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COMPARATIVE PHYSICOCHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF BULL AND BOAR SEMEN

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Abstract

There are numerous specialized studies of the physicochemical and biochemical aspects of bull and boar semen, but this paper highlights additional enzymatic activity of superoxide dismutase and glutathione peroxidase enzymes that behave as sperm defense mechanism against of oxygen free radicals(the protection of membrane lipids against oxidative damage). Such a comparative study was performed from a physicochemical (pH, buffering capacity) and biochemical (seminal fructose content, total protein, total lipids, total cholesterol and lactate dehydrogenase enzymatic activity, glutamate dehydrogenase, superoxide dismutase, glutathione peroxidase and acrosin activity) point of view for 10 samples of boar and bull seminal plasma. The investigation showed smaller values of total protein, fructose, lipids and total cholesterol in the boar seminal plasma samples than in the bull seminal plasma samples (the decrease was significant, P <0.05). LDH and GDH activity in bull sperm is increased compared to the boar (P< 0.05), which leads to the idea that the anaerobic degradation of fructose (fructolise) in which LDH is involved, as well as the oxidation of the amino acids where GDH is involved are both processes that take place more intensively in bull sperm than in the boar one. Acrozine of boar sperm cells showed an increased activity compared with that of bull sperm (P< 0.05), because the intracellular pH of boar sperm (7.3-7.9) is closer to the optimal action pH for this enzyme (8,0). SOD activity in both the sperm extract and the seminal plasma of the bovines is increased compared to the ones from boar samples(P<0.01), which explains the higher resistance to lipid peroxidation of the bovine sperm compared to all other animal species. Regarding glutathione peroxidase activity, similarly to SOD activity, it is greatly increased in the extract of bovine sperm compared to bovine seminal plasma. In the boar samples, no traces of glutathione peroxidase could be found.

Keywords: *boar semen, superoxide dismutase, glutathione peroxidase*

INTRODUCTION

Advanced research activity in the field of domestic animals sperm biochemistry have been the subject of many specialized works which have shown the important role of biochemical semen quality on livestock birth growth ratio and prolificacy (Cristea, 1999).

On the other hand, biochemical assessment of sperm from different breeding individuals facilitates the understanding of many different transformations that occur during semen preservation until artificial insemination (Bailey, 2012). The need for introducing a physicochemical and biochemical control of sperm arises, along the usual microscopic control, in the standard spermiogram (Cristea, 1999; Diaconescu, 2001).

The most important physicochemical parameters of semen are: volume, color, pH and buffering capacity (determined and highlighted as a metabolic feature).

In order to move, sperm need energy that can be obtained either by anaerobic catabolism of carbohydrates (fructose) or by oxidation of substrates in the presence of oxygen or by endogenous lipid catabolism (Tamba, 1998; Bailey, 2012). Due to the partial electric charge of the amino acids, seminal plasma proteins contribute to the determination of the pH and to buffering it, and by the albumin the osmotic pressure is adjusted in the sperm, and it helps maintain the integrity of the membrane (Diaconescu, 2001). Many of the proteins have bio catalytic properties, so they are enzymes and they represent the key to intermediary metabolism. There is a wide variety of enzymes active in sperm, some having an important role in the energy generating processes (lactate dehydrogenase and sorbitol dehydrogenase), in the protein metabolism process (glutamate dehydrogenase and acrozine) and especially in the protective lipid peroxidation processes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) (Dejica, 2000).

It is known that from all animal species, the bull sperm, followed by the boar one, is the

most resistant to freezing in liquid nitrogen (Bailey, 2012).

Therefore, this paper aimed to conduct a physicochemical study (pH, buffering capacity) and biochemical (seminal fructose content, total protein, total lipids, total cholesterol and enzyme activity of lactate dehydrogenase, glutamate dehydrogenase, superoxide dismutase, glutathione peroxidase and acrozinic activity) of the bull semen compared with the boar one.

MATERIALS AND METHODS

For the physicochemical and biochemical characterization, the following number of samples were used: 10 bull ejaculates (5 of 5 Friesian breed brown), 10 breeds Landrace boar ejaculate (5 samples), Hampshire (5 samples).

Semen samples were collected from clinically healthy animals and sperm harvesting was done with artificial vagina in both species.

Fresh semen was centrifuged at 2000 g for 20 minutes. Seminal plasma was decanted.

In order to obtain the extract of sperm, after removal of seminal plasma, gametes were washed two times with Ringer solution pH = 6.6, and then mixed/crushed with silica sand, and re-suspended in PBS buffer or saline solution, centrifuged at 25,000 g , at t = 4°C, for 20 minutes so that the supernatant contains 0.5 g% protein.

Both in seminal plasma as well as on the extraction of sperm following determinations were performed:

PH measurement: were performed obtaining potentiometric pH values with two decimal precision;

Buffer capacity measurement: in 0.15 ml of seminal plasma 0.05 ml 0.1 N HCl was added. The pH was measured before and after the addition of the acid. The difference is the buffering capacity;

The seminal fructose measurements based on its reaction with concentrated hydrochloric acid, the resulting hydroxymethyl - furfural which, with resorcino, forms a complex condensation whose intense red color is estimated by photocalorimetry at λ = 540nm (Diaconescu, 2001).

Measurement of total protein by Biuret method: over the variable seminal plasma volume a 5 ml of Biuret reagent was added and after 30 minutes the purple color intensity was estimated by photocalorimetry at λ = 570 nm using as base etalon a standard a solution of 1% bovine serum albumin (Merck). The results were expressed in grams of protein on 100 g seminal plasma (Iordăchescu and Dumitru, 1988)

Measurement of total lipid: total lipids were extracted from both plasma and sperm suspension by homogenization with a mixture of chloroform: methanol (2:1). From the methanol and chloroform extract, the total lipids were determined on the basis of the reaction with the compound fosfovanilina.

The red colored substances was estimated at λ = 530 nm (Diaconescu, 2001). Results were expressed as mg of total lipid /100 ml seminal plasma and sperm extract.

Measurement of total cholesterol: from the chloroform & methanol extract, the total cholesterol (free + esterified) was determined by the Zlatkis-Zak-Boyle method using ferric chloride. The red compound whose intensity of color was read at λ = 570 nm.

Results were expressed in mg colesterol/100 ml seminal plasma and sperm extract (Diaconescu, 2001).

Measurement of lactate dehydrogenase activity (LDH): LDH catalyzes the reduction reaction of pyruvate to lactate, by NADH means (H^+), the reaction rate is determined by subtracting the optical density at 340 nm following the oxidation of NADH (H^+). The results were expressed in $mUI/10^8$ sperm (Rajan, 2011);

Measurement of glutamate dehydrogenase activity (GDH): GDH catalyzes the reaction of oxidative deamination of α-glutamate to α-ketoglutarate. The reaction speed is determined by reducing the optical density at λ= 340 nm due to NADH oxidation (H^+). Calculation and expression of results were performed as in point 7 (Rajan, 2011).

Measurement of superoxide dismutase activity (SOD): SOD activity was determined by a method which is based on the ability of SOD to inhibit the reduction of the tetrazolium salt (NBT2+) due to the superoxide radicals, till it reaches blueformazan (solution). The

color intensity was estimated by photocalorimetry at λ = 560 nm. Results were expressed in UI/10^{10} spermatozoa (Michalski, 1996).

Measurement of glutathione peroxidase activity (GPX) is based on the Plagia&Valentine method (Iordăchescu and Dumitru, 1988);

Measurement of acrozine activity: was performed using the Schwert and Takenaka method. The results were expressed in UI/10^{8} spermatozoa (Strzezek et al., 1992).

RESULTS AND DISCUSSIONS

Physicochemical and biochemical parameters measured in seminal plasma of the studied species are shown in Table 1.

Table 1. Values for the main physicochemical and biochemical parameters determined in the bull and boar seminal plasma

Biochemical parameters	Species	
	Bull	Boar
PH ($\overline{X} \pm s_{\overline{X}}$)	6.87 ± 0.02	7.35 ± 0.04
Buffering capacity ($\overline{X} \pm s_{\overline{X}}$)	$1.17 \pm 0,02$	$1.89 \pm 0,07$
Protein (g%ml) ($\overline{X} \pm s_{\overline{X}}$)	$7.80 \pm 0,19$	$2.30 \pm 0,09$
Fructoze (mg/100 ml) ($\overline{X} \pm s_{\overline{X}}$)	713 ± 25.70	78 ± 1.20
Total lipids (mg/100 ml) ($\overline{X} \pm s_{\overline{X}}$)	59.10 ± 4.89	5.60 ± 0.10
Total cholesterol (mg/100 ml) ($\overline{X} \pm s_{\overline{X}}$)	25.49 ± 1.60	2.08 ± 0.16

*Values are shown as mean±standard deviation of 10 samples

By analyzing the pH values and those of buffering capacity, we can see that they fall within the limits described in the domain literature (7.57 for bull and 6.7 for boar);

In boar we found lower fructose values (P<0.001), knowing that boar spermatozoa have a predominantly respiratory activity and the fructolize index is very low compared to the bull.

The protein content had the highest value in bull samples (7.80 ± 0.19 versus 2.30 ± 0.09) and is correlated with the field research which revealed that 90 % of the total nitrogen of bull seminal plasma is of protein nature (Cristea and Rotar, 1999).

From the analysis of the total lipid concentration, large differences are observed between the bull and boar ($\hat{t} > t_{\alpha}$ = 0,01) and in general, low levels are recorded, that correlate with the field literature (67 mg/100 ml in bull and 5 mg/100 ml in boar samples) (Cristea and Rotar, 1999).

Studies have shown that seminal plasma lipids originate primarily from prostatic secretion and are concentrated mainly in the sperm membrane (23% in the tail, 6% in the intermediate piece and 7% at the ends) .As for the concentration of total cholesterol, boar seminal plasma has the lowest values compared with the bull one (the decrease was significant, P < 0.05), which is positively correlated with total lipid content.

Table 2 shows the values of intracellular enzyme activity for LDH, GDH and acrozine, in Brown and Friesian breeds for cattle and Landrace bread for swine.

Table 2. Values of intracellular enzyme activity for LDH, GDH and acrozine, in Brown and Friesian breeds for cattle and Landrace bread for swine

Biochemical parameters	Specie and breed		
	Cattle		Swine
	Brown $\overline{X} \pm s_{\overline{X}}$	Friesian $\overline{X} \pm s_{\overline{X}}$	Landrace $\overline{X} \pm s_{\overline{X}}$
LDH (mUI/10^{8}spz)	4.65 ± 0.14	4.10 ± 0.17	3.60 ± 0.06
GDH (mUI/10^{8}spz)	3.39 ± 0.13	2.80 ± 0.14	2.29 ± 0.06
Acrozine (N.F.U.I/10^{8}spz)	1787.78 ± 9.15	1766.70 ± 8.34	1893.01 ± 7.83

*Values are shown as mean ± standard deviation of 10 samples

Analysis of these experimental values revealed the following:

- activity of LDH, GDH and acrozine in the sperm did not differ significantly between Brown and Friesian cattle breeds ($\hat{t} < t_{\alpha}$ = 0,05 which $\hat{t}$ = 2,262) and are, in general, comparable with the literature (Cristea and Rotar, 1999);
- In the same breed (both in cattle and swine) the highest enzymatic activity is that of the LDH, which is correlated with the importance of this enzyme in fructolize (Kohsaka et al., 1992);
- Between species, the measurements showed

that LDH and GDH activities of bull sperm are increased compared to the boar ones ($P < 0.05$), which leads to the idea that anaerobic degradation of fructose involving LDH, as well as the oxidation of the amino acids involving GDH, are processes that take place more intensively in bull sperm than in the boar one. It is known that boar sperm have a predominantly respiratory activity and low fructolize index versus bull sperm. Meanwhile, acrozine of boar sperm showed increased activity compared to that of bull sperm ($P < 0.05$), because intracellular pH of boar sperm (7.3-7.9) is closer to the optimum activity pH of this enzyme (8.0), compared to the intracellular pH of bull sperm (6.4-6.5) (Cristea and Rotar, 1999).

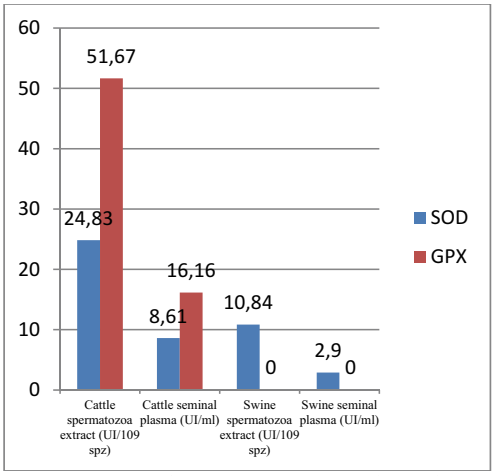

Figure 1. SOD and GPX activity measured in a plasma extract and in fresh sperm, from boar and bull. Values are shown as mean ± standard deviation of 10 samples

Figure 1 presents the main values of enzymatic activity for superoxide dismutase (SOD) and glutathione peroxidase (GPX), measured in a plasma extract and in fresh sperm, from boar and bull.

The analysis of the experimental results shows that the highest enzyme activity of SOD is the intracellular one, compared with the one from seminal plasma, both in the boar and bull, which confirmed that the SOD prevails in the cytoplasm and mitochondria of sperm.

Regarding GPX activity, like that of SOD, is greatly increased in the sperm extract compared to the seminal plasma for bull, and in boar the presence of glutathione peroxidase could not be revealed. Furthermore, research in this area revealed the presence of GPX only in bull, goat and ram sperm (Cristea and Rotar, 1999). The bovine seminal plasma shows a significantly increased GPX activity compared to SOD, which means that this enzyme performs an intense activity in bull sperm to reduce the harmful effect of H_2O_2.

CONCLUSIONS

The pH and the buffering capacity fall within the limits described in the literature for the two species investigated.

Research has shown values lower values of fructose, total protein, total lipids and cholesterol in all boar seminal plasma samples, compared with the ones of the bull (significant decrease of $P < 0.05$); LDH and GDH activity in bull spermatozoa were elevated as compared to the boar ones ($P< 0.05$), which correlates with the significantly lower index of fructose in the boar, versus the bull one;

Acrozine from boar sperm showed increased activity compared to that of bull spermatozoa;

SOD and GPX activity from both bovine sperm extract and seminal plasma is increased compared with the boar samples ($P < 0.01$), which explains the greater resistance to lipid peroxidation of bovine semen compared to all other animal species.

Data obtained from the comparative study of the physicochemical and biochemical parameters in bull and boar semen shows that these parameters are species particularities (related).

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