Folia biologica (Kraków), vol. **56** (2008), No 1-2
doi:10.3409/fb56_1-2.97-101

Zn and Pb Concentration in Seminal Plasma in Reference to Selected Parameters of Semiological Assessment of Bull Semen

Bogdan JANICKI and Dorota CYGAN-SZCZEGIELNIAK

Accepted October 10, 2007

JANICKI B., CYGAN-SZCZEGIELNIAK D. 2008. Zn and Pb concentration in seminal plasma in reference to selected parameters of semiological assessment of bull semen. Folia biol. (Kraków) **56**: 97-101.

The Zn and Pb concentration was determined in 45 samples of bull semen from three Artificial Insemination Stations (A,B,C) differing in degree of environmental pollution. Bull semen was diluted with Biociphos-Plus to such an extent as to obtain 16 million sperm cells in an insemination dose. The research aimed at assessing the correlation between zinc, lead and other parameters influencing the semiological assessment of bull semen. The content of zinc and lead was determined with the use of the flame method and an absorption spectrophotometer (AAS). Positive, statistically significant correlations between zinc concentration and semen motility were observed in station A at r = 0,61 and station C at r = 0,614. In station B these correspondences were not statistically confirmed. Positive correlation at r = 0,583 was observed in case of zinc and the ejaculate volume only in station B. No statistically significant correlation was observed between lead concentration and the analyzed parameters.

Key words: Bull, zinc, lead, semen concentration, semen motility.

Bogdan JANICKI, Dorota CYGAN-SZCZEGIELNIAK, Department of Small Ruminant Biology and Environmental Biochemistry, University of Technology and Life Sciences, Faculty of Animal Breeding and Biology, Mazowiecka 28, 85-084, Bydgoszcz, Poland.
E-mail: janicki@utp.edu.pl

The dynamic development of agriculture as well as intensive urbanization contributes to environmental pollution, which is followed by infiltration of some elements into the food chain of people and animals. The accumulation level of mineral elements and heavy metals in tissues depends on such factors as their amount, exposition time, animal reproductive period, their age and breed. Some metals are necessary for life; others do not have a specified biological function and may be beneficial for health or cause disturbances at different levels in an organism (MASSANYI *et al.* 2003). It should be stressed that the level of the influence of metals on the biological system of animals depends on the interaction between their different forms (BIRES *et al*. 1991).

Zinc is a stabilizer in the process of spermatogenesis (ABBASI *et al.* 1980; TIKKIWAL *et al*. 1987) and a superoxide dismutase coenzyme, protecting semen from the harmful effects of free oxygen radicals (KASPERCZYK *et al.* 2001), and heavy metals such as cadmium and lead (ABBASI *et al.* 1980; KASPERCZYK *et al.* 2001). This element plays an active part in the metabolism of carbohydrates and supports the immunological system (MASSANYI *et al.* 2003). Zinc deficiency leads to disorders in testicle development and disturbances in the process of spermatogenesis (CIGANKOVA *et al.* 1998; MASSANYI *et al.* 2004).

Lead, in turn, is a heavy metal common in the natural environment, derived from natural and anthropogenic sources. Accumulated in tissues, it may cause numerous neurological, hematological and reproductive disorders (MASSANYI *et al.* 2004). The long-term influence of lead may decrease semen quality and its chromatin structure.

An increased content of heavy metals in an organism is correlated with a decrease of male, i.e bull and ram, reproductive coefficients (MASSANYI *et al.* 2004). A similar influence was observed in human semen, where 'healthy semen' subjected to egzogenic lead, gave similar results, i.e. a decrease in semen motility and number which was closely connected with a decrease of insemination capability (BENOFF *et al.* 2000).

The aim of this research was to determine the zinc and lead concentration in semen samples from three Artificial Insemination Stations and the cor-

relation between their concentration and selected semen parameters.

Material and Methods

The research subjects consisted of bulls from three different stations (A, B and C) belonging to Artificial Insemination Stations. The economy of the region of station A is dominated by food, chemical, electromechanical, timber and paper-making industries. Agricultural lands constitute 65% of the area. Station B has an agricultural profile. The characteristic landscape features of station C are woods, meadows, moors and lakes.

In each station, 15 bulls were examined in order to determine the lead (Fig.1) and zinc (Fig. 2) concentrations, as well as other parameters such as the number of sperm cells in 1 ml volume (Table 1), semen wave motion, and progressive semen movement before and after reproduction (Table 2). The bulls from stations A and B were between 6-8 years of age and the animals from station C were between 2-5 years of age.

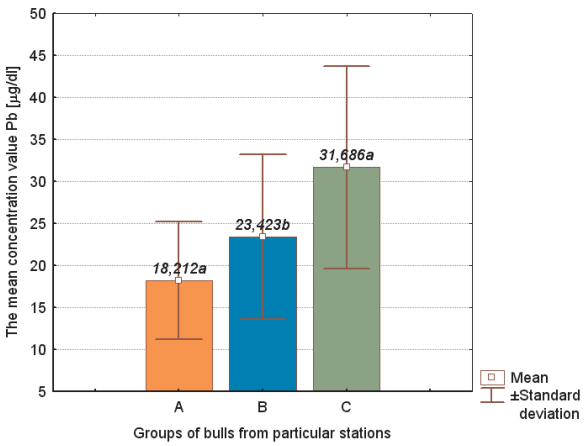

Fig.1. Pb concentration in bull semen in stations A, B and C. The statistical differences between the means marked with the same letters (a,a) are significant at (P<0.05) and those marked with different letters (a,b) are not significant.

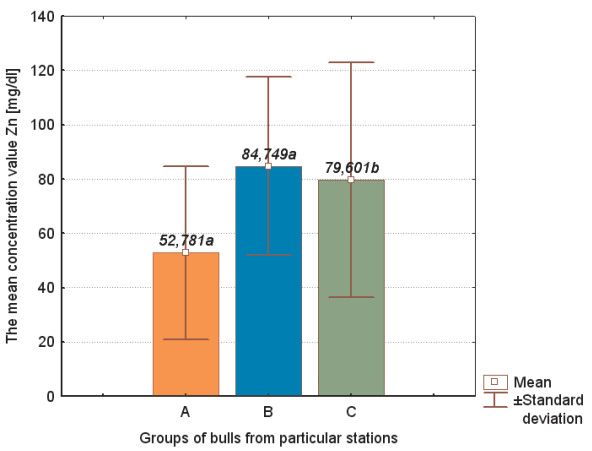

Fig. 2. Zn concentration in bull semen in stations A, B and C. The statistical differences between the means marked with the same letters (a,a) are significant at (P<0.05) and those marked with different letters (a,b) are not significant.

Due to the autumn period of semen collection, the bull feed dose consisted of feeds collected in the given area. The bulls from station A were fed on hay in the amount of 8 kg per 24 hours, grain mixture (wheat-rye, barley, rolled grains) in the doses of 4 kg per 24 hours, middlings (about 2 kg per 24 hours), oat (about 0.5 kg per 24 hours) and the mineral complement Protamino KM (about 0.5 kg per 24 hours). The bulls from station B received hay (about 2 kg per 24 hours), hay silage (about 5 kg per 24 hours) and calf carter containing 5% premix Primasan SANO (about 2 kg per 24 hours). The bulls from station C were fed on grass hay silage (about 3 kg per 24 hours) and straw at will or, interchangeably, calf carter SOMB in the amount of 5 kg per 24 hours and hay at will. It should be stressed that no forage analyses were conducted with reference to the occurrence of Zn and Pb.

The semen was collected into an artificial vagina, according to standard methods used in SHIUZ. The material obtained with the use of this method resulted from the natural process of ejaculation, and therefore the analytical value of the sample was undisputable. The semen containing the dilutent Bicophos-Plus was placed in special plastic straws (0.25 ml) and kept in liquid Nitrogen, then refrozen and rotated at a speed of 3000xg and, after separating semen plasma, frozen until marking. Immediately before assessment, the plasma was refrozen diluted ten times and analyzed.

The Zn content was determined with the use of the flame method and the Solar 969 Unicam absorption spectrophotometer with a deuterium correction. Before assessing 0.2 cm^3 of the analyzed sample, 1.8 cm^3 was diluted with de-ionized water, and in order to obtain the model curve, Merck water models of 100 ppm and 200 ppm concentrations were used. The external control consisted of Merck models 50 ppm and 150 ppm concentration (KASPERCZYK *et al.* 2001). The Pb content was determined with the use of an AAS Solar 939 QZ spectrophotometer, with a deuterium correction, a graphite cuvette GF 90 and the automatic sample feeder FS 90. Calibration was carried out according to the Norwegian models SERO AS. Pb was determined for the wave length of 286.3 nm (HOVATTA *et al.* 1998). The analysis of the composition of the Bicophos-Plus dilutent was also carried out with the use of the same method. The appropriate calculations and adjustments were made with reference to Zn and Pb concentrations in the dilutent. The tests used for statistical analysis were ANOVA variance analysis (Kruskal-Wallis test) and the Spearman test used for determining correlation.

Table 1

The volume and concentration of sperm cells in bull semen in stations A, B, C. The statistical differences between the means in small print are significant ($P \leq 0.05$)

Station A			Station B			Station C		
Bull number	Concentration in thous/mm^3	Ejaculate volume (ml)	Bull number	Concentration in thous/mm^3	Ejaculate volume (ml)	Bull number	Concentration in thous/mm^3	Ejaculate volume (ml)
1A	759	5	1B	1420.5	5	1C	2080	13
2A	940	4.5	2B	1032	4.5	2C	2170	5
3A	397	8	3B	658	5	3C	1480	13
4A	559	4	4B	1420	3.25	4C	1660	15
5A	533	4.25	5B	1688.5	3.5	5C	1780	13
6A	715	6	6B	899.5	7	6C	1460	12
7A	1417	7	7B	847	4.75	7C	1116	4
8A	1299.5	4.5	8B	1378.5	5	8C	1030	3
9A	1266.5	3.5	9B	1052.5	4.75	9C	2050	4
10A	1013.5	4.75	10B	1007	4	10C	2280	5
11A	837	3.25	11B	1308	3	11C	1810	4
12A	982	4.75	12B	1585	4	12C	930	5
13A	1093	7	13B	1414	5.25	13C	1360	3
14A	1251	2.5	14B	729	8	14C	1120	6
15A	1433	7	15B	1000	4.75	15C	2070	8
$\bar{x}$	966.367 a	5.067	$\bar{x}$	1162.633a	4.783	$\bar{x}$	1626.4a	7.533
SD	329.821	1.596	**SD**	317.355	1.312	**SD**	450.46	450.46

Table 2

The percentage of sperm cells in progressive movement and sperm cell mass mobility. The statistical differences between the means marked with the same letters are significant ($P \leq 0.01$); + + + intensive wave motion and whirl formation; + + lively wave motion; + slow motion; – no motion

Station A				Station B				Station C			
Bull number	Motility (%) In fresh semen	Motility after refreezing (%)	Mass motility	Bull number	Motility (%) In fresh semen	Motility after refreezing (%)	Mass motility	Bull number	Motility (%) In fresh semen	Motility after refreezing (%)	Mass motility
1A	70	60	+ +	1B	70	50	+ +	1C	80	50	+ + +
2A	80	50	+ + +	2B	70	50	+ +	2C	80	50	+ + +
3A	70	50	+ +	3B	70	50	+ +	3C	70	50	+ +
4A	70	50	+ +	4B	70	50	+ +	4C	90	50	+ + +
5A	70	50	+ +	5B	70	50	+ +	5C	90	50	+ + +
6A	70	60	+ +	6B	70	50	+ +	6C	80	50	+ + +
7A	80	50	+ + +	7B	60	50	+ +	7C	70	50	+ +
8A	75	60	+ + +	8B	75	50	+ +	8C	60	50	+ +
9A	80	50	+ + +	9B	70	50	+ +	9C	80	50	+ + +
10A	75	50	+ + +	10B	70	50	+ +	10C	80	50	+ + +
11A	70	60	+ +	11B	70	50	+ +	11C	80	50	+ + +
12A	80	60	+ + +	12B	70	50	+ +	12C	70	60	+ +
13A	80	50	+ + +	13B	70	50	+ +	13C	70	50	+ +
14A	70	50	+ +	14B	70	50	+ +	14C	70	50	+ +
15A	80	60	+ + +	15B	70	50	+ +	15C	80	50	+ + +
$\bar{x}$	74.67 A	54.0 A		$\bar{x}$	69.67 B	50B		$\bar{x}$	76.67C	50.67C	
SD	4.81	5.07		**SD**	2.97	0		**SD**	8.16	2.58	

Results and Discussion

A widely accepted system of bull breeding and bull preparation in test stations promotes a thorough selection before qualifying bulls in semen production stations (KASPERCZYK *et al.* 2001). The semen quality assessment methods comprise the analysis of many parameters such as the colour of an ejaculate, its volume, consistence, number of sperm cells in 1 ml (concentration), motility and sperm cell morphology (MADEJA *et al.* 2003). Besides vestigial elements contained in semen, zinc, for many years, has been an object of interest due to its high concentration in semen. However, research on the correlation between the sperm cell number, motility or semen volume and zinc content in semen liquid or blood serum has not given unambiguous results (CARRERAS & MENDOZA 1990).

In this study, the zinc concentration in bull semen showed a positive, statistically significant correlation with semen motility characterized by progressive movement in station A at r = 0,694. Similar correspondences for Zn were confirmed by other authors in research concerning its influence on human semen parameters (KASPERCZYK *et al.* 2001; PANDY *et al.* 1983). In the remaining stations, no statistically significant correlation between zinc and motility was observed.

However, statistically significant positive correlations between Zn and semen concentration in station A at r = 0.881 and C at r = 0.873 were observed. In station B these correspondences were not statistically confirmed. A significant correlation between Pb concentration and the analyzed parameters was not observed. The obtained data correspond with results on human semen (HOVATTA *et al.* 1998).

Variance analyses for the semen samples from three different stations were conducted. Semen did not differ statistically in terms of obtained volume (Table 1), but differed statistically in terms of the Pb concentration between station A and C (Fig. 1). This indicates that the sampling site and slight age difference do not have a statistically significant influence on the analyzed parameter. However, statistically significant differences were observed between zinc concentration in station A and B (which may have resulted from the differences in the content of mineral complements added to feed) and bull semen motility in three different stations (Table 2).

Basic bull semen semiological analysis was conducted with the aim of determining the intensity of sperm cell motility in relation to the percentage of sperm cells characterized with progressive movement. All other types of movement were treated as anomalous. An assessment of sperm cells mass motility is very important for the semen insemination value; however it is not the main criterion (WIERZBOWSKI 1996). It is assumed that the sperm cell mass assessment corresponds with the insemination ability, which is one of the criteria used for preparing insemination doses for production. In bulls from stations A and C, progressive movement before freezing retained values in the range of 80-100%, which means that almost all sperm cells move, and in the range of 60-80% (Table 2). Progressive movement in fresh bull semen from station B (Table 2) exhibited a range of 60-80% for all individuals. Good semen should contain over 70% sperm cells in progressive movement. Ejaculates below 70% progressive movement may be used only in well-founded cases; however those under 50% should not be used. All the examined bull semen from station A equaled or exceeded 70%. The ejaculates from 93% of animals from stations B and C showed that ≥ 70% of sperm cells were characterized by progressive movement.

The statistical assessment of the above mentioned parameters between fresh and frozen semen indicated a statistically significant decrease in the progressive movement in relation to fresh semen. The assessment of wave motility depends on the concentration and percentage of sperm cells showing progressive movement (WIERZBOWSKI 1996). Good quality semen should be characterized with intensive wave motility (KUPFERSCHMIED 1996), which may be observed in about 50% of bull semen from stations A and C (Table 2). In the remaining individuals lively wave motion was observed.

In this study positive correlations between Zn concentration and the motility sperm of cells characterized by progressive movement were observed, which has also been confirmed by other authors (KASPERCZYK *et al.* 2001; SKANDAM *et al.* 1978; STANKOVIC & MICKACDEVIC 1976). The bull semen from three different stations bore statistically significant differences in reference to Zn concentration as well as semen motility before and after refreezing.

The Biociphos-Plus composition and dilution were taken into consideration in the appropriate calculations of Zn and Pb concentrations in the plasma of semen. Zn concentration in the prepared dilutent remained at a level of 0,39 mg/dl and Pb level was below the detection limit.

References

ABBASI A. A., PRASAD A. S., ROBBAIR P., DUMUCHELLE G. 1980. Experimental zinc deficiency in man. J. Bab. Clin. Med. **96**: 544-547.

BENOFF S., JACOB A., HURLEY I. 2000. Male infertility and environmental exposure to lead and cadmium. Hum. Reprod. Update **6** :107-121.

BIRES J., KOVAC G., VRZGULA L. 1991. Mineral profile of serum in experimental copper intoxication of sheep from industrial emissions. Vet. Hum. Toxicol. **33**:489-491.

CARRERAS A., MENDOZA C.1990. Zinc levels in seminal plasma of fertile and infertile men. (In:Andrologia, vol.1. S. Wierzbowski, Platan, National Research Institute of Animal Production, Cracow): 279-283.

CIGANKOVA V., MESAROS P., BIRES J., LEDECKY V., CIGANEK J., TOMAJKOVA E. 1998. Morphological structure of the testes in stallions at zinc deficiency. Slov. Vet. J. **23**: 97-100.

HOVATTA O., VENÄLÄINEN E. R., KUUSIMÄKI L., HEIKKILÄ J., HIRVI T., REIMA I. 1998. Aluminium, lead and cadmium concentrations in seminal plasma and spermatozoa, and quality in Finnish men. Hum. Reprod. **13**: 115-119.

KASPERCZYK A., KASPERCZYK S., DZIWISZ M., BIRKNER E., ZALEJSKA-FIOLKA J., WALECKA C., WINIARSKA H., BIRKNER J. 2001. The influence of Zinc and Selen concentration on the quality of human semen. Bromat. Chem. Toksykol. **34**: 233-237.

KUPFERSCHMIED H. U. 1996. Bulls as producers of semen for insemination. (In:Andrologia, vol.1. S. Wierzbowski, Platan, National Research Institute of Animal Production, Cracow): 126-142.

MADEJA Z., WAROCZYK M., STRABEL T., LECHNIAK D. 2003. The usefulness of hyposomic tests of semen swell in assessing bulls' and boars' semen quality. Medycyna Wet. **59**: 1115-1118

MASSANYI P., TRANDZIK J., NAD P., KORENEKOVA B., SKALICKA M., TOMAN R., LUKAC N., STRAPAK P., HALO M., TURCAN J. 2003. Concentration of copper, iron, zinc, cadmium, lead nad nickel in boar semen and relation to the spermatozoa quality. J. Environ. Sci. Health A Tox Hazard Subst. Environ. Eng. **38**: 2643-51.

MASSANYI P., TRANDZIK J., NAD P., KORENEKOVA B., SKALICKA M., TOMAN R., LUKAC N., HALO M., STRAPAK P. 2004. Concentration of copper, iron, zinc, cadmium, lead, and nickel in bull and ram semen and relation to the occurrence of pathological spermatozoa. J. Environ. Sci. Health A Tox Hazard Subst. Environ. Eng. **39**: 3005-14.

MASSANYI P., TRANDZIK J., NAD P., TOMAN R., SKALICKA M., KORENEKOVA B. 2003. Seminal concentrations of trace elements in various animals and their correlations. Asian J. Androl. **5**: 101-104.

PANDY V. K., PARMESHWARAN M., SOMAN S. D., DACOSTA J. C. 1983.Concentrations of morphologically normal, motile spermatoza: Mg, Ca and Zn. Sci. Total Environ. **27**: 49-52.

SKANDAM K. P., SKANDHAM S., MEHTA Y. B. 1978. Semen electrolytes in normal and infertile subjects II: zinc. Experientia **34**: 1476-1480.

STANKOVIC H., MICKACDEVIC D. 1976. Zinc and copper in human semen. Clin. Chim. Acta. **70**: 123-125.

TIKKIWAL M., AJMERA R. L., MATHUR N. K. 1987. Effect of zinc administration on meminal zinc and fertility of oligospermic males. Indian. J. Physiol. Pharmacol. **31**: 30-34.

WIERZBOWSKI S. 1996. Physiology and pathology of bulls' sexual activities. (In: Andrologia, vol.1. S. Wierzbowski, Platan, National Research Institute of Animal Production, Cracow): 95-99.