JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

THE EFFECT OF PATULIN ON RABBIT SPERM MOTILITY AND PROGRESSIVE MOTILITY

Monika Schneidgenová[1], Anna Kalafová[1], Jana Emrichová[1], Katarína Zbynovská[1], Peter Petruska[1], Lubomír Ondruska[2], Rastislav Jurcík[2], Lubica Chrastinová[2], Marcela Capcarová[1]*

Address(es): Ing. Monika Schneidgenová, PhD.,
[1]Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.
[2]Animal Production Research Centre Nitra, Hlohovecka 2, 949 01 Nitra, Slovak Republic.

*Corresponding author: monika.schneidgenova@uniag.sk

ARTICLE INFO

Received 14. 10. 2013
Revised 20. 11. 2013
Accepted 8. 1. 2014
Published 1. 2. 2014

Regular article

OPEN ACCESS

ABSTRACT

The aim of our study was to compare the motility and progressive motility of rabbit sperm after patulin intramuscular administration twice a week for two weeks. Adult male rabbits (n= 30) were used in experiment. Animals were divided into two groups: control group (C) without patulin exposure and experimental group (E) with addition of patulin (10 μg.kg^{-1} of body weight). Semen collection was performed using an artificial vagina. All samples were analysed using CASA (Computer Assisted Semen Analysis) system and following parameters were evaluated: percentage of motile spermatozoa and percentage of progressive motile spermatozoa. The sperm motility ($p<0.05$) as well as the progressive motility ($p<0.05$) was significantly lower in E group when compared to the control.

Keywords: rabbit, motility, progressive motility, patulin

INTRODUCTION

Patulin is a mycotoxin produced by several *Penicillium*, *Aspergillus* and *Byssachlamys* species. Patulin can be produced on different food products including fruits, grains, cheese, cured meats, but in natural situations patulin is exclusively found in apple and apple products (**Mortimer *et al.*, 1985; Harrison, 1989; Paster *et al.*, 1995**). There are a number of research studies related to reproductive and developmental toxicity, carcinogenicity, mutagenicity and immunotoxicity of patulin (**Becci *et al.*, 1981; Choudhary *et al.*, 1992; Smith *et al.*, 1993; Llewellyn *et al.*, 1998; Alves *et al.*, 2000**). Patulin has a strong affinity for sulfhydryl group, which explains why it inhibits the activity of many enzymes (**Askar, 1999**). The seminal characteristics are affected by many factors (breed, feeding, health status, rearing condition, season and collection
The aim of present study was to analyse motility and progressive motility of rabbit's sperm after patulin intramuscular administration twice a week for two weeks.

MATERIAL AND METHODS

Animals

Adult male rabbits (n= 30), maternal albinotic line (crossbreed Newzealand white, Buskat rabbit, French silver) and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in experiment. Rabbits were healthy and their condition was judged as good at the commencement of the experiment. Water was available at any time from automatic drinking troughs. Groups of adult animals were balanced for age (150 days) and body weight (4 ± 0.5 kg) at the beginning of the experiment. Adult rabbits were fed diet of a 12.35 MJ.kg^{-1} of metabolizable energy (Table 1) composed of a pelleted concentrate. Animals were divided into two groups: control group (C) and experiemtnal group (E) with addition of patulin (10 μg.kg^{-1} of body weight). Animals from E group received patulin through intramuscular injection twice a week for two weeks.
In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by ethical committee.

Semen collection and analysis

Semen collection was performed using an artificial vagina. 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: percentage of motile spermatozoa (motility >5 μm/s) and percentage of progressive motile spermatozoa (motility >20μm/s.

Statistical analysis

The data used for statistical analyses represent means of values obtained in blood collection. To compare the results, t-test was applied to calculate basic statistic characteristics and to determine significant differences among the experimental and control groups. Statistical software SIGMA PLOT 12.0 (Jandel, Corte Madera, CA, USA) was used. Differences were compared for statistical significance at the level $P< 0.05$.

Table 1 The chemical composition (g.kg^{-1}) of experimental diet.

Component	
Dry mater	926.26
Crude protein	192.06
Fat	36.08
Fibre	135.79
Non-nitrogen compounds	483.56
Ash	78.78
Organic mater	847.49
Calcium	9.73
Phospophorus	6.84
Magnesium	2.77
Sodium	1.81
Potassium	10.94
Metabizable energy	12.35 MJ.kg^{-1}

RESULTS AND DISCUSSION

The results of effect of patulin on rabbit sperm motility and progressive motility are summarized in Table 2. Motility was significantly lower in E group (59.85 %; $p<0.05$) as compared with the control group C (82.17 %). Similarly, progressive motility was significantly lower in E group (44.78; $p<0.05$) as compared with the control group C (73.12%).

Table 2 The percentage of motility and progressive motility of rabbit's spermatozoa after patulin administration.

Group	C	E
MOT (%)		
x	82.17	59.85*
min	74.57	37.58
max	91.20	86.66
SD	5.85	19.22
PROG (%)		
x	73.12	44.78*
min	62.71	19.14
max	80.21	70.93
SD	6.52	20.84

Legend: C – control group, E – experimental group with patulin exposure, , MOT (%) – motility spermatozoa, PROG (%) – progressive motility spermatozoa, x – mean, min – minimum value, max – maximum value, SD – standard deviation,
* - means significant difference $p < 0.05$

Selmanoğlu (2006) examined the effects of patulin on the epididymis, seminal vesicle and prostate tissues. While sperm counts increased in patulin-treated rats for 60 days, sperm counts in patulin-treated rats for 90 days decreased compared to the corresponding control group. Patulin affected sperm morphology of growing male rats. In histological examinations of the testes of rats treated with patulin, oedema, fibrosis and local Leydig cell hyperplasia in the interstitial tissue, and disorganization of seminiferous tubule epithelium were observed. The thyroid of rats treated with patulin revealed lymphoid cell infiltration and enlargement of interstitial tissue between follicles, and degenerated colloid. (**Selmanoğlu, Koçkaya, 2004**)
Becci *et al*. (1981) reported that patulin did not affect some reproductive parameters such as mating success, litter size, fertility, gestation, viability and lactation indices, and pup weight in Wistar rats exposed to 0, 0.1, 0.5, or 1.5 mg/kg bw/day of patulin for 4 weeks before mating, and dosed pregnant females through gestation and lactation.
Meistrich *et al*. (1985) have reported that a sample containing a high percentage of abnormal sperm is indicative of impaired fertility.
Results of **Selmanoğlu and Koçkaya (2004)** revealed that while patulin caused an increase (66.6%) in testosterone levels and a decrease (17.3%) in T4 levels of rats treated for 60 days, there was no change in the other hormone levels compared to those of the control group. When patulin treatment was extended to 90 days, increased serum testosterone (75%) and LH levels (146%) were observed.

CONCLUSION

In our experiment, we found that patulin administered to animals at a dose of 10 $\mu g.kg^{-1}$ significantly decreased motility and progressive motility of rabbit's sperm. Patulin had therefore a negative effect on sperm endpoints of rabbits.

Acknowledgments: This work was financially supported by VEGA scientific grants 1/0084/12 ,VEGA project No. 1/0532/11, VEGA 1/0022/13 , APVV-0304-12 and KEGA 030 SPU-4/2012 and by European Community under project no 26220220180: Building Research Centre „AgroBioTech".

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