino rats elevated testicular malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), and decreased the activities of testicular antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase. The GSH/GSSG ratio also

decreased significantly. Time series analysis revealed transitory oxidative stress and expression of inflammatory mediators such as interleukin-1beta (IL-1beta), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) from 3 h to 12 h after LPS. Testicular expression of steroidogenic acute regulatory (StAR) protein decreased to 24 h, in correlation with damage to spermatogenesis. These data are consistent with oxidative stress as a major causal factor in altered steroidogenesis, spermatogenesis, and perhaps male infertility during endotoxin-induced acute inflammation.

According to described patterns of endotoxemia in male mammals we analysed our results. Recovery interval in our study and consequently endotoxemia depended on basic condition and healing process and varied from 72 h (claw correction only) to 30 days (surgical removal of blisters and ulcera followed by supportive therapy). In 2 bulls surgery was repeated after 70 days since ulcera reoccurred. According to clinical and laboratory findings only bulls on which repeated surgery was performed needed and received NSAID therapy to remove pain. They received flunixin-meglumine 2.5 mg/kg IV/24 h during 3 consecutive days. According to laboratory data, bulls after surgery and during supportive therapy (n = 5) had altered haemogram and biochemical parameters specific for reversible renal damage and endotoxaemia (altered total proteins, creatinin, urea, lymphopenia) for more than 30 days although they clinically appeared healthy. Also, there wasn't any álteration in spermiogram although we checked spermiograms weekly during 6 months following surgery. It is in contrast to results published by DANEK (2003) in stallions, who noticed alterations of spermatogenesis, cortisol and testosterone level due to mild longterm endotoxemia. Furthermore, all obtained data for control group (haematological, endocrinological and spermiological) were within already published reference values for breed and productive type of the animals (FOOTE et al., 1976; LUMSDEN et al., 1980; DOORNENBAL et al., 1988; STALHAMMAR et al., 1994; LUMSDEN, 1998; SAACKE et al., 1998; RODRIGUEZ-MARTINEZ, 2003). There wasn't any noticeable difference for bulls (n = 2) that were reoperated and received strong antibiotic and NSAID therapy for 3-5 days. Still, as mentioned above, all bulls that were cured for ulcera and blisters had mild endotoxemia although appeared healthy, returned back to semen collection and gave ejaculat of acceptable quality. Since subclinical endotoxemia alters fertility features in stallions, dogs and rats it would be logical to expect simmilar effect in bulls. However, further research is needed to determine "breaking point" that links endotoxemia and spermatogenesys in bulls.

Conclusions

According to results of this trial it is easy to see that superficial wounds (caused by inappropriate box, sharp and too short stenchions) influence health and welfare of the Holstein-Friesian bulls much more seriously than it seems, according to clinical

findings and spermiogram only. In the same time, although bulls suffered long-term mild endotoxaemia and received supportive therapy, this didn't have influence neither on sperm production nor stress level of the animal. However, further research is needed to determine precise long-term influence of such conditions on health, welfare and reproduction in Holstein-Friesian bulls.

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SAŽETAK

U radu je razmatran utjecaj stresa, upale i boli uzrokovanih hromošću na zdravlje i rasplođivanje bikova holštajnsko-frizijske pasmine. U prvu, pokusnu skupinu (n = 5) spadali su bikovi koji su imali patološke promjene na nogama dok su drugu skupinu sačinjavali zdravi bikovi (n = 6) na kojima smo samo proveli rutinsku kontrolu i korekciju papaka. Na početku istraživanja provedena je kirurška korekcija i liječenje papaka zajedno s rutinskom kontrolom. Potom su sve životinje u istraživanju dnevno praćene tijekom 90 dana. Jednom tjedno uzimani su im uzorci krvi za određivanje hemograma i biokemijskih profila te su određivane razine kortizola i testosterona. Bikovi su sukladno procesu oporavka vraćani u postupak uzimanja ejakulata pri čemu im je određivan spermiogram. Klinički i laboratorijski nalazi očitovali su razlike između skupina bikova i ukazali su na pojavu blage endotoksemije u pokusnoj skupini (limfopenija, poremećene vrijednosti ukupnih proteina, povišen kreatinin i urea u krvi). Na osnovi naših rezultata razvidno je da i naoko zdrave životinje, očuvanog libida i optimalne plodnosti mogu zbog promjena na papcima dugo biti u blagoj endotoksemiji što bi se dugoročno moglo odraziti na njihovo zdravlje, dugovječnost i upotrebu u rasplodu.

Ključne riječi: bik, stres, hromost, endotoksemija, spermiogram