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EFFECTS OF SELECTED EPIGENETIC FACTORS ON THE RABBIT EJACULATE QUALITY

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The aim of our study was to monitor the impact of heavy metals and hyperthermia on the basic rabbit semen characteristics. Rabbit males (n=31) of New Zealand White line were exposed to different doses of nickel and zinc administered to feed mixture (P1 group – 17.5 mg $NiCl_2$/kg, P2 group – 35.0 mg $NiCl_2$/kg, P3 group – 17.5 mg $NiCl_2$/kg + 30.0 mg $ZnCl_2$/kg and P4 group – 35.0 mg $NiCl_2$/kg + 30.0 mg $ZnCl_2$/kg; experiment I) and to high ambient temperature (36 ± 3°C; experiment II), and then compared to control C. Semen samples collected from each buck were analysed using CASA system in order to evaluate the concentration and motility parameters of rabbit spermatozoa. In the first experiment, highly significant differences were observed in the motility of spermatozoa between P3 and P1, P4 groups and C ($p<0.001$). P3 group had the lowest progressive motility of spermatozoa in comparison to groups P1, P4 and C ($p<0.001$). In the second experiment, the lowest motility was noted in the last experimental collection in comparison to the 1st collection ($p<0.01$). The highest progressive motility was found in the 1st collection comparing to the control collection and the 3rd collection ($p<0.05$). In conclusion, our study showed that the quality of rabbits semen and subsequently also fertility may be potentially negatively affected by exposure to heavy metals and hyperthermia.

Key words: CASA, heavy metals, hyperthermia, rabbit sperm

INTRODUCTION

Rabbits are an attractive alternative model to produce recombinant protein and to study biological functions and human diseases (Bozse *et al*., 2003). It is favoured mainly due to its reproductive traits. There are many factors influencing the quality and quantity of rabbit semen such as breed (Amin *et al*., 1987), individual (Castellini, 1996), age (Gogol *et al*., 2002), season (Bodnar *et al*., 2000), photoperiod (Theau-Clement *et al*., 1995), nutrition (Fodor *et al*., 2003), collection

rhythms (Nizza *et al*., 2003) and transgenesis (Chrenek *et al*., 2007a and 2007b). Therefore, it is important to define a sexual regime for the male to be able to provide large volumes of high quality semen.

Reproductive organs are sensitive indicators of tissue injury by environmental toxicants as a nickel, zinc etc. This system is important not only from the viewpoint of conservation of the individual, but also the species; perhaps this is why majority of toxicants significantly affect the reproductive system (Lukac *et al*., 2007).

Nickel is toxic to reproductive systems, yielding aberrant sperm and deformed uteri in exposed animals. In rats and mice, dietary nickel (1 mg/kg) can damage sperm, reducing its normal motility and physiological functions (Yokoi *et al*., 2003), perhaps because of oxidative damage (Kasprzak *et al*., 2003; Doreswamy *et al*., 2004). On the other hand, zinc (Zn) is environmentally ubiquitous and essential for life (Sandstead and Au, 2007). Zinc is an essential trace element and serves as the active centre of approximately 300 enzymes. Therefore, zinc deficiency may be associated with a variety of clinical features such as hypogeusia, hyposmia, growth retardation, dermatitis, alopecia, gonadal hypofunction, abnormal pregnancy, susceptibility to infections, delayed wound healing, impaired glucose tolerance, and increased carcinogenesis (Yanagisawa, 2008).

Spermatogenesis is sensitive to a variety of chemical and physical stressors. Testicular hyperthermia has been known to have a deleterious effect on male fertility since the time of Hippocrates and is a well-recognized cause of impaired sperm production (Dada *et al*., 2003).

The aim of our study was to monitor the impact of heavy metals and hyperthermia on the basic rabbit semen characteristics.

MATERIAL AND METHODS

Animals

Sexually mature (4 – 5 months old) and clinically healthy rabbit males (n=31) of New Zealand White (NZW) line reared in a partially air-conditioned hall of a local rabbit farm at APRC (Animal Production Research Centre) Nitra were used in the experiment. The males were housed in individual cages, under a constant photoperiod of 14h of daylight. Temperature and humidity in the building were recorded continuously by means of a thermograph positioned at the same level as the cages (average relative humidity and temperature during the year was maintained at 60±5% and 17±3^{o}C). The rabbits were fed *ad libitum* with a commercial diet (KV; TEKRO Nitra, s.r.o.) and water was provided *ad libitum* with nipple drinkers.

Nickel and zinc treatment

Animals were divided into five groups: control group C and 4 experimental groups P1, P2, P3 and P4 (5 animals in each group). Experimental animals of P1 and P2 groups received nickel and animals of P3 and P4 groups received nickel + zinc supplement to the feed mixture for 90 days in the following amounts: P1

group – 17.5 mg $NiCl_2$/kg, P2 group – 35.0 mg $NiCl_2$/kg, P3 group – 17.5 mg $NiCl_2$/kg + 30.0 mg $ZnCl_2$/kg and P4 group – 35.0 mg $NiCl_2$/kg + 30.0 mg $ZnCl_2$/kg.

Hyperthermia

Experimental conditions with a defined temperature (36 ± 3°C) were simulated in a closed breeding area, with an installed heat aggregate and temperature sensor. Experimental animals (6 males) were placed in separate sectors of the breeding cages with *ad libitum* feeding and watering systems. The control group of animals were the same as in experimental group (6 males), but the ejaculate parameters were evaluated before exposure to high ambient temperature.

The treatment of the animals was approved by the Ministry of Agriculture and Rural Development of the Slovak Republic no. SK P 28004 and Ro 1488/06-221/3a.

Semen collection and analysis

Semen collection was performed using an artificial vagina in all experiments. In the first experiment, where the effect of nickel and zinc addition to the feed mixture on reproductive parameters of male rabbits was observed, the ejaculate was collected once a month throughout the duration of the experiment (90 days). In the second experiment, where the impact of high ambient temperature on sperm quality was observed, ejaculates were collected once a week throughout the period of the experiment (21 days, 3 collections). The control semen collection was performed before exposing animals to high ambient temperature.

All samples were analysed using CASA (Computer Assisted Semen Analysis) system – SpermVision (Minitüb, Tiefenbach, Germany) combined with Olympus BX 51 microscope (Olympus, Japan) and following parameters were evaluated: concentration ($x10^9$ cells per mL); percentage of motile spermatozoa (motility >5 μm/s) and percentage of progressively motile spermatozoa (motility >20 μm/s).

Statistics

For comparing results the analysis of variance and Scheffe test were used in order to calculate basic statistical characteristics and to determine significant differences between experimental and control groups in the SAS 6.02 statistical software (SAS Institute Inc., U.S.A.). Data were presented as mean ± SD (standard deviation). P-values at $p<0.05$ were considered as statistically significant.

RESULTS

The results of basic ejaculate characteristics with statistical differences between control group (C) and experimental groups (P1, P2, P3, P4), after nickel and zinc administration to the feed mixture for rabbits are presented in Table 1.

Table 1. CASA analysis of the rabbit ejaculate parameters after exposure to different nickel and zinc doses in the feed mixture

	Group				
Parameter	P1	P2	P3	P4	Control
Ejaculate volume (mL)	0.55±0.35	0.73±0.25	0.60±0.57	0.73±0.25	1.00±0.92
Sperm concentration ($x10^9$ cells per mL)	1.52±0.16^{c}	1.12±0.36	0.60±0.32^{a,d}	1.30±1.08^{b}	0.88±0.51
Motility (%)	83.39±2.66^{e}	71.24±10.98^{a}	55.37±24.39^{b,f}	84.87±8.63^{e}	83.12±9.56^{e}
Progressive motility (%)	75.40±3.20^{a,e}	54.58±18.26^{b,c}	39.78±30.26^{f}	77.80±12.60^{d,e}	73.92±15.27^{a,e}

^{a} vs ^{b} – statistically significant at $p<0.05$; ^{c} vs ^{d} – statistically significant at $p<0.01$; ^{e} vs ^{f} – statistically significant at $p<0.001$

Table 2. CASA analysis of the rabbit ejaculate parameters after high ambient temperature exposure

	Ejaculate collection			
Parameter	Control	1.	2.	3.
Ejaculate volume (mL)	0.71±0.55	0.50±0.14	0.30±0.14	0.55±0.21
Sperm concentration ($x10^9$ cells per mL)	1.06±0.65	1.18±0.48	0.91±0.82	1.12±0.67
Motility (%)	44.38±30.77	64.96±11.79^{c}	55.80±26.33	33.95±18.89^{d}
Progressive motility (%)	26.44±27.80^{a}	50.11±10.42^{b}	31.64±37.99	19.49±15.24^{a}

^{a} vs ^{b} – statistically significant at $p<0.05$; ^{c} vs ^{d} – statistically significant at $p<0.01$

There were no significant differences in the ejaculate volume between experimental groups and control group. Significant differences were noted in sperm concentration between P3 and P4 group ($p<0.05$) and between P1 and P3 groups ($p<0.01$). Significant differences were also observed in the motility of spermatozoa between experimental groups P2 and P3 ($p<0.05$), whereas between P3 and groups P1, P4 and C highly significant differences ($p<0.001$) were found. The progressive motility of rabbit spermatozoa was significantly different between experimental groups P2 and P1, as well as the control group ($p<0.05$), and between groups P2 and P4 ($p<0.01$). P3 group had significantly lowest progressive motility of spermatozoa in comparison to groups P1, P4 and C ($p<.001$; Figure 1).

Table 2 shows the basic ejaculate parameters of rabbits after exposure to high ambient temperature. No significant differences ($p>0.05$) were found in the case of ejaculate volume and sperm concentration among all ejaculate collections. In the 1st and 2nd collection the motility of spermatozoa showed increasing tendency in comparison to the ejaculate collected from the control group, although the differences were not significant ($p>0.05$). The lowest motility

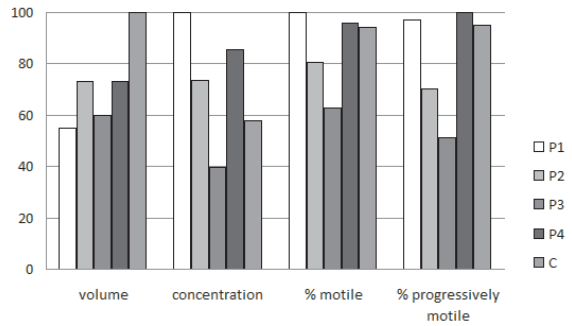

Figure 1. Differences in basic ejaculate characteristics among observed experimental groups and control group

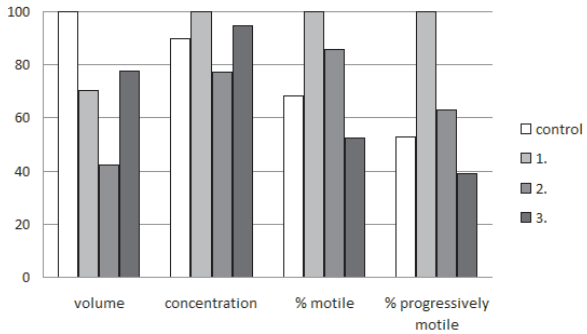

Figure 2. Differences in basic ejaculate characteristics between observed ejaculate collections

was noted in the last experimental collection in comparison to the 1st collection ($p<0.01$). The highest progressive motility was found in the 1st collection comparing to the control collection and the 3rd collection ($p<.05$; Figure 2).

DISCUSSION

The reproductive health of animals could be affected by a number of endogenous as well as exogenous factors, such as exposure to heavy metals (Hansen *et al*., 2010) and ambient temperature (Schwalm *et al*., 2007). As it was published previously, the exposure of animals to xenobiotics caused various alterations of zootechnical parameters (Kalafová *et al*., 2009), as well as imbalance in internal milieu (Capcarova *et al*., 2010). According to Sevi *et al*. (2001), exposure of the animals to high ambient temperature has the adverse effect on the organism. It has been reported that the main consequences of high ambient temperature exposure are cerebral ischemia (Lin and Lin, 1992), loss of sensation, deep hyperthermia, coma (Shih *et al.*, 1984), hormonal alterations (West, 2003), liver and heart damages (Yan *et al*., 2009) and necrosis of animal tissues (Huang *et al*., 2009). When rabbits were exposed to chronic hyperthermic stress, the most sensitive animals, especially younger rabbits could not regulate their internal milieu and hyperthermic collapse appeared resulting in increasing mortality (Mashaly *et al*., 2004).

This *in vivo* study was aimed at the investigation of the changes in rabbit sperm motility after exposure of rabbits to different nickel and zinc doses in the feed mixture and exposure to high ambient temperature.

Our previous studies have shown that nickel and zinc at defined doses significantly influenced the activity of selected enzymes (GGT, AST, GLDH) in blood serum of rabbits (Kalafová *et al*., 2010a), but have not significant effects on rabbit meat quality (Kalafová *et al*., 2011) and concentrations of calcium, sodium and magnesium in muscle tissue of rabbits (Kalafová *et al*., 2010b). Lukac *et al*. (2010) reported that *in vitro* exposure to nickel caused a decrease in bovine spermatozoa motility. The progressive motility of spermatozoa showed an inhibiting tendency. Our *in vivo* study partially confirmed these previous *in vitro* results. The progressive motility showed a decreasing tendency depending on nickel dose (group P1, P2 and P3), but it was rapidly improved by zinc addition to the rabbit feed mixture (P4 group; Figure 1). It is known that zinc plays an essential role in spermatogenesis and fertility (Wong *et al*., 2001). Poor Zn nutrition may be an important risk factor for low quality of sperm and idiopathic male infertility (Colagar *et al*., 2009). In this paper the addition of nickel and zinc had no effect on the ejaculate volume. Dissanyyake *et al*. (2010) reported that count, motility, viability, pH and viscosity of human semen are affected by variations of seminal plasma zinc. In another report there was no statistically significant relationship between zinc in seminal plasma or serum and semen quality parameters. Zinc levels did not influence sperm capacity to penetrate cervical mucus *in vitro* or *in vivo*, and did not affect subsequent fertility (Eggert-Kruse *et al*., 2002).

Trudeau and Sanford (1986) reported higher values of boar semen parameters in winter in comparison to summer. It means that higher ambient

temperature can affect sperm characteristics, which was also confirmed in our experiment with rabbits. Animals exposed to high ambient temperature showed increased sperm motility and altered semen parameters (Lue *et al*., 2000; Brecchia *et al*., 2010). On the other hand, a number of authors observed decreased values of the rabbit sperm motility in the summer in comparison to winter (El-Masry *et al*., 1994; Nizza *et al*., 2003; Safaa *et al*., 2008). In our experiment the ejaculate volume and sperm concentration of rabbits were not significantly affected by high ambient temperature, whereas motility and progressive motility of rabbit spermatozoa showed increasing tendency in the first two experimental collections. However, the last collection had the lowest motility parameters from all experimental collections including the control semen collection, which can indicate a partial adaptation of rabbits to ambient temperature, but ultimately, the long-term exposure to high ambient temperature may have a negative effect on rabbit sperm motility (Figure 2).

The seminal characteristics are affected by many factors (breed, feeding, health status, rearing condition, season and collection frequency) and there is a wide variety in semen traits (Alvarino, 2000). Our study showed that the quality of semen parameters and subsequently also fertility of rabbits may be negatively affected by exposure to heavy metals and hyperthermia.

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UTICAJ POJEDINIH EPIGENETSKIH FAKTORA NA KVALITET EJAKULATA KUNIĆA

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SADRŽAJ

Cilj našeg rada je bio praćenje uticaja teških metala i hipertermije na osnovne karakteristike ejakulata kunića. Mužjaci NZW (New Zealand White) kunića (n=31) su bili hranjeni smešama su različitim količinama nikla i cinka (P1 grupa – 17,5 mg $NiCl_2$/kg, P2 grupa – 35,0 mg $NiCl_2$/kg, P3 grupa – 17,5 mg $NiCl_2$/kg + 30,0 mg $ZnCl_2$/kg and P4 grupa – 35,0 mg $NiCl_2$/kg + 30,0 mg $ZnCl_2$/kg; eksperiment I) i visokim temperaturama (36 ± 3 ^oC; eksperiment II), a zatim su njihovi na-

lazi upoređivani sa nalazima dobijenim u kontrolnoj grupi C. Uzorci ejakulata su bili analizirani CASA sistemom da bi se procenila koncentracija i parametri pokretljivosti spermatozoida kunića. U prvom eksperimentu su primećene značajne razlike u pokretljivosti spermatozoida između P3 i P1, P4 i C grupa ($p<0,001$). Grupa P3 je imala najnižu progresivnu pokrtetljivost spermatozoida u poređenju sa grupama P1, P4 i C ($p<0,001$). U drugom eksperimentu najniža pokretljivost je zabeležena u poslednjoj eksperimentalnoj grupi i te razlike su bile statistički značajne u poređenju sa prvom grupom uzoraka ($p<0,01$). Najviša progresivna pokretljivost je registrovana u prvoj grupi, a razlike su bile ststistički značajne u poređenju sa kontrolnom i trećom grupom ($p<0,05$). Izloženost teškim metalima i hipertermiji može da ima negativan uticaj na kvalitet sperme, a kasnije i na plodnost kunića.