

Pathological Findings of Post-anesthetic Myopathy Associated With Type 1 Polysaccharide Storage Myopathy in a Percheron Horse

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ABSTRACT

Background: Post-anesthetic myopathy is the most common complication associated with general anesthesia in horses. Polysaccharide storage myopathy (PSSM) is characterized by an abnormal accumulation of glycogen and glycogen-related polysaccharides in the skeletal muscle, which is categorized in type 1 (PSSM1) and type 2 (PSSM2). The purpose of this study is to report the clinical, pathological and molecular findings in a Percheron mare with post-anesthetic myopathy associated with a PSSM1.

Case: A 9-year-old Percheron mare was submitted to a caesarean section due to clinical dystocia during labor. Xylazine was employed during pre-anesthesia, followed by induction with ketamine and diazepam, while anesthetic maintenance was obtained with isoflurane. The mare showed good recovery, however 24 h later, sternal recumbency and hyperthermia (41°C) were observed. The mare was euthanized, and a necropsy was performed. Samples of multiple tissues were collected and routinely processed for histology. At necropsy, segments of skeletal muscles had bilateral pale areas. The kidneys had old and recent infarcts. The heart had whitish areas in the myocardium. The brain showed focally extensive reddish areas, with flattening of gyri. Histologically, skeletal muscle fibers had in the sarcoplasm multiple homogeneous globular clear eosinophilic formations, in addition to mild hyaline necrosis. In the heart and in the kidney, there were extensive areas of acute coagulative necrosis. The brain showed marked multifocal fibrinoid degeneration of vessels and hemorrhage. Refrigerated liver samples were submitted to DNA extraction to detect mutations in the GYS1 (type 1 PSSM) and RyR1 genes (malignant hyperthermia). A positive result for a homozygous dominant mutation in GYS1 (type 1 PSSM) was observed, while the mutation responsible for malignant hyperthermia was not identified.

Discussion: The diagnosis of post-anesthetic myopathy associated with PSSM was obtained by the presence of amylase resistant polysaccharide complex inclusions, glycogen subsarcolemmal aggregates, and central cytoplasmic corpuscles containing glycogen through PAS-amylase resistant histochemical technique, associated to the myopathy microscopical features. Microscopic findings were related to clinical history, and the diagnosis of PSSM underlying post-anesthetic myopathy was determined. The predisposition of the Percheron horse has been described as an inherited predisposition leading to PSSM susceptibility, as was observed in the present case. We speculated that the anesthetic procedure resulted in the precipitation of the drug and a presentation of an acute anesthetic myopathy, while the muscle damage most likely occurred due to the ischemia caused by systemic hypotension. In addition to these lesions, other lesions were considered related to the use of the anesthetics, which may predispose to vasculogenic injuries. This horse was diagnosed as being homozygous dominant for the GYS1 gene, which causes a gain-of-function and results in glycogenolysis with glycogen accumulation in myofibers. Horses that are homozygous for the GYS1 gene may exhibit more severe histological changes in the skeletal muscle fibers, such as necrosis, anisocytosis, endomysial fibrosis, and fatty infiltration. In PSSM, there is a bilateral involvement of the skeletal muscles with areas of degeneration of whitish or greyish coloration, as well as pale muscle with whitish streaks due to coagulative necrosis and edema. In our study, we observed bilateral skeletal muscle lesions and cardiomyocyte necrosis. Post-anesthetic myopathy, along with skeletal muscle lesions, may predispose to vasculogenic injuries, with kidney and brain lesions in horses. Dominant homozygosis for the GYS1 gene with consequent PSSM1 disease probably aggravated the condition in this Percheron, with more severe histological muscular lesions. Our study should bring attention to the use of anesthetics in horses with PSSM1, especially in the Percheron breed.

Keywords: horses, metabolic disease, PSSM, periodic acid-Schiff, skeletal muscle diseases.

INTRODUCTION

Post-anesthetic myopathy is the most common complication associated with general anesthesia in horses, occurring in up to 7% of anaesthetized horses [12]. In heavy animals, compression tissue hypoperfusion may occur and lead to ischemia with muscle necrosis [5]. Horses with myopathies, such as hyperkaliaemic periodic paralysis and the accumulation of polysaccharides are particularly likely to develop this type of myopathy [2].

Polysaccharide storage myopathy (PSSM) is characterized by an abnormal accumulation of glycogen and glycogen-related polysaccharides in the skeletal muscle fibers [3,14]. PSSM is categorized in type 1 (PSSM1) and type 2 (PSSM2), with PSSM1 being caused by a gain-of-function mutation in the skeletal muscle glycogen synthase 1 (GYS1) gene, resulting in glycogenosis with an excessive accumulation of glycogen. This mutation has an autosomal dominant inheritance pattern, and has been previously identified in horses along with the histopathological features of PSSM [8]. Horses with PSSM from undetermined reasons are categorized as PSSM2 [11].

The purpose of this study is to report the clinical, pathological and molecular findings in a Percheron mare with post-anesthetic myopathy associated with a PSSM1.

CASE

A 9-year-old Percheron mare was submitted to a caesarean section due to clinical dystocia during labor. According to the veterinarian, xylazine was employed during pre-anesthesia, followed by induction with ketamine and diazepam, while anesthetic maintenance was obtained with isoflurane. The surgical procedure lasted for approximately 4 h. The mare showed good postoperative recovery, however 24 h later, sternal recumbency and hyperthermia (41°C) were observed. The following day, the mare was euthanized due to an unfavorable prognosis, and a necropsy was performed immediately after death.

Samples of multiple tissues and organs were collected during necropsy, fixed in 10% neutral buffered formalin solution and routinely processed for histology. Tissue sections were cut at 3 µm and stained with hematoxylin and eosin (HE). Serial sections of skeletal muscle were submitted to histochemical examination with periodic acid-Schiff (PAS), with and without amylase digestion, according to a previously described protocol [15].

At necropsy, the semitendinosus, semimembranosus, biceps brachii, biceps femoris, and gluteus medius skeletal muscles showed multifocal pale areas (Figure 1). The kidneys showed multifocal irregular areas that were not limited, which were sometimes wedge-shaped with a whitish color (previous infarcts), and occasionally formed reddish halos (recent infarcts). The heart showed diffuse whitish areas in the left and right ventricular myocardium. The brain showed focally extensive areas of reddish coloration, which were occasionally yellowish, with flattening of the cerebral gyri in the temporal and parietal right lobe.

Histologically, the skeletal muscles had multifocal areas within the sarcoplasmic myocytes with multiple homogeneous globular clear eosinophilic formations, which were often distributed on the periphery of the sarcoplasm (Figure 2B). There was flocculate and mild hyaline necrosis of the muscle fibers, and small foci with muscle regeneration and satellite cells. In the heart, there were extensive areas of acute coagulative necrosis, characterized by hypereosinophilic cardiomyocytes with a homogeneous cytoplasm, karyorrhexis, and karyolysis (Figure 2A). There were also multifocal areas of mineralization with floccular necrosis, mild proliferation of connective tissue, and mild infiltration of neutrophils. The Purkinje fibers showed swelling and vacuolization, and mild infiltration of neutrophils and macrophages. The kidneys showed multifocal areas of coagulative necrosis (infarcts) and fibrinoid vessel degeneration associated with fibrin deposition in adjacent areas. The brain showed marked multifocal fibrinoid degeneration of blood vessels with marked hemorrhage associated; adjacent to that, there was mild neutrophil infiltration with fibrin thrombi formations. There was also marked edema around the vessels, in addition to necrotic neurons, which were retracted and hypereosinophilic. The leptomeninges had marked hemorrhage and thrombosis. In the grey matter, there were astrocytes arranged in pairs with swollen nuclei (Alzheimer's type II astrocytes). Upon histochemical examination of PAS, there was a marked hypereosinophilia (PAS-positive) in the skeletal muscle and Purkinje fibers in the heart. There were also areas with marked deposition of hypereosinophilic floccular intracytoplasmic materials (PAS-positive) with an intense amount of strongly eosinophilic material deposited inside the sarcoplasm, which were interpreted as glycogen aggregates (Figure 2C). On the histological sections of the brain, perivas-

cular PAS-positive material and marked vascular fibri-
noid degeneration were evident. In the PAS procedure
previously treated by alpha-amylase, subsarcolemmal
and central intracytoplasmic accumulation, as well as
diffuse homogeneous eosinophilic material arranged
in a floccular manner were noted. These materials are
resistant to amylase digestion and, thus, are compatible
with the accumulation of polysaccharides (Figure 2D).

Refrigerated liver samples were submitted to
DNA extraction to detect mutations in the GYS1 (type
1 polysaccharide storage myopathy - PSSM) and RyR1
genes (responsible for malignant hyperthermia). Liver
DNA extraction was performed using the Illustra™
GenomicPrep Blood Mini Spin Kit¹, according to the
manufacturer's instructions. The extracted DNA was
tested for purity (A260/280) and quantified using a
NanoDrop spectrophotometer². Primers were designed
using the Primer Express software program³ from the
GYS1 gene sequence (Gene ID: 100054723) and the
RyR1 gene sequence (Gene ID: 100034090) stored
at Genbank. The primers amplified a 279 bp product
including the point mutation region (c.926G>A) on
the GYS1 gene, which was previously described as
being responsible for PSSM1 [8]. A 208bp product,
including the point mutation region (c.7360C>G) on
the RyR1 gene, was obtained [1]. Polymerase chain
reaction (PCR) was optimized for a final volume of
25 µL. The amplified product length was analyzed
using 1.5% agarose gel electrophoresis stained with
GelRed⁴ and then compared to the LowRanger 100 bp
DNA ladder molecular weight marker⁵. The amplified
sample was purified with a NucleoSpin® Gel and PCR
clean-up⁶, according to the manufacturer's instructions.
Subsequently, 10 µL of the purified PCR product
and 5 µL of the reverse primer from both genes were
sequenced using the BigDye® Terminator v3.1 Cycle
Sequencing Kit and the ABI 3500 Genetic Analyser
Sequencer³. After sequencing, quality control of the
sequences and electroespherograms were performed
using the Sequencing Analysis 5.3.1 software⁷. In a
second step, the sequences and electroespherograms
were analyzed using the Sequencer 5.1 program⁸ to
evaluate the nucleotide sequence.

When the test was performed for the mutation
in the GYS1 gene, a positive result for a homozygous
dominant mutation was observed, which is responsible
for type 1 PSSM (Figure 3). The mutation responsible for
malignant hyperthermia was not identified in this animal.

DISCUSSION

The diagnosis of post-anesthetic myopathy
associated with PSSM was obtained by the presence of
amylase resistant polysaccharide complex inclusions,
glycogen subsarcolemmal aggregates, and central cyto-
plasmic corpuscles containing glycogen through PAS-
amylase resistant histochemical technique, associated to
the myopathy features such as variant fiber size and an in-
ternalized nucleus [3,13,17]. Microscopic findings were
related to clinical history, and the diagnosis of PSSM
underlying post-anesthetic myopathy was determined.

PSSM primarily affects horses at an average
age between 8-10 years [16], and often varies according
to the breed of the animal [11]. The predisposition of
the Percheron horse has been described as an inherited
predisposition leading to PSSM susceptibility [15], as
was observed in the present case, where the mare's epi-
demiology corroborates with previous findings [15,16].

Regarding the mare's clinical signs described
herein, due to the acute and progressive clinical course
of permanent lateral recumbency, euthanasia was elec-
ted. Still, the absence of muscle dysfunction in cases of
PSSM in horses with polysaccharide resistant amylase
may occur when those animals are not exercising or are
not actively working [4,15]. In the mare here reported,
we speculated that the anesthetic procedure resulted in
the precipitation of the drug and a presentation of an
acute anesthetic myopathy, while the muscle damage
most likely occurred due to the ischemia caused by the
systemic hypotension, which eventually has led to tissue
and/or muscle hypoxia. In addition to the muscle ischemic
lesions, other lesions were considered related to the use
of the anesthetics, which may predispose to vasculogenic
injuries, and, in this case, led to kidney and brain lesions.

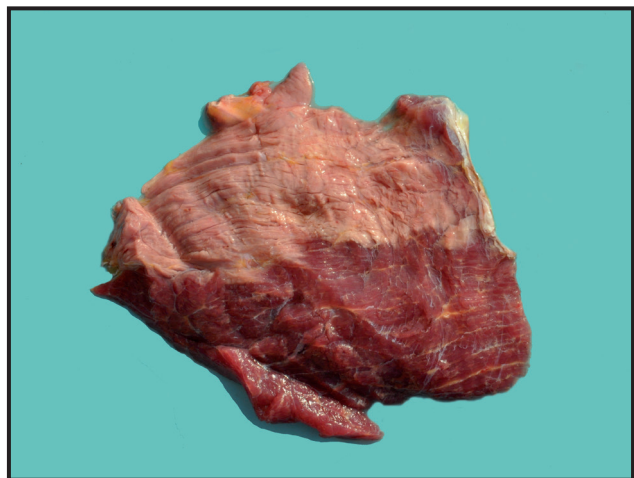


Figure 1. Skeletal muscle. Transition of affected and normal striated skeletal muscle, showing severe focally extensive pale area.

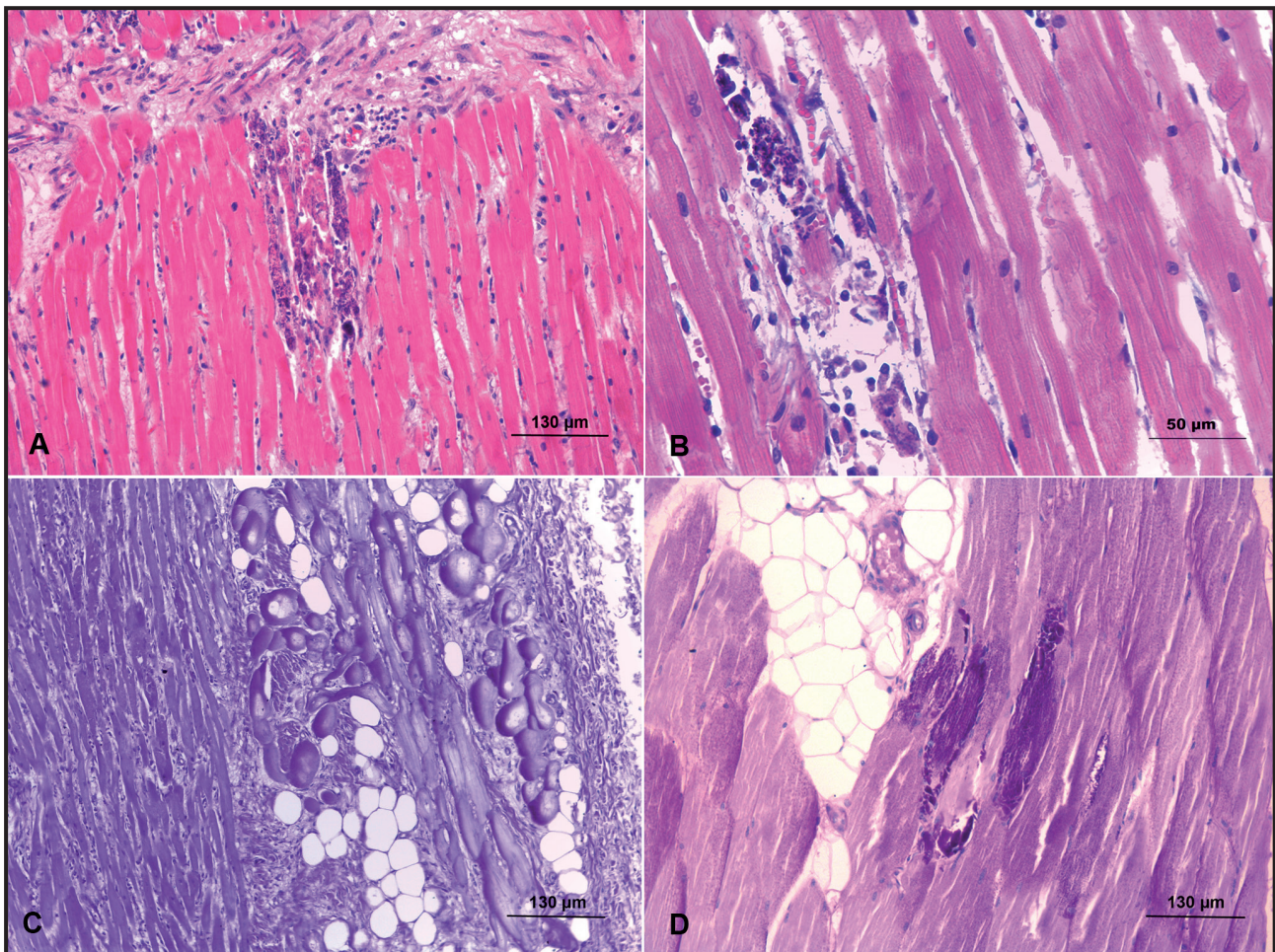


Figure 2. Pathological findings of post-anesthetic myopathy associated with type 1 polysaccharide storage myopathy in a Percheron horse. A- Heart. Focal area of coagulative necrosis, characterized by hyper-eosinophilic cardiomyocytes with a homogeneous cytoplasm, karyorrhexis and karyolysis. [HE, obj. 20x]. B- Skeletal muscle. Focal area with flocculate necrosis of the muscle fibers with deposition of granular material. [HE, obj. 40x]. C- Skeletal muscle. Intracytoplasmic and subsarcolemmal glycogen accumulations. [PAS, obj. 20x]. D- Central intracytoplasmic accumulation, as well as diffuse homogeneous eosinophilic material arranged in a floccular manner, resistant to amylase digestion (compatible with the accumulation of polysaccharides). [PAS-amylase resist stain, obj. 20x].

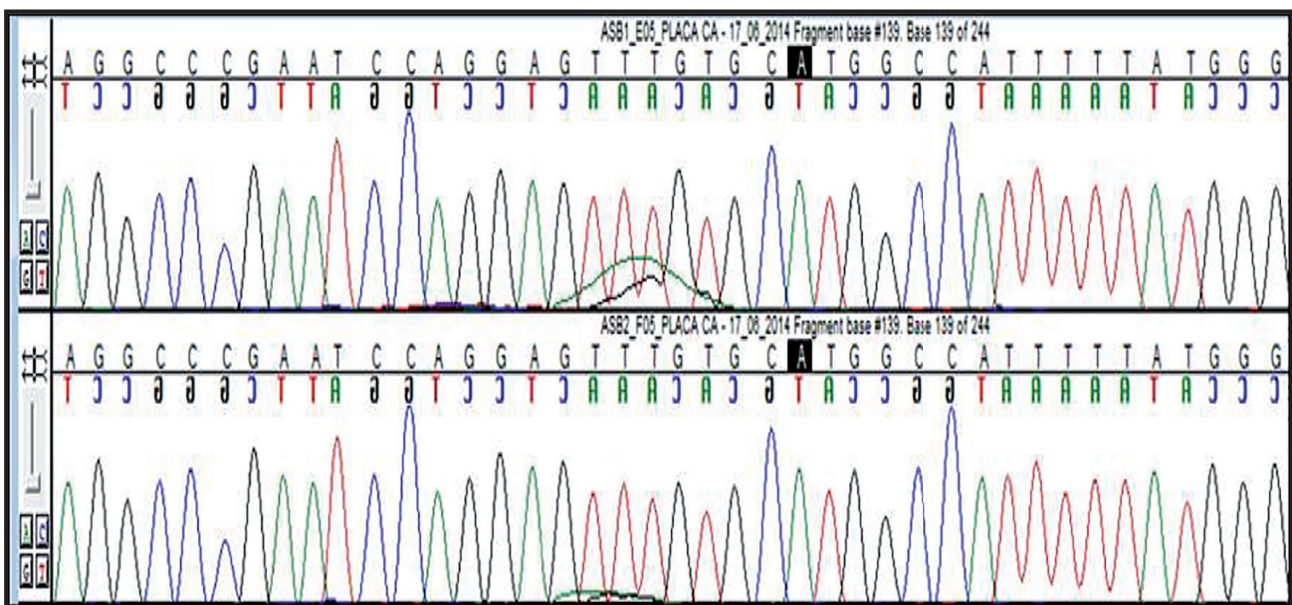


Figure 3. Electrosequencing. DNA extracted from liver (ASB 1) and kidney (ASB 2). In a normal animal (wild type) there is a “G” in the region in bold, and in both samples ASB 1 and ASB 2 it was observed an “A”. This occurred replacing a guanine by adenine (c.926G> A), which characterized that the animal tested is dominant homozygous (affected) for PSSM1.

Post-anesthetic myopathy may be caused, among other causes, by the weight of the animal [10], which is an important predisposing factor observed with the recumbency and that may also be associated to the myopathy in this case. Some studies reported that this condition may occur even with excellent anesthesia or after anesthesia without any complications, and it is characterized by affecting one or more muscle groups, with a variety of clinical signs [9,10]. A form of malignant hyperthermia has also been proposed as a cause of anesthetic-related myopathy in horses, however most of these affected horses develop the myopathy during the recovery rather than during the anesthesia [2], which differs from what this mare presented. Also, through PCR the mutation responsible for malignant hyperthermia was not identified in this animal.

In the genetic test for PSSM1, the horse was diagnosed as being homozygous dominant for the GYS1 gene, which is a gain-of-function mutation in the skeletal muscle that results in glycogenolysis with glycogen accumulation in myofibers. Horses that are homozygous for the GYS1 gene may exhibit more severe histological changes in the skeletal muscle fibers, such as necrosis, anisocytosis, endomysial fibrosis, and fatty infiltration [4]. Recently, a mutation in the GYS1 gene and the resulting p.Arg309His substitution in the skeletal muscle isoform of glycogen synthase was identified in 52.3% of Quarter Horses diagnosed with PSSM [8]. Heterozygosity for the mutation is sufficient to cause PSSM; however, the penetrance is clearly

affected by environmental factors, including diet and exercise, and possibly by the breed [7,8]. McCue *et al.* [8] detected a GYS1 mutation from nine different breeds across North America and Europe. In addition, the mutation was found to be the highest in the North American Percheron and in the Belgian Draft breeds.

Post-anesthetic myopathy, along with skeletal muscle lesions, may predispose to vasculogenic injuries, with kidney and brain lesions in horses. Dominant homozygosity for the GYS1 gene with consequent PSSM1 disease probably aggravated the condition in this Percheron, with more severe histological muscular lesions. Our study should bring attention to the use of anesthetics in horses with PSSM1, especially in the Percheron breed.

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REFERENCES

- 1 Aleman M., Nieto J.E. & Magdesian K.G. 2009.** Malignant hyperthermia associated with ryanodine receptor 1 (C7360G) mutation in Quarter Horses. *Journal of Veterinary Internal Medicine*. 23(2): 329-334.
- 2 Cooper B.J. & Valentine B.A. 2016.** Muscle and tendon. In: Maxie M.G. (Ed). *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th edn. St. Louis: Elsevier, pp. 164-249.
- 3 De La Corte F.D., Valberg S.J., MacLeay J.M. & Mickelson J.R. 2002.** Developmental onset of polysaccharide storage myopathy in 4 Quarter Horse foals. *Journal of Veterinary Internal Medicine*. 16(5): 581-587.
- 4 Firshman A.M., Valberg S.J., Bender J.B., Annandale E.J. & Hayden D.W. 2006.** Comparison of histopathologic criteria and skeletal muscle fixation techniques for the diagnosis of polysaccharide storage myopathy in horses. *Veterinary Pathology*. 43(3): 257-269.
- 5 Klein L. 1990.** Anesthetic complications in horses. *The Veterinary Clinics of North America: Equine Practice*. 6: 665-692.
- 6 Löfstedt J. 1997.** White muscle disease of foals. *The Veterinary Clinics of North America: Equine Practice*. 13(1): 169-185.
- 7 McCue M.E., Anderson S.M., Valberg S.J., Piercy R.J., Barakzai S.Z., Binns M.M., Distl O., Penedo M.C.T., Wagner M.L. & Mickelson J.R. 2010.** Estimated prevalence of the type 1 polysaccharide storage myopathy mutation in selected North American and European breeds. *Animal Genetics*. 41(suppl. 2): 145-149.

- 8 McCue M.E., Valberg S.J., Lucio M. & Mickelson J.R. 2008. Glycogen synthase 1 (GYS1) mutation in diverse breeds with polysaccharide storage myopathy. *Journal of Veterinary Internal Medicine*. 22(5): 1228-1233.
- 9 Muir W.W. & Hubbel J.A.E. 2009. *Equine anesthesia: monitoring and emergency therapy*. 2nd edn. St. Louis: Saunders, 515p.
- 10 Nixon A.J. 1996. *Equine Fracture Repair*. Philadelphia: Saunders, 384p.
- 11 Söderqvist E., Svanholm C., Nautrup O.S. & Leifsson P.S. 2013. Equine polysaccharide storage myopathy - a necropsy study of 60 Danish horses. *Dansk Veterinærtidskrift*. 96(2): 26-31.
- 12 Tylor P.M. 1984. Risk of recumbency in the anaesthetised horse. *Equine Veterinary Journal*. 16(2): 77-78.
- 13 Valberg S.J., Townsend D. & Mickelson J.R. 1998. Skeletal muscle glycolytic capacity and phosphofructokinase regulation in horses with polysaccharide storage myopathy. *American Journal of Veterinary Research*. 59(6): 782-785.
- 14 Valentine B.A. 2005. Diagnosis and treatment of equine polysaccharide storage myopathy. *Journal of Equine Veterinary Science*. 25(2): 52-61.
- 15 Valentine B.A. & Cooper B.J. 2005. Incidence of polysaccharide storage myopathy: necropsy study of 225 horses. *Veterinary Pathology*. 42(6): 823-827.
- 16 Valentine B.A. & Cooper B.J. 2006. Development of polyglucosan inclusions in skeletal muscle. *Neuromuscular disorders*. 16(9-10): 603-607.
- 17 Valentine B.A., Habecker P.L., Patterson J.S., Njaa B.L., Shapiro J., Holshuh H.J., Bildfell R.J. & Bird K.E. 2001. Incidence of polysaccharide storage myopathy in draft horse-related breeds: a necropsy study of 37 horses and a mule. *Journal of Veterinary Diagnostic Investigation*. 13(1): 63-68.