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Short communication

SPERMATOZOA QUALITY OF THE TRANSGENIC RABBIT OFFSPRING

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ABSTRACT

The objective of this study was to evaluate the spermatozoa quality of the F_4 - F_5 generation of transgenic rabbit males carrying the mWAP-hFVIII gene in comparison to non-transgenic individuals and previous transgenic generations. Semen samples from 4 transgenic rabbits and 10 randomly selected non-transgenic males of the same breed and age were collected once a week (5 ejaculates per male were analyzed) using an artificial vagina. Each sample of fresh ejaculate was evaluated using CASA system for concentration and motility parameters. The ejaculate volume was the same in both groups of animals, but the sperm concentration was significantly higher ($p<0.001$) in transgenic bucks than in non-transgenic ones. There was not statistically significant difference in motility of spermatozoa, but the progressive motility was significantly lower ($p<0.001$) in transgenic males when compared to non-transgenic ones. We found significant differences also in other CASA parameters: DAP, LIN, BCF ($p<0.05$) and DSL, VSL ($p<0.01$). In comparison to our previous results (F_1 - F_3), the spermatozoa quality in the offspring (F_4 - F_5) was similar, although the sperm concentration increased twofold, but the ejaculate volume and sperm motility were roughly the same. Our results showed good spermatozoa quality of the F_4 and F_5 generations of transgenic rabbit males without any reproductive disorders caused by transgenesis. Consequently the ability to produce offspring for the purpose to create stable transgenic line was retained.

Key words: transgenic rabbit; spermatozoa quality; CASA

INTRODUCTION

Successful reproduction of transgenic animals is still problematic. The best way to evaluate the success of transgenesis is to produce subsequent generations of transgenic animals, by mating founders of transgenic line with non-transgenic individuals. In this way it is reasonable to compare the development of offspring of transgenic genotype with standard genotype offspring in the same litter (Chrenek *et al*., 2006). Reproductive capabilities of transgenic rabbit founders affect the creation of stable lines of transgenic offspring, which can be used to produce different biologically active recombinant proteins, enzymes or to improve meat and milk quality and quantity or enhance resistance of such transgenic animals against disease (Chrenek *et al*,. 2007a).

Reproductive disorders have been observed in transgenic mice, pigs and sheep (Rexroad *et al*., 1989; Pursel *et al*., 1990; Bartke *et al*., 1992; Meliska and Bartke,

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1997; Maleszewski *et al*., 1998). The decrease in fertility or the infertility of transgenic males has been related to altered copulatory behaviour (Meliska and Bartke, 1997), structural defects and defective sperm function (Maleszewski *et al*., 1998). Semen analysis, including the assessment of sperm concentration, motility and the percentage of the normal forms present, remains the standard procedure for evaluating the fertility potential of semen samples (Bryla *et al*., 2010).

In this study, we report the spermatozoa characteristics of the F_4 - F_5 generation of transgenic rabbit males in comparison to non-transgenic individuals and previous transgenic generations.

MATERIALS AND METHODS

Animals

Transgenic founders with the mWAP-hFVIII gene were produced as described by Chrenek *et al*. (2005). In our experiments we used four transgenic rabbits of the F_4 and F_5 generation. These were obtained by breeding of transgenic founders with non-transgenic rabbits of the same New Zealand White (NZW) breed. Ten randomly selected non-transgenic males of the same breed and age were used as a control. The males were housed in individual cages, under a constant photoperiod of 14 h of light day in a partially air-conditioned hall of a local rabbit farm at APRC Nitra. 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 were maintained at 60±5 % and 17±3°C). The rabbits were fed *ad libitum* with a commercial diet (KV; TEKRO Nitra, Ltd.) and water was provided *ad libitum* with nipple drinkers.

Semen collection and analysis

Semen samples from each buck (transgenic and non-transgenic) were collected once a week (5 ejaculates per male were analyzed) using an artificial vagina. Each sample of fresh ejaculate was evaluated using CASA (Computer Assisted Semen Analysis; MiniTüb, Tiefenbach, Germany) system combined with Olympus BX 51 microscope (Olympus, Japan). Following parameters were evaluated: concentration (10^9 cells per ml); percentage of motile spermatozoa (motility > 5 µm/s), percentage of progressively motile spermatozoa (motility > 20µm/s), DAP (distance average path, µm), DCL (distance curved line; µm), DSL (distance straight line, µm), VAP (velocity average path, µm/s), VCL (velocity curved line, µm/s), VSL (velocity straight line, µm/s), STR (straightness - VSL:VAP), LIN (linearity - VSL:VCL), WOB (wobble - VAP:VCL), ALH (amplitude of lateral head displacement, µm) and BCF (beat cross frequency, Hz).

Table 1: CASA parameters of the transgenic hFVIII (F_4 - F_5 generations) and non-transgenic rabbit males

PARAMETER	Transgenic males (n=4)	Non-transgenic males (n=10)
Ejaculate volume (ml)	0.60±0.05	0.60±0.19
Sperm concentration (x 10^9 cells.ml^{-1})	1.86±1.70e	0.94±0.95f
Motility (%)	64.56±24.95	70.21±22.31
Progressive motility (%)	44.46±27.50e	59.83±26.38f
DAP (µm)	18.05±7.77a	20.36±6.98b
DCL (µm)	41.11±16.70	43.08±12.95
DSL (µm)	13.36±6.20c	15.94±6.07d
VAP (µm.s^{-1})	41.91±18.54	46.24±16.54
VCL (µm.s^{-1})	94.03±38.60	96.77±28.83
VSL (µm.s^{-1})	31.11±14.76c	36.25±14.09d
STR	0.69±0.19	0.73±0.19
LIN	0.31±0.11a	0.35±0.12b
WOB	0.42±0.14	0.47±0.38
ALH (µm)	3.63±1.42	3.38±1.36
BCF (Hz)	26.64±9.03a	29.54±9.59b

a vs. b – statistically significant at $p<0.05$
c vs. d – statistically significant at $p<0.01$
e vs. f – statistically significant at $p<0.001$

Statistics

The average spermatozoa quality of transgenic and non-transgenic rabbit males was observed. Obtained results were statistically evaluated using SAS 6.02 statistical software (SAS Institute Inc., U.S.A.). Data are expressed as mean ± SD (standard deviation). P-values at $p<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Basic semen traits of transgenic and non-transgenic bucks from our experiment are presented in Table 1. The ejaculate volume was the same in both groups of animals, but the sperm concentration was significantly higher ($p<0.001$) in transgenic bucks than in non-transgenic rabbits, similarly as in our previous experiment (Chrenek *et al*., 2007b). Rabbit ejaculate volume is usually in the range of 0.1 - 1.5 ml, and sperm concentration per ml is 0.5 x 10^9 - 3.5 x 10^9 (Hamner, 1970). After testing reproduction traits of standard males, Yousef *et al*. (2003) reported that common rabbit ejaculate volume is 0.7 ml (+0.16) and sperm concentration is 3 x 10^9 per ml. Our results (Table 1) are close to values in the literature.

Spermatozoa motility is one of the important parameters of semen quality. Visual evaluation of the motility by an operator is rather subjective; therefore for objective evaluation of the motility the use of the CASA system (Fig. 1) is necessary. Motility parameters, determined by this method, in combination with spermatozoa morphology analysis can provide more accurate information about the fertilizing potential of rabbit spermatozoa (Lavara *et al*., 2005). In our study, there was no statistically significant difference in motility of spermatozoa between transgenic and non-transgenic animals. On the other hand, the progressive motility

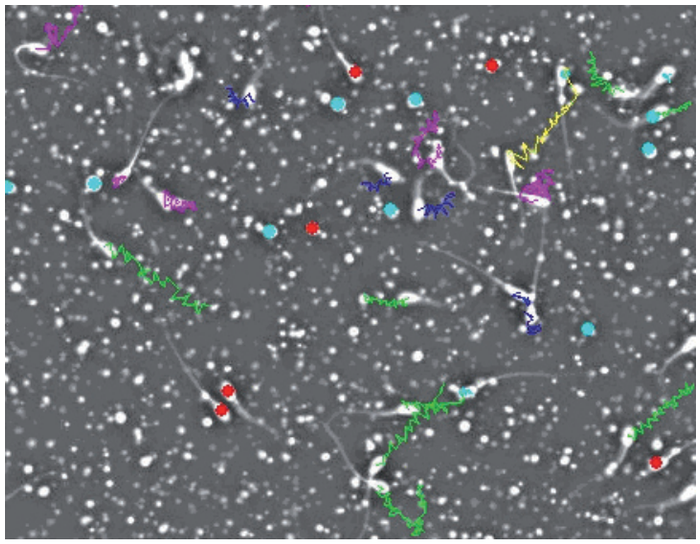

Fig. 1: Representative output from CASA analysis of transgene rabbit spermatozoa

Table 2: Basic ejaculate characteristics of the different generation of transgenic rabbits

Generation of transgenic rabbits (transgene)	TRAIT				Reference
	Libido (s)	Ejaculation	Concentration volume (ml)	Motility (%) (x 10^9 cells.ml^{-1})	
F_1 - F_3 (hPC^+)	34.00±4.18	0.93±0.40	1.26±0.26	65.00±7.91	Chrenek et al., 2006
F_1 - F_3 (hPC^-)	25.00	0.25	0.41±0.08	72.50±3.54	Chrenek et al., 2006
F_1 - F_3 (hFVIII)	7.90±1.00	0.62±0.04	0.69±0.02	65.00±1.00	Chrenek et al., 2007b
F_4 - F_5 (hFVIII)	-	0.60±0.05	1.86±1.70	64.56±24.95	Present study

hPC^+ - human protein C positive spermatozoa; hPC^- - human protein C negative spermatozoa; hFVIII - human Factor VIII

was significantly lower ($p<0.001$) in transgenic males in comparison to non-transgenic ones. We found significant differences also in other CASA parameters: DAP, LIN, BCF ($p<0.05$) and DSL, VSL ($p<0.01$) between the transgenic and non-transgenic group. There were not significant differences in other motility parameters (DCL, VAP, VCL, STR, WOB, and ALH) between analyzed groups (Table 1). On the contrary, Lukac *et al.* (2009) observed in the similar study better motility parameters in transgenic rabbit (F_2 - F_3) spermatozoa versus non-transgenic rabbit spermatozoa. It may be due to the smaller number of transgenic individuals used in our experiment.

The present study evaluated the spermatozoa quality of transgenic bucks (F_4 - F_5 generation) carrying the mWAP-hFVIII gene. In comparison to our previous results (F_1 - F_3 generation; Chrenek *et al*., 2007b), the offspring maintained similar spermatozoa quality. Although the sperm concentration increased twofold, but the ejaculate volume and sperm motility were roughly the same (Table 2). Similar motility of spermatozoa was observed for transgenic rabbit males (F_1 - F_3) with hPC (human protein C) positive spermatozoa (Chrenek *et al.*, 2006), although the other semen traits were different from those measured in present study.

Presented data did not prove the effect of transgenesis on ejaculate volume, concentration and motility of rabbit spermatozoa, although there were differences in some other motility parameters between transgenic and non-transgenic group. Overall, the presence of the transgene in the genome of these animals does not appear to interfere with normal semen production. Similar results have been reported either for transgenic rabbits (Chrenek *et al*., 2007b) or transgenic cattle (Richt *et al*., 2006) and goats (Jackson *et al*., 2010).

CONCLUSIONS

Our results showed good spermatozoa quality of the F_4 and F_5 generations of transgenic rabbit males without any reproductive disorders caused by transgenesis. Consequently the ability to produce offspring for the purpose to create stable transgenic line was retained.

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