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REPRODUCTIVE CHARACTERISTICS OF TRANSGENIC RABBIT MALES WITH HUMAN PROTEIN C GENE

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ABSTRACT

Basic objective of this research was to compare sperm characteristics (ejaculate volume, motility and sperm concentration), libido and reproductive capabilities of transgenic and non-transgenic rabbit males of the same age. First generation of transgenic rabbits was created by microinjection of foreign gene construct, consisting of mouse whey acid protein regulation gene and human protein C structural gene (mWAP-hPC), into pronucleus of fertilized eggs. Next generations of transgenic rabbits were obtained after mating of transgenic females with non-transgenic males. Significant differences in ejaculate volumes and concentrations between transgenic rabbits with hPC positive and negative sperms were observed. No significant differences were found in ejaculate volume and sperm concentration between average values of non-transgenic and transgenic males.
Our results show that the transgenesis with mWAP-hPC gene construct does not affect reproductive traits of rabbit males with positive sperm and therefore do not disturb transgene transmission to offspring.

Key words: transgenic rabbit sperm, ejaculation volume, human protein C, sperm concentration

INTRODUCTION

Pronuclear microinjection is the most common technique used to generate transgenic animals. First papers describing microinjection were published more than two decades ago (Gordon et al., 1980; Palmiter et al., 1982). Despite the fact that this is not a new method, at best only 15% of micromanipulated embryos give rise to newborn animals and only about 1.5% of the preimplanted embryos result in transgenic offspring (Viglietta et al., 1997). Moreover, transgenic offspring often die before attaining reproductive age (Dragin, 2003).

Besides damages influenced by manipulation, embryo gains additional DNA that can also compromise its development. Due to these facts, 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. In this study transgenic rabbits were used as a model for genome alteration research because of their good reproductive qualities and short generation interval. The objective of this research was to compare sperm characteristic (ejaculate volume, sperm concentration and motility) and reproductive capabilities (fertility rate and transgene transmission) of transgenic and non-transgenic males in order to reveal the consequence of transgenesis on reproductive capacity of rabbit males.

MATERIALS AND METHODS

Biological material

Rabbits of New Zealand and Californian breed of 4-5 month old at 3,5-4kg body weight were housed in individual steel cages in controlled environment (constant temperature and light-dark regime). Food and water were supplied ad libitum. Founder rabbits of transgenic line were generated by microinjection of foreign DNA (mWAP-hPC construct) into pronucleus of fertilized egg as described earlier (Chrenek et al., 2002).

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Transgenic males (selected based on PCR analysis, Chrenek et al., 2002) were crossed with non-transgenic females of the same breed to test fertility rate and transgene transmission.

Fertilizing capacity of spermatozoa

The embryos were flushed from the oviduct of mated females at 20hpc (1-cell stage) and cultured in k-DMEM medium + 10%FCS (both Gibco BRL) in the CO2 incubator (5%CO2 and 39°C), until blastocyst stage (96h PC).

Libido and semen analysis

Libido (reaction time) is estimated as the time elapsed between introduction of female into the male's cage and the ejaculation. Semen was collected using artificial vagina once per week, for three subsequent weeks. Physiological traits of semen the volume, motility (evaluated visually as the percentage of straight moving sperm cells), sperm concentration (number of spermatozoa/ml) were counted for each sample using Burker chamber.

Statistics

Standard t-test was used to compare ejaculation volume and sperm concentration of different groups. Average values of experimental and control groups were tested between each other.

RESULTS

Basic ejaculate characteristics of analysed transgenic rabbit males are presented in tab. 1. The results show that libido (from 30±10s up to 40±15s) and motility (from 55±15 up to 75±40%) of transgenic males with hPC positive sperm are similar to transgenic male with hPC gene negative sperm. Ejaculate volume and concentration was significantly higher (t 0,05) in transgenic rabbit males (average 0.93 vs 0.27ml) with hPC positive sperms than transgenic male with hPC negative sperms (tab. 3). Very significant differences (t 0,01) were observed in sperm

Table 1: Libido and basic ejaculate characteristics of transgenic rabbit males

Transgenic rabbits (hPC)*	Libido (s)	Ejaculation volume (ml)	Motility (%)	Concentration (ml)
Transgenic male with hPC positive sperm				
♂ 47	35±22 s	1.300±38.50	70±33	$1.26x10^9$
♂ 60	30±21 s	1.100±37.40	75±40	$1.38x10^9$
♂ 85	40±15 s	0.800±33.45	60±28	$1.61x10^9$
♂ 104	35±18 s	0.300±32.25	55±15	$0.92x10^9$
♂ 109	30±10 s	1.150±37.10	65±20	$1.15x10^9$
Transgenic male with hPC negative sperm				
♂ 81	25±12 s	0.250±25.20	70±35	$3.45x10^8$
♂ 97	25±15 s	0.250±35.50	75±30	$4.6x10^8$

*three samples from each male were analyzed

Table 2: Reproductive traits of transgenic rabbit males

Transgenic rabbits hPC*	Number of collected embryos	Number of fecunded eggs (%)	Number of newborn offspring /%	Transgenic offspring /born alive (%)
Transgenic male with hPC positive sperm				
♂ 47	70	68/70(97)	27	11/27 (41)
♂ 60	60	60/60(100)	20	6/20 (30)
♂ 85	60	56/60(93.3)	17	3/17(18)
♂ 104	55	55/55(100)	12	4/12 (33)
♂ 109	40	40/40(100)	9	2/9 (22)
Transgenic male with hPC negative sperm				
♂ 81	35	35/35(100)	20	0/20 (0)
♂ 97	30	28/30(94)	25	0/25 (0)

*Detection of transgene (hPC) integration only by PCR

concentration between these groups.

An average ejaculate volume in herd of non-transgenic rabbit males was 0.550±28.60 ml and sperm concentration was 1.2 x 10^9. Comparison of sperm concentration and ejaculate volume showed that there were no significant differences between non-transgenic herd (SARC Nitra) and transgenic male averages (tab. 3).

Fertilizing capacity of spermatozoa

Fertilizing capacity of each transgenic male was estimated on the basis of a number of embryos after mating with non-transgenic females and liveborn offspring (tab. 2). No significant difference in egg fertilization between transgenic rabbit male with and without hPC positive sperms was found. However, transgenic males of no. 47, 60, 85, 104 and 109 transmitted the ransgene on offsprings in the range between 18 - 41% via transgenic sperms, but males of no. 81 and 97 didn´t bring about transgenic alive offspring.

DISCUSSION

Present work compared some reproductive traits between transgenic males with and without hPC positive sperm. Rabbits offer an advantageous experimental model because they have characteristic sexual behavior including libido and ejaculate volume of male (Ambriz et al., 2002). Lower libido, motility of sperm was revealed only in the male no. 47. Generally, there are several factors which have depressing effect on libido, such as the temperature in rabbit building (Alvarino et al., 2000), extensive rhythm of ejaculate collection (Nizza et al., 2003) and/or a consequence of feed deprivation (Fodor et al., 2003).

Rabbit ejaculate volume is usually in the range is of 0.1 – 1.5 ml, and sperm concentration per ml is 0.5x 10^9 - 3.5x 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 3x10^9/ml. Results of ejaculate volume in our experiments are close to values in the literature, but sperm concentration of transgenic male in our experiment is lower.

Many genetic and epigenetic factors are influence reproductive traits of animals. Lower concentration of sperm can be due to specific farm conditions, because there are no significant differences between standard herd and transgenic male averages, except for two males (no. 81 and 97) that gave hPC negative sperm. These two males also had significantly lower ejaculate volume, and very significant reduction in sperm concentration compared to transgenic bucks that gave hPC positive sperm. Possible explanation is that not every embryonic cell in early stage of development accepted foreign gene construct, or it can be detected by DNA repair mechanism. Mosaics are a common genetic abnormality in animal DNA (Ločniškar, 2002). It is well known that even the smallest of differences in DNA can cause immune responce in an of organism, so it can be concluded that lower values of sperm concentration and ejaculation volume can be a part of similar process.

Our results show that the integration of mWAP-hPC gene construct and expression of hPC does not affect reproductive traits of transgenic rabbit males with hPC positive sperm and the ability of transgene transmission to offsprings. To establish new transgenic line, an individual evaluation and selection of transgenic animals is required.

Table 3: Statistical analysis of transgenic rabbit sperm

Transgenic males		Ejaculation volume (ml)	Sperm concentration (/1ml of ejaculation)	Production of sperms
average $\bar{y}_1$		0.74	8.86 x 10^8	n
average + $\bar{y}_2$		0.93	1.10 x 10^9	hPC positive
average - $\bar{y}_3$		0.27	4.00 x 10^8	HPC negative
$t_{\bar{y}\bar{y}_1}$	t(0,05)=2.447 t(0,01)=3.707	1.0847 ns	2.0281 ns	
$t_{\bar{y}_1\bar{y}_2}$	t(0,05)=2.776 t(0,01)=4.604	1.0722 ns	2.1428 ns	
$t_{\bar{y}\bar{y}_2}$	t(0,05)=2.776 t(0,01)=4.604	2.1444 ns	1.0000 ns	
$t_{\bar{y}_2\bar{y}_3}$	t(0,05)=2.571 t(0,01)=4.032	3.6592 s	6.708 vs	

ns – no significant diference; s – significant differences; vs – very significant diferences

ẏ– average from non-transgenic male from SARC Nitra, $\bar{y}_1$ - average from all analysed transgenic males, $\bar{y}_2$ - average from of transgenic males transmitting transgene (no. 47, 60, 85, 104 and 109) and $\bar{y}_3$ - average from transgenic males which did not transmit transgene (no. 81 and 97)

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