TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2016, vol. 1, No 1(1): 61–65

A TRIAL TO TESTIFY THE SAFETY OF VACCINAL MYXOMA VIRUS ON SPERMATOGENESIS IN RABBITS

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ABSTRACT

The aim of our study was to monitor in dynamics the impact and safety of attenuated vaccinal myxoma virus strain on some rabbit semen characteristics. Male rabbits of New Zealand White line were vaccinated (on D0) with attenuated myxomatosis vaccine with standard dose (group A, n=10) and compared in dynamic to control unvaccinated animals (group B, n=6). The samples were collected using an artificial vagina twice – on D20 and D50 post vaccination. Semen samples were analyzed using CASA system in order to evaluate the concentration, motility and velocity parameters of rabbit spermatozoa. Our study showed that there is no negative influence in basic spermatogenic parameters and the quality of rabbits' semen is not negatively affected by standard myxomatosis vaccination.

Key words: myxoma virus, rabbit, vaccination, spermatogenesis, CASA.

Introduction

The members from the Poxviridae family are large DNA viruses which can infect mammals and insects. These viruses are 69, divided in two subfamilies and in 28 genera, ten of which affect mammals (King et al., 2012). Typically, these viral infections include formation of skin lesions and papules but some of them are generalized potentially fatal infections (human's small pox, rabbit's myxomatosis).

Some of the poxviruses have the potential to affect also the male reproductive system and to cause orchitis, epididymitis, impaired spermatogenesis and infertility (smallpox virus, vaccinia virus, chickenpox virus, myxoma virus) (Riggs, Sanford, 1962; Mikuz, Damjanov, 1982; Dejucq, Jegou, 2001).

Myxomatosis is generalized virus infection which affects mainly Oryctolagus cuniculus. It has two different clinical forms – nodular myxomatosis which is characterized with formation of tumor-like myxomas in different parts of the body, and non-myxomatosed myxomatosis– mainly with respiratory symptoms (Best, Kerr, 2000, Farsang et al., 2003). Lethality in nonimmune rabbits affected from high virulent myxoma virus is more than 90 % (Fenner, Ratcliffe, 1965). It is known from long time that myxoma virus affect the male reproductive system (Hurst, 1937; Fenner, Woodroofe, 1953). There are inflammatory and necrotic changes in the testicles and the virus can be isolated from them and from the ejaculate. Six months after infection 50 % of the male rabbits are still sterile (Sobey, Turnbull, 1956). Recent trials proved that even field attenuated myxoma viruses can cause interstitial orchitis, epididymitis, impaired spermatogenesis and temporally infertility (Fountain et al., 1997). In non-myxomatosed form of the disease the virus cannot be isolated from nasal and conjunctival secretions, from monocytes, ovaries, but can be found in the testicles (Marlier et al., 2000). These date give us an idea for myxoma virus influence of the reproductive system.

Prevention from myxomatosis is based on vaccination mainly with attenuated mono- and bivalent vaccines (Lemiere 2000; Marlier, 2010). The EU legislations require proofs for safety and

immunogenicity of vaccines. Important part is lack of effects on the animal's reproductive system (Regulation (EC) No 726/2004, Directive 2001/82/EC, Directive 2009/9/EC).

The aim of our study was to monitor in dynamics the impact and safety of attenuated vaccinal myxoma virus strain on some rabbit semen characteristics.

Material and Methods

Animals

The trial was carried out in a rabbit farm in the Institute of Animal Science, Kostinbrod. The experiment included 16 clinically healthy mature male rabbits (5 months old) of New Zealand White line. Till the moment of the experiment the rabbits were unvaccinated but regularly dehelminthed. During the period the animals were in separated cages and bred under equal circumstances. The animals were fed with nourishing fodder, hay and root crops. Food and water were given ad libitum. All procedures followed the good clinical practice.

Experiment

The animals were divided in two groups: Group A /n=10/ with myxomatosis vaccine, single application, standard dose according to the manufacture requirement and Group B (n=6) – control group, with physiologic saline application. Spermogram parameters were investigated in dynamics on 20th (D20) and 50th (D50) post vaccination. The parameters between the two groups were compared.

Vaccine

The vaccine used was legalized, homologue, monovalent, lyophilized, attenuated myxomatosis vaccine, which includes in a single dose Poxvirus myxomatosae attenuatum – min 103.3 TCID50 max 105.8 TCID50. Vaccines were stored and diluted according to the manufacture requirements.

Semen collection, storage and transportation

Samples were collected with artificial vagina in D20 and D50. The proper storage of semen is of crucial significance when the samples are to be examined later than an hour after collection. The temperature shock should be minimized. A special storage solution is necessary for the vitality preservation. There are several suitable solutions mentioned in literature. In this experiment Tris Buffer was used (Boiti et al., 2005). After the sample was taken it was transferred in a warmed sterile Eppendorf cuvettes and diluted 1:1 with warmed Tris Buffer solution. The samples were stored and transferred to the laboratory under controlled temperature 18–20 ^{o}C.

Semen analysis

All samples were analysed using CASA (Computer Assisted Semen Analysis) system – Sperm Class Analyzer (Microptic, Spain) combined with microscope Nikon Eclipse E200 (Nikon, Japan) in no more than 2 hours after collection. We analyzed three different drops from every sample.

Statystical analysis

For statistical analysis and determination of significant differences was used SAS 6.02 statistical software (SAS Institute Inc., U.S.A.). The results are presented as means ± standard deviation (SD). P-values at $p<0.05$ were considered as statistically significant.

Results and Discussion

The following parameters from CASA analysis are presented: ejaculate concentration (106/ml); spermatozoa motility – static (%), non-progressive motile (%), progressive motile (%); velocity rate – rapid (%), medium (%), slow (%).

The experiment duration took into consideration the literature date about rabbit spermatogenesis. According to different authors this takes from 48 to 52 days (Swierstra, Foote, 1965; Morton, 1988).

Results from CASA analysis of some of the ejaculate parameters at D20 are presented in Table 1.

Table 1: CASA analysis of some of the rabbit ejaculate parameters, D20

	Group A, n=10	Group B, n=6
	X ± SD	X ± SD
Concentration 10^6/ml	604.43 ± 252.55	565.59 ± 206.8
Static (%)	58.5 ± 16.66	42.67 ± 22.11
Non-progressive motile (%)	36.8 ± 13.53	49.12 ± 16.47
Progressive motile (%)	4.61 ± 4.87	8.20 ± 5.85
Velocity – Rapid (%)	2.88 ± 3.09	7.74 ± 9.68
Velocity – Medium (%)	7.24 ± 5.74	16.64 ± 10.64
Velocity – Slow (%)	29.43 ± 7.03	32.89 ± 3.81

Statistically significant at $p<0.05$

There is no significant difference between experimental and control groups in both examinations. The literature date for spermatozoa count per milliliter shows reference rate – 250–600 106/ml (Boiti et al., 2005). The concentration in New Zealand White line is 416.72 ± 9.16 x 106 (Campos et al., 2014). The results from our examination and the literature date are similar. Normally only mature sperm cells are present in the ejaculate in about 40–42 days. During this long production period, the vaccine may show some negative effects on sperm quality, but such effects were not observed.

The percent of progressive motile spermatozoa is important parameter for male fertility. The reference rage in rabbits is 30–90 % (Boiti et al., 2005). The results in our examination showed severe aberration without clear explanation. The motility parameters show decreased per cent progressive motile in Group A in comparison to Group B in D20 but without statistically significance.

There is decreased percent of rapid and medium velocity spermatozoa in Group A in comparison to Group B in D 20 without statistically significance.

Results from CASA analysis of some of the ejaculate parameters at D50 are presented in Table 2.

Table 2: CASA analysis of some of the rabbit ejaculate parameters, 50 days

	Group A, n=10 X± SD	Group B, n=6 X± SD
Concentration 10^6/ml	437.1± 156.12	315.05± 103.63
Static (%)	49.52± 18.73	47.81± 8.84
Non-progressive motile (%)	45.12 ± 14.57	47.38± 5.26
Progressive motile (%)	5.38 ± 4.22	4.83± 3.62
Velocity – Rapid (%)	3.99 ± 4.11	1.52± 0.72
Velocity – Medium (%)	8.52 ± 4.96	8.56± 3.14
Velocity – Slow (%)	34.99 ± 5.24	42.12± 5.34

Statistically significant at $p<0.05$

The results from the experimental and control groups do not have any significant difference.

There are several possible explanations for the observed decrease in progressively motile spermatozoa only in D20. Vaccine can affect temporarily the mitochondrial function. Mitochondria are abundant in spermatozoa and provide adenosine triphosphate, necessary to maintain progressive motility (Evenson et al., 1982). It is known that retroviruses may affect mitochondrial function by causing mDNA depletion (Diehl et al., 2003). There is no such available date for poxviruses.

Different factors such as heat and cold exposure, pH and osmolality changes, oxidative damage can also affect motility (Castellini et al., 2003; Chrenek et al., 2011). It can be also affected by periods of sexual inactivity – male rabbits that have not ejaculated for prolonged periods often have poor motility on the first ejaculate, but much better on the second ejaculate collected soon thereafter. The same can be observed also in rabbits which are not still sexually active.

Conclusion

The trial testified that vaccination with attenuated myxoma virus in standard dose does not affect negatively ejaculate parameters. The vaccine application is safety for the male reproductive capability.

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