COMPARISON OF INNER ORGANS WEIGHT AND SOME HEMATOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS OF TRANSGENIC AND NONTRANSGENIC RABBITS.

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ABSTRACT

Chosen anatomical and physiological parameters were compared in F2 generation transgenic rabbits produced by microinjection of WAP-hFVIII gene construct (10 animals) and rabbits of initial population in conformable age and average weight (20 animals). The animals were bred in equal conditions similar to intensive industrial ones. The transgenic rabbits of average weight 2,81 kg and control animals of average weight 2.83 kg were slaughtered. Before slaughtering the blood for haematological and biochemical analysis was taken from the central ear artery. Autopsy and pathological examination of vital organs were performed. The laboratory scales with the accuracy of 0.01 g were used to determine weight of chosen organs: heart, lungs, liver, kidney right, kidney left, spleen, both adrenal glands. Haematological values (white blood cells WBC, red blood cells RBC, blood haemoglobin HGB, haematocrit HGT, mean corpuscular volume MCV, mean corpuscular haemoglobin MCH, mean corpuscular haemoglobin concentration MCHC, platelets PLT) were determined by Sysmex KX-21Nä Automated Haematology Analyzer. Biochemical parameters (total protein, glucose, urea, creatinine and aspartat-aminotransferase AST, alanine-aminotransferase ALT and gamma-glutamyltransferase activities GMT) were assessed by laboratory analyzer COBAS INTEGRA 400 plus using Roche diagnostic tests. Pathological examination of vital organs showed relatively more pathological changes in transgenic rabbits. Our results showed no significant differences of chosen inner organs weight between transgenic and nontransgenic animals except of weight of lungs (transgenic rabbits 14.06g, nontransgenic 16.82; $P < 0.01$). We observed statistically significant higher values of WBC (transgenic $12.37.10^9$/l – nontransgenic $9.42.10^9$/l; $P<0.001$) and PLT ($567.10.10^9$/l – $481.64.10^9$/l; $P<0.05$), and lower values of MCH (19.85 pg – nontransgenic 21.01pg; $P<0.01$) and MCHC(29.49 g/dl – 30.30 g/dl; $P<0.05$) in blood of transgenic rabbit.Significant differences were found in the concentration of urea depending on the group (transgenic 6.78 $mmol.l^{-1}$ and nontransgenic 5.23, $P<0.001$), creatinine (transgenic 73.20 $\mu mol.l^{-1}$and nontransgenic 62.66, $P<0.01$), and total protein (65.78 $g.l^{-1}$and61.97 $g.l^{-1}$, $P<0.05$).Highly significant differences were obtained also in AST (transgenic 0.41 $\mu kat.l^{-1}$ and nontransgenic 0.27, $P<0.001$), and in GMT (transgenic 0.17 $\mu kat.l^{-1}$ and nontransgenic 0.10, $P<0.001$) activity.

Key words: transgenic rabbits, inner organs weight, haematological values, serum biochemistry.

INTRODUCTION

Transgenic rabbits have proved to be suitable bioreactors for the production of recombinant protein both on an experimental and a commercial scale (BŐSZE *et al.,* 2003*).* The experiments in our laboratory have been oriented to creation of transgenic rabbit line with recombinant human factor VIII (rhFVIII) production in milk. However, there are many obstacles that need to be worked out in technology of recombinant proteins production in the rabbits. A number of factors influence the success of the generation of transgenic animals: low pregnancy rate, low litter size, cannibalism, low positive rate within the pups, higher mortality of transgenic offspring, uncontrolled expression (ectopic) and mosaic founders that are incapable of germline transmission. (KALASHNIKOVA *et al*., 1994; PETROVIČOVÁ, 2000; MERTENS and RÜLICKE, 2000; FAN and WATANABE, 2003). Our study was focused on comparison of chosen anatomical and physiological characteristics in transgenic and nontransgenic rabbits.

MATERIAL AND METHODS

Animals

The experiment was carried out on the New Zealand White and California rabbits from rabbit farm at the Research Institute for Animal Production in Nitra.
Transgenic rabbits were produced by microinjection of WAP-hFVIII gene construct into male pronucleus of fertilized eggs from superovulated does (CHRENEK *et al*., 2004). The gene construct was kindly provided by Dr. H. Lubon (American Red Cross, Maryland, USA). Subsequently, transgenic founders were mated to obtain F1 and F2 generation.
In the experiment were used transgenic rabbits remounted from two litters of F2 generation(4 and 6 young). They were compared with rabbits of initial population at conformable age and average weight.
The rabbits were housed in closed rabbit house in flat – deck wire cages. The animals were fed a commercial pellet (crude protein 16.5 – 17.5%, fiber 14 – 16%, fat 3 – 4%, ME 10 – 11 MJ/kg) ad libitum. The water was provided all time by automatic watering system. The breeding conditions were similar to the intensive industrial ones.

Sample collection

The transgenic animals weighting between 2.63 to 3.09 kg (on average 2,81 kg) and control animals of average weight 2.83 kg were slaughtered.
Blood for haematological and biochemical analysis was taken from the central ear artery before slaughtering (between 10.00 to14.00 hours). For the determination of haematological values, as white blood cells (WBC), red blood cells (RBC), blood haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), K-EDTA was added to the tubes as anticoagulant. Serum was used to determination of total protein, glucose, urea, creatinine and aspartat

aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase activities (GMT).
Autopsy and pathological examination of vital organs were performed after slaughtering. We used the laboratory scales with the accuracy of 0.01 g to determine weight of selected organs: heart, lungs, liver, kidney right, kidney left, spleen, both adrenal glands.

Blood samples analysis

Haematological parameters were determined by Sysmex KX-21Nä Automated Haematology Analyzer, and biochemical parameters by laboratory analyzer COBAS INTEGRA 400 plus using Roche diagnostic tests. For HGB determination was used the non – cyanide haemoglobin analysis method. HCT was measured by RBC pulse high detection method. Total protein content of the sample was assayed by biuret reaction. Content of glucose was measured by enzymatic reference method with hexokinase. Kinetic test with urease and glutamate dehydrogenase was used for determination of urea. Creatinine was analysed by buffered kinetic Jaffé reaction without deproteinization. ALT, AST activity was defined according to IFCC (AST without pyridoxal 5' - phosphate). For GMT determination was used the method based on kinetic procedure using the carboxy substrate L – y – glutamyl – 3 – carboxy – 4 – nitroanilide.

Statistical method

We calculated the basic statistic characteristics. T-test was used to calculating the differences between the control and transgenic population. We used the statistic pocket of SAS program: procedure MEANS, TTEST, CORR. (SAS Inc., 2001).

RESULTS AND DISCUSSION

Pathological examination of vital organs showed some pathological changes in both transgenic and nontransgenic rabbits. We detected relatively more pathological changes in transgenic rabbits (Table 1).
Arithmetic means, standard deviations, coefficient of variation, maximum, minimum and significant differences of selected inner organs weight for each group are given in Table 2. Our results show no significant differences between transgenic and nontransgenic animals in most of the studied parameters. The only significant difference, at the level of significance $P < 0.01$ was found in the weight of lungs (transgenic rabbits 14.06g, nontransgenic 16.82). As agonal haemorrhages were attendant in the lung of some animals, it is not possible to exclude influence of slaughtering blood aspiration at all. The obtained weights of liver, spleen, adrenal glands, kidneys and heart were a little higher in comparison with results of KOZMA et al. (1974).
The haematological values of transgenic and nontransgenic rabbit groups are presented in Table 3.We observed statistically significant higher values of white blood cells (transgenic $12.37.10^9$/l – nontransgenic $9.42.10^9$/l; $P<0.001$) and platelets ($567.10.10^9$/l – $481.64.10^9$/l; $P<0.05$) in blood of transgenic rabbits.On the contrary, in transgenic

Table 1. Pathological changes in vital organs of transgenic and nontransgenic rabbits

Animals	N	Number of cases	Pathological changes
Transgenic rabbits	10	2 (20%)	tracheobronchitis haemorrhagica, hyperemia renis, steatosis hepatis
		1 (10%)	splenomegalia, steatosis hepatis, hyperaemia renis
		1 (10%)	metritis purulenta, steatosis hepatis
		6 (60%)	without pathological changes
Non-transgenic rabbits	20	1 (5%)	dilatatio cordis, bronchopneumonia mucopurulenta chronica, atelectasis pulmonum
		1 (5%)	steatosis hepatis, gastroenteritis catarrhalis acuta, meteorismus
		1 (5%)	necrosis hepatis,
		1 (5%)	hyperemia pulmonum, hyperemia renis, splenomegalia
		16 (80%)	without pathological changes

N – number of animals

Table 2. Comparison of body and inner organs weight (g) in transgenic and nontransgenic rabbits

Trait		N	M	MD	v%	min.	max.	Statistical significance
Body	T	10	2808	0.15	5.18	2630	3090	
	Nt	20	2826	0.18	6.32	2620	3200	
Heart	T	10	9.14	0.25	2.77	8.73	9.61	
	nt	20	8.70	1.23	14.22	7.32	12.48	
Lungs	T	10	14.06	1.77	12.59	11.81	17.19	++
	nt	20	16.82	2.34	13.91	12.38	19.82	
Liver	T	10	83.99	6.09	7.25	74.85	92.63	
	Nt	20	89.64	10.16	11.33	79.00	110.35	
Kidney right	T	10	9.54	0.52	5.49	8.63	10.26	
	Nt	20	9.86	0.86	8.74	8.64	11.74	
Kidney Left	T	10	9.75	0.59	6.03	8.74	11.00	
	nt	20	10.13	0.84	8.30	9.05	11.79	
Spleen	T	10	1.40	0.51	36.26	0.66	2.12	
	Nt	20	1.65	0.53	32.14	0.93	3.50	
Adrenal Glands	T	10	0.30	0.07	24.25	0.21	0.41	
	Nt	20	0.39	0.23	58.88	0.20	1.28	

- number of animals, M – mean, MD standard deviation, v% - coefficient of variation, min. – minimum, max. – maximum, t – transgenic, nt – nontransgenic rabbits

rabbit blood were detected lower values of mean corpuscular haemoglobin (transgenic 19.85 pg – nontransgenic 21.01pg; P<0.01) and mean corpuscular haemoglobin concentration (29.49 g/dl – 30.30 g/dl; P<0.05). Determined values of HCT and HGB are comparable with the results of KOZMA et al. (1974).In comparison with results of BERSÉNYI et al. (2003), the obtained values of WBC and PLT of transgenic rabbits were higher, counter values of MCV and MCHC were lower.

Table 3. Comparison of selected haematological parameters in transgenic and nontransgenic rabbits

Trait		N	M	MD	v%	min.	max.	Statistical significance
WBC (10^9/l)	T	10	12.37	1.61	12.98	9.20	15.7	+++
	Nt	25	9.42	1.82	19.36	5.80	13.50	
RBC (10^{12}/l)	T	10	6.08	0.63	10.37	4.39	6.57	
	Nt	25	5.93	0.46	7.74	4.42	6.44	
HGB (g/dl)	T	10	12.07	1.05	8.70	9.40	13.30	
	Nt	25	12.28	1.23	10.06	7.90	14.10	
HCT (%)	T	10	40.70	2.88	7.07	34.70	45.20	
	Nt	25	41.04	2.45	5.98	36.20	45.40	
MCV (fl)	T	10	67.00	4.59	6.86	63.50	79.00	
	Nt	25	69.35	4.53	6.53	63.10	87.30	
MCH (pg)	T	10	19.85	0.74	3.75	18.90	21.40	++
	Nt	25	21.01	1.23	5.90	19.00	24.90	
MCHC (g/dl)	T	10	29.49	0.89	3.00	27.10	30.30	+
	Nt	25	30.30	0.79	2.64	28.50	31.90	
PLT (10^9/l)	T	10	567.10	81.55	14.38	482.0025	726.00	+
	Nt	25	481.64	173.53	29.20	1.00	753.00	

N. M. MD. v%. min.. max. – as in Table 2.

Leukocytosis accompanied by thrombocytosis in transgenic rabbits could refer to a pasteurelosis (RUBLE et al., 1999).

Comparison of selected biochemical blood serum parameters in transgenic and nontransgenic rabbits is given in Table 4. Significant differences were found in the concentration of urea depending on the group (transgenic 6.78 mmol.l^{-1} and nontransgenic 5.23, P<0.001), creatinine (transgenic 73.20 µmol.$l^{-1}$and nontransgenic 62.66, P<0.01), and total protein (65.78 g.l^{-1}and61.97 g.l^{-1}, P<0.05).Highly significant differences were obtained also in AST (transgenic 0.41 µkat.l^{-1} and nontransgenic 0.27, P<0.001), and in GMT (transgenic 0.17 µkat.l^{-1} and nontransgenic 0.10, P<0.001) activity. Determined biochemical values of transgenic rabbit blood indicate the chronic liver damage and the worse renal function (VRZGULA et al, 1982).

It was our objective to find out if transgenesis in young rabbits affects their physical development and state of health. According to article of MERTENS and RÜLICKE(2000) altering the genotype has no health consequences for about 90% of transgenic animals, but the remaining 10% have a reduced viability or impaired health at the phenotypic

level.Each newly created transgenic strain has the potential to cause poor health and suffering in the animals. As described by FAN and WATANABE (2003) condition and state of health in donors and recipients also belong among factors which affect success in production of recombinant proteins by transgenic rabbits, divided into definable and non-definable. We observed transgenic and non-transgenic young of clinically healthy mothers of one population, reared in identical conditions. State of health in transgenic young was worse. Findings correspond to results of KALASHNIKOVA et al. (1994) and PETROVIČOVÁ (1999), who mentioned higher morbidity in transgenic young. Neither the fact can be omitted that the state of health in young is affected not directly by transgenesis but e.g. by the surgical intervention in recipients at insertion of embryos (although we did not observe postoperative complications). As viability and good state of health in transgenic young are the basic precondition of creation of transgenic populations and production of recombinant proteins in milk of rabbits it is necessary to deal with this problem in the future also.

Table 4. Comparison of selected biochemical blood serum parameters in transgenic and nontransgenic rabbits

Trait		N	M	MD	v%	min.	max.	Statistical significance
Glucose ($mmol.l^{-1}$)	T	10	7.53	0.66	8.36	6.88	8.87	
	nt	29	7.92	1.06	14.01	6.19	11.50	
Urea ($mmol.l^{-1}$)	T	10	6.78	0.85	12.54	5.64	8.61	+++
	nt	29	5.23	0.86	16.35	3.70	6.96	
Creatinine ($\mu mol.l^{-1}$)	T	10	73.20	9.08	12.40	58.00	85.00	++
	nt	29	62.66	8.35	13.33	44.00	78.00	
Total protein ($g.l^{-1}$)	T	10	65.78	5.28	8.02	58.10	73.80	+
	nt	29	61.97	4.28	6.90	55.10	74.10	
AST ($\mu kat.l^{-1}$)	T	10	0.41	0.08	18.44	0.29	0.57	+++
	Nt	29	0.27	0.06	21.47	0.18	0.43	
ALT ($\mu kat.l^{-1}$)	T	10	1.12	0.15	13.42	0.90	1.44	
	nt	29	1.01	0.20	19.30	0.69	1.39	
GMT ($\mu kat.l^{-1}$)	T	10	0.17	0.04	26.01	0.09	0.24	+++
	Nt	29	0.10	0.03	32.70	0.04	0.17	

N, M, MD, v%, min., max. – as in Table 2

CONCLUSION

The results of this article point out that the examined transgenic rabbits reacted more sensitively than rabbits of initial population in the equal environmental conditions. However, the studied group was rather small, it necessitates additional exploration.

REFERENCES

BERSÉNYI A., FEKETE S.GY., SZÖCS Z., BERTA E. 2003. Effect of ingested heavy metals (Cd, Pb, and Hg) on haematology and serum biochemistry in rabbits. *Acta Veterinaria Hungarica* **51**: 297 – 304.

BŐSZE ZS., HIRIPI L., CARNWATH J.W., NIEMANN H. 2003. The transgenic rabbit as model for human diseases and as a source of biologically active recombinant proteins. *Transgenic Research* **12**: 541 – 553.

CHRENEK P., VASICEK D., MAKAREVICH A., JURCIK R., SUVEGOVA K.,BAUER M., RAFAY J., BULLA J., HETENYI L., ERICKSON J., PALEYANDA R.K. 2004. Integration rates of exogenous DNA into the rabbit genome using single and double pronuclei microinjection. *Transgenic Research,* **13**, *February, abstract.*

FAN J., WATANABE, T. 2003. Transgenic rabbits as therapeutic protein bioreactors and human disease models.*Pharmacology & Therapeutics.* **99**: 261 – 282.

KALASHNIKOVÁ L.A., KOZIKOVA L.V., RASHEED S.T., BULLA J., RAFAY J. 1994.Mortality of transgenic animal offspring. *Proc Eur. Conf. Embryo Techn. & Genet Engin. In Cattle & Sheep, Kraków, Poland,* p. 212.

KOZMA C., MACKLIN W., CUMMINS L.M., MAUER R. 1974. The Anatomy, Physiology, and the Biochemistry of the Rabbit. In: WEISBROTH H., FLATT

R.E., KRAUS A.L.: The Biology of the Laboratory Rabbit, Academic Press New York, San Francisco, London, pp. 50 – 73.

MERTENS C., RÜLICKE T. (2000): Phenotype Characterization and Welfare assessment of Transgenic Rodents (Mice). *Journal of Applied Animal Welfare Science (JAAWS)* **3**: 127 - 139.

PETROVIČOVÁ, I. 1999. Analýza metód a postupov tvorby transgénnych králikov. *Autoreferát dizertačnej práce, SPU Nitra.*

RUBLE R.P., CULLOR J.S., BROOKS D.L. 1999. The Observation of Reactive Thrombocytosis in New Zealand White Rabbits in Response to Experimental *Pasteurella multocida* Infection. *Blood Cells, Molecules, and Diseases.* **25**:95 – 102.

SAS/STAT. 1999-2001, Version 8.2., SAS Institute Inc., Cary, NC, USA.

VRZGULA L. et Al. 1982. Poruchy látkového metabolizmu hospodárskych zvierat a ich prevencia, Príroda Bratislava, 492 pp..