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Lactate dehydrogenase as a possible indicator of reproductive capacity of boars

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ABSTRACT

The aim of this study was to determine the activity of lactate dehydrogenase (LDH) and its isoenzymes in the blood serum and seminal plasma of boars from the breeding and insemination centre with regard to their age. Significant quantitative differences in the presence of isoenzymes between blood serum and seminal plasma were recorded. Isoenzyme LDH-C4 in the seminal plasma of breeding boars of both groups was identified in the position between the third and fourth fraction of LDH, although boars from the breeding centre showed significantly lower concentrations. Refrigeration had a negative influence on the activity of LDH isoenzymes in the blood serum after 7 days and in seminal plasma after 3 days of storage. The work extends the knowledge in an insufficiently examined field of veterinary medicine: the distribution and physiological function of LDH in boars focusing on isoenzyme LDH-C4 in seminal plasma.

Key words: Boars, Isoenzymes, Lactate dehydrogenase, LDH-C4, Seminal plasma

Lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27) (LDH) is a tetrameric enzyme which exists in vertebrates in 3 basic homotetrameric forms: heart H4 (LDH 1), muscle M4 (LDH 5) and C4 (LDH-X) (Wu *et al.* 1987). Three structurally different polypeptide chains forming homotetrameric LDH molecules are encoded by 3 distinct genes, which are termed LDH-B (for the sequence of the H subunit), LDH-A (sequence – M subunit) and LDH-C (sequence for subunit C) (Tsoi and Li 1994). Somatic LDH isoenzymes exist in 5 structural forms (LDH 1 to 5) (Wu *et al.* 1987). The individual isoenzymes have different representation in the cells based on their structural and biochemical properties. Molecular properties and catalytic characteristics of isoenzymes were extensively studied (Tsoi and Li 1994), which has enabled their metabolic function to be explained, as well as the probable cause of their relatively different distribution and concentration in the tissues. LDH-C4 was known as a major isoenzyme of sperm synthesized in the testes during spermatogenesis; however Wang *et al.* (2013) recorded the presence of LDH-C4 also in somatic cells of different tissues in black-lipped pika. The catalytic activity of LDH-C4 is significantly different from other LDH isoenzymes and fulfils a specialized function for the metabolic requirements of adult males (O'Flaherty *et al.* 2005, Yan *et al.* 2009). According to Alvareaz and Storey (1984) more than 80% of LDH-C4 is located in the cytosol of germ cells. This isoenzyme has a specific function related to the metabolic processes by which the stem cells obtain their energy (O'Flaherty *et al.* 2002). Tsujii *et al.* (2002) observed that LDH-C4 is an important marker of seminiferous epithelium activity in the diagnostics and treatment of male infertility. The *in vitro* studies proved that LDH-C4 is also involved in sperm capacitation (Duan and Goldberg 2003).

The aim of this study was to determine the activity of total LDH and its isoenzymes (LDH 1 to 5) in the seminal plasma of breeding boars from the breeding and insemination center focusing on the LDH-C4 fraction, which is closely related to the reproductive capacity of males. We also studied the activity of LDH and its isoenzymes in the blood serum and seminal plasma depending on the length of refrigeration storage.

MATERIALS AND METHODS

Experimental animals: The first group of animals consisted of 1 to 4 years old, 6 breeding boars (insemination centre – IC), of different breeds (Landras, Slovak Large White, Landras, Yorkshire × Pietrain) from the Petrovany insemination centre weighing from 180 to 320 kg. The second group consisted of 6 breeding boars (breeding centre

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– BC) of Landras and Slovak Large White breed, 1-year old, weighing from 180 to 190 kg from the breeding centre in Vysny Zipov. The boars were in good condition, with individual treatment, fed on standard feed mixtures KKZ (for breeding boars).

Sampling: The ejaculate was obtained from male animals after previous stimulation. Blood was collected without the addition of anticoagulants. Ejaculate and blood were centrifuged after standing for 1h at 1500 × g for 15 min. Samples were processed in fresh state (on the day of collection) and chilled (on the third and seventh day). The sperm concentration in 1 ml and sperm activity in seminal plasma or sperm supernatant of boars of both groups was examined microscopically. The concentration of total LDH in samples was determined spectroscopically and concentration of its isoenzymes (LDH 1 to 5) using electrophoresis.

Assay of total lactate dehydrogenase activity: The concentration of total LDH (µkat/l) activity was assayed using commercial diagnostic kits with automatic biochemical analyser at laboratory temperature. Before analysis, 300 µl of reagents (phosphate buffer with concentration of 50 mmol/l and pH 7.5; pyruvate with concentration 0.6 mmol/l; NADH with concentration 0.18 mmol/l) were mixed with 5 µl of the sample and absorbance was measured at 340 nm.

Assay of lactate dehydrogenase isoenzymes activity: For electrophoretic study, 10 µl of blood serum and seminal plasma was used for each separation. A Hydrasys device was used for the determination of LDH isoenzyme activity (µkat/l). The samples were separated using Hydragel 7 ISO-LDH commercial electrophoretic kits on alkaline buffered (pH 8.4) agarose gels. Each gel contained agarose (0.8 g/dl) and alkaline buffer (pH 8.40 ± 0.05). The substrate contained phenazine methosulfate (PMS), nicotinamide adenine dinucleotide (NAD) and nitro blue tetrazolium (NBT) diluted in solution containing lithium lactate. The blocking solution contained acetic acid (5%) and citric acid (0.5%). Dried gels were prepared for visual examination and densitometry to obtain accurate relative quantification of individual zones. The image of electrophoretic migration was scanned with light transmission and automatically converted into an optical density curve presentation. Then photographs of the gels were taken. Qualitative evaluations of the gels were done directly from electrophoretograms, and densitometric curves of separations were created by means of photo densitometer scanning at 570 nm and evaluated using PHORESIS software.

Statistical analysis: Statistical analysis of the results were performed using the GraphPad Prism 3.0 statistical program for Windows. All data are means with S.E.M. Changes in values in individual groups during storage were compared with each other using one-way ANOVA (repeated measures ANOVA) test. To compare the statistical differences depending on time, Tukey's comparison test was used. For comparison between the groups the MANN WHITNEY test was used. Differences from Day 0 and between groups were marked with superscript letters and declared to be significant at levels of $P< 0.05$, $P< 0.01$, $P< 0.001$.

RESULTS AND DISCUSSION

Lactate dehydrogenase-C4 is involved in metabolic processes of sperm in the seminal plasma and is closely related to reproductive performance of males (Yan *et al.* 2009, Zhang and Lin 2009). Low concentration of LDH-C4 isoenzyme is associated with decreased sperm counts and reduced motility and fertility in men (Fadiloðlu *et al.* 1998, Sawane *et al.* 2002) and mice (Odet *et al.* 2008, Yan *et al.* 2009). Isoenzyme LDH-C4 is required for the energy metabolism of sperm. It catalyzes the oxidation of lactate, present in seminal plasma, in germ cells as well as in the female reproductive tract, thereby maintaining sperm motility (Storey 2008).

From the literature, the position of isozyme LDH-C4 in boars is not known. We found that LDH-C4 isoenzyme (Fig. 1) occurred in the position between LDH 3 and LDH 4 isoenzymes. Such observations showed for the first time the position of isoenzyme LDH-C4 in seminal plasma of boars using electrophoretic analysis. Our observations are consistent with the observations of breeding rams (Sutiakova *et al.* 2003) and human males (Javed *et al.* 1994). In contrast, in mice it was found at the position of LDH 5 and in dogs between LDH 4 and LDH 5 isoenzymes (Javed *et al.* 1994). We suppose therefore that the localization of LDH-C4 is species-specific. Interspecies differences in the activity of isoenzyme LDH-C4 are explained by the fact that testes contain different types of cells, each of which produce a different isoenzyme spectrum (Abdelmory 1999).

The highest concentration of LDH-C4 (Table 1) was recorded in a 1-year-old breeding boar from the IC with a small number of jumps, but high sperm motility. Lower values of LDH-C4 in this group were found in older boars with higher number of jumps and similar sperm activity.

Table 1. Mean values of reproductive parameters of boar's semen from insemination center (IC) and breeding center (BC)

	BC	IC	IC
Age	1 year	1 year	2 to 4 years
Sperm motility (%)	79	85	83
Sperm concentration (10^3/l)	276	275	343
The number of hops	-	17	120
LDH-C4 (µkat/l)	0.15	0.48	0.33

Table 2. The concentration of total lactate dehydrogenase activity in blood serum and seminal plasma, expressed in µkat/l (mean ± SEM)

		Blood serum	Seminal plasma
Day 0	IC	15.55 ± 1.633	10.03 ± 0.6535
	BC	18.01 ± 0.8324	8.966 ± 0.2837
Day 3	IC	15.1 ± 1.559	8.76 ± 0.5609
Day 7	IC	13.45 ± 1.531	5.148 ± 0.5364

The lowest activity of LDH-C4 was noticed in a boar's seminal plasma from the BC.

The concentrations of total LDH activity of boar's semen and blood serum are shown in Table 2. Distribution of LDH isoenzymes is specific for individual tissues and body fluids. Concentration of LDH isoenzymes in seminal plasma is not sufficiently known; we recorded maximum activity of LDH 4 isoenzyme in the seminal plasma of breeding boars from the IC. In the seminal plasma of both groups, concentration of LDH isoenzymes was observed in the following order: LDH 4 > LDH 5 > LDH 1 > LDH 3 > LDH 2 > LDH-C4. LDH isoenzyme activity in the seminal plasma of boars in both groups is shown in Fig. 1. The concentration of LDH-C4 isoenzyme ($P< 0.01$) and LDH 4 ($P< 0.05$) was higher in normozoospermic boars from the IC compared to oligozoospermic boars from the BC. These observations showed that testicles and sperms themselves are the source of LDH, which is consistent with the results of García Díez *et al.* (1992). Semen volume, sperm count, and sperm morphological changes are important indicators of performance of male fertilization, but not the only one (Gagnon *et al.* 1982). Enzyme composition including LDH-C4 isoenzyme (Yan *et al.* 2009) and mineral composition

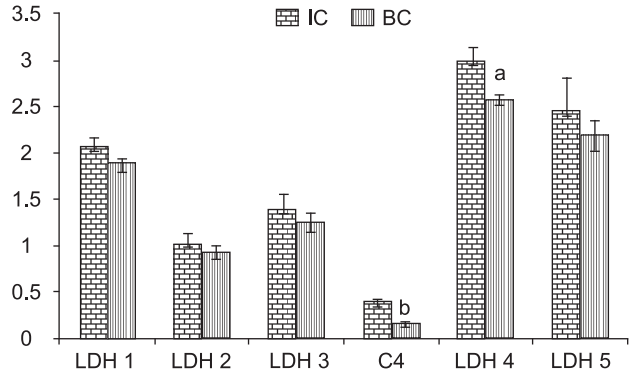

Fig. 1. Average concentrations of LDH isoenzymes (LDH 1 to 5) in the seminal plasma of breeding boars from insemination centre (IC) and breeding centre (BC), expressed in µkat/l (mean ± SEM). [a], $P< 0.05$; [b],$P< 0.01$; BC to IC.

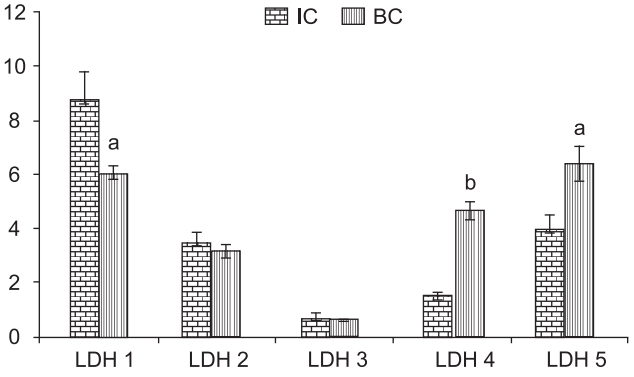

Fig. 2. Average concentrations of LDH isoenzymes (LDH 1 to 5) in the blood serum of breeding boars from insemination centre (IC) and breeding centre (BC), expressed in µkat/l (mean ± SEM). [a],$P< 0.05$; [b],$P< 0.01$; BC to IC.

of seminal plasma appears to be more important indicators of fertility (Asadpour 2012).

In the blood serum of both groups the concentration of LDH isoenzymes was recorded in the following descending order: LDH 1 > LDH 5 > LDH 4 > LDH 3 > LDH 2. We found that LDH 1 isoenzyme was the most represented, which is consistent with the results of LDH isoenzyme activity in the blood serum of breeding rams (Šutiaková *et al.* 2003), cattle (Šutiaková *et al.* 1998, Asefa Asmare *et al.* 1999), sheep, pigs, and rabbits (Heinová and Blahovec 1994). The total activity of LDH in the blood serum showed no differences between individuals in the studied groups. The results of LDH isoenzyme activity in the blood serum of boars are shown in Fig. 2. The activity of LDH 1 ($P< 0.05$) and LDH 2 ($P< 0.01$) in the blood serum was higher in boars from the IC group compared to the BC, while the activity of LDH 4 ($P< 0.01$) and LDH 5 ($P< 0.05$) were higher in boars from the BC.

The concentration of LDH isoenzymes in the blood serum was significantly affected by the duration of cold preservation. In the blood serum (Fig. 3) the concentration of LDH 4 and LDH 5 reduced ($P< 0.05$) after 7 days of refrigeration, which is in contrast with the observations of Lindner and Hatzipanagiotou (1993), who observed an increase in these isoenzymes after storage at 20° to 22 °C at the same length of storage. In the seminal plasma (Fig. 4) the activity of isoenzymes LDH 1 and 2 ($P< 0.05$) decreased after 3 days of cooling; and the concentration of isoenzymes LDH 1, 4 and 5 ($P< 0.001$) and LDH 2 ($P< 0.05$) decreased after 7 days of cooling compared to day 3.

Comparison of the concentration of total LDH and its

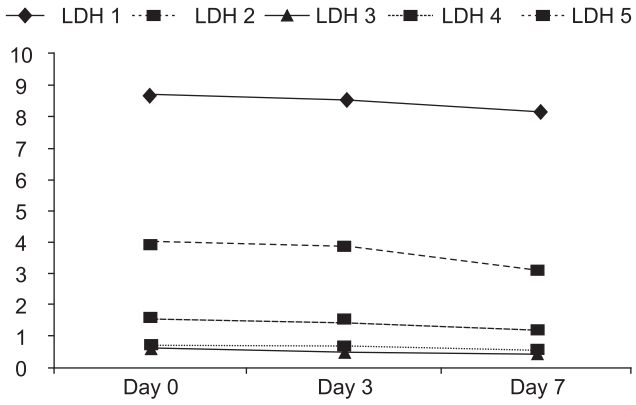

Fig. 3. Dynamics of changes in concentrations of LDH isoenzymes (LDH 1 to 5) in the blood serum recorded during storage of samples, expressed in µkat/l (mean ± SEM). [a], $P< 0.05$; [b], $P< 0.01$; day 7 to day 0.

isoenzymes between blood serum and seminal plasma of boars in both groups is shown in Figs 5 and 6. The concentration of total LDH was higher ($P< 0.01$) in both groups in the blood serum compared to seminal plasma. In the blood serum of IC boars, the concentration of LDH 1 ($P< 0.01$) was higher compared to the seminal plasma, but the activity of LDH 3 ($P< 0.05$) and LDH 4 ($P< 0.01$)

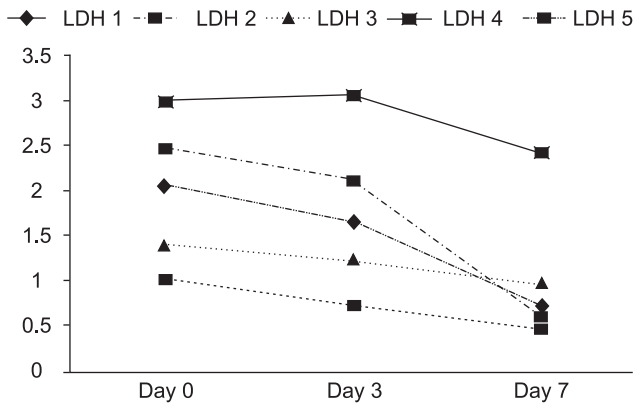

Fig. 4. Dynamics of changes in concentrations of LDH isoenzymes (LDH 1 to 5) in the seminal plasma recorded during storage of samples, expressed in µkat/l (mean ± SEM). [a],P< 0.05; [b], P< 0.01; day 7 to day 3 and day 3 to day 0.

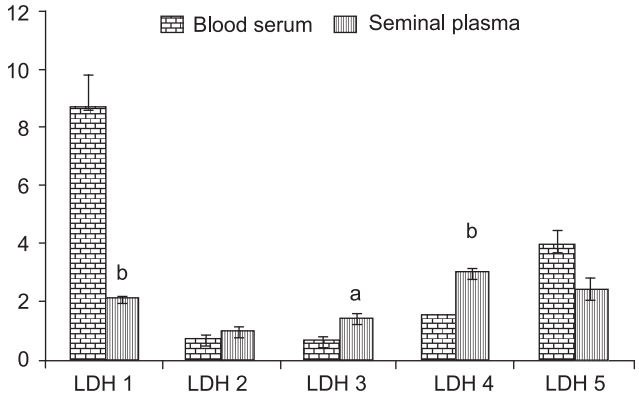

Fig. 5. Differences in the activity of LDH isoenzymes between blood serum and seminal plasma of breeding boars from insemination centre, expressed in µkat/l (mean ± SEM). [a], P< 0.05; [b], P< 0.01; seminal plasma to blood serum.

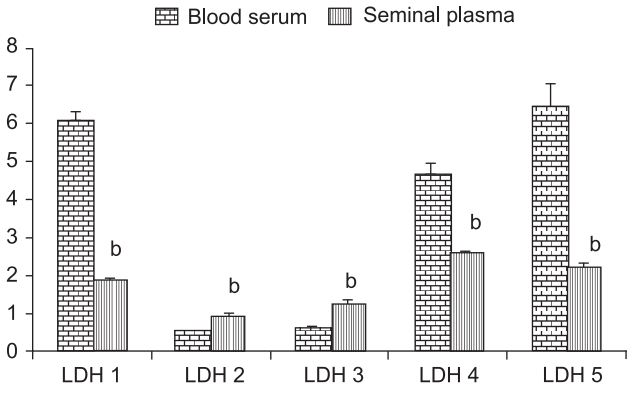

Fig. 6. Differences in the activity of LDH isoenzymes between blood serum and seminal plasma of breeding boars from breeding centre, expressed in µkat/l (mean ± SEM). [a], P< 0.05; [b], P< 0.01; seminal plasma to blood serum.

isoenzymes was higher in the seminal plasma in comparison to blood serum. In the blood serum of BC boars, increased concentrations (P< 0.01) of LDH 1, 4 and 5 were found. In contrast, in the seminal plasma the activities of LDH 2 and 3 were higher (P< 0.01).

Measuring the activity of LDH and its isoenzymes in blood serum and seminal plasma, we found different representation of isoenzymes in breeding boars from both the insemination and the breeding center. In seminal plasma, the presence of LDH-C4 isoenzyme was observed in a position between LDH 3 and LDH 4, similar to humans and rams. The activity of isoenzyme LDH-C4 was higher in boars from the insemination centre. The concentration of LDH-C4 isoenzyme in the seminal plasma is considered to be affected by the animals' age but not breed. Length of refrigeration storage had significant impact on the activity of LDH isoenzymes in the blood serum after seven days and in the seminal plasma after only three days of storage. This work explains the physiological function of LDH and its isoenzymes, mainly LDH-C4 in the seminal plasma of animals, for improvement of methods for laboratory diagnostics of fertility.

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