

SHORT COMMUNICATION: INFLUENCE OF VITAMINS C AND E ON SPERM MOTILITY OF RABBIT BUCKS

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Abstract: The objective of the study is to investigate the effect of vitamin C and E supplementation on rabbit sperm motility. Forty INAT breed bucks aged 8.5 mo were divided into 2 groups: control group (C) and treated group (T) receiving vitamins C (1 g/L) and E (1 g/L) incorporated in the drinking water. Semen was collected using an artificial vagina over 6 wk (C, total ejaculates=81; T, total ejaculates=76). The massal motility (MM) was evaluated in the fresh and raw semen. Then, the individual motility at 0 (IM₀), 2 (IM₂) and 4 h (IM₄) after semen collection was determined in diluted semen and conserved at +4°C for the 2 groups. MM was higher in T than in C group ($P=0.0012$). However, the individual motilities IM₀, IM₂ and IM₄ did not vary between the 2 groups. In conclusion, the supplementation of vitamins C and E in the drinking water for rabbit influenced only the MM in both fresh and raw semen.

Key Words: vitamin C , vitamin E, sperm motility, rabbit bucks.

INTRODUCTION

Vitamin C (ascorbic acid) concentration in the organism depends on the diet. *In vitro*, vitamin C protects cells from oxidation of substrates such as proteins, fatty acids, and DNA (Pincemail *et al.*, 1998). Vitamin E (α -tocopherol) is implicated in neurological and immune functions and protects the cells from potential deleterious effects of free radicals. Vitamin E is also involved in the control of enzyme activity to stabilise biological membrane cells (Feki *et al.*, 2001). In rabbits, Youssef *et al.* (2003) have shown that vitamin C and E improved rabbit male fertility by increasing sperm concentration and total motile sperm and decreasing abnormal and dead sperm. In fact, antioxidants supplementation on drinking water has been observed as an interesting application to improve sperm motility in rabbits (Mangiagalli *et al.*, 2012). Based on these references; we undertook this study to investigate the short-term effect of vitamins C and E in drinking water on semen motility of INAT breed rabbit bucks, which were shown to have a low sperm motility in a previous study (Najjar *et al.*, 2009).

MATERIALS AND METHODS

Animals and treatments

The experiment took place in the experimental facility of the National Institute of Agronomy of Tunisia for 6 wk (W1 to W6), from mid April to late May 2011. Forty INAT bucks aged 8.5 mo were divided into 2 groups: control group (C; n=20, total ejaculates=81) receiving drinking water without vitamins; and treated group (T; n=20, total ejaculates=76) receiving vitamins C (1 g/L) and E (1 g/L) in the drinking water. Daily water consumption was 250±50 mL/male. Vitamin C and E were purchased from Medivet laboratory (Tunisia).

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The INAT bucks line was obtained from the crossbreeding between a local Tunisian population and 2 French breeds, the Californian and the white New Zealand, over 6 generations.

Semen collection and evaluation

Semen was collected twice a week from the 2 groups of bucks using an artificial vagina (IMV, France) and a receptive female as describe by Boussit (1989).

Immediately after semen collection, the massal motility (MM) was determined in the fresh raw semen, using Petitjean scale (0 to 9) which is based on the observation of the mass sperm motility under photonic microscope ($\times 10$): scale 0=no sperm to 9=stormy movements (Boussit, 1989). Individual motility (IM) was determined after semen dilution with the GALAP extender (IMV, France), using the Adrieu scale (0 to 4), which is based on observation of the individual sperm motility under a photonic microscope ($\times 40$): scale 0=no movement to 4=sperm move rapidly along a small diameter propeller (Boussit, 1989). The IM was determined at $+4^{\circ}\text{C}$ in semen stored for 0 (IM_0), 2 (IM_2) and 4 h (IM_4) after semen collection.

Statistical Analysis

Data analysis was performed using SAS software (SAS Institute Inc., Cary, NC, USA). For each male, the 2 collected ejaculates were combined for each week. Thirty-nine and forty-four seminal samples were eliminated respectively from the C and T groups as they were heavily contaminated with urine. Treatment and week effect on semen parameters motility was performed using a general linear model (GLM). Comparison of means was performed using the Duncan test and the threshold of significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Results showed that the massal motility varied from 5.8 ± 0.7 in week 1 to 8.1 ± 1 in week 6 for the T group and from 5.7 ± 2 in week 3 to 6.5 ± 1.9 in week 4 for the C group (Figure 1). Significant differences due to the treatment ($P = 0.0012$) and the collection week ($P = 0.0028$) were found.

Individual motility at collection (IM_0) varied from 3.1 ± 0.6 in week 1 to 3.7 ± 0.4 in week 6 for T group and from 2.9 ± 0.8 in week 3 to 3.3 ± 0.6 in week 6 for C group ($P = 0.4780$; Table 1). The individual motility at 2 h after collection (IM_2) varied from 2 ± 0.7 in week 4 to 2.8 ± 0.7 in week 5 for T group and from 2 ± 0.7 in week 3 to 3 ± 0.4 in week 2 for C group ($P = 0.7155$). The individual motility at 4 h after collection (IM_4) varied from 1.5 ± 0.5 in week 3 to 2.2 ± 0.5 in week 6 for T group and from 1.6 ± 0.9 in week 3 to 2.3 ± 0.6 in week 2 for C group ($P = 0.5172$).

The main results in this study showed that supplementation vitamins C and E in the drinking water improved the massal motility, especially from 3rd week. In fact, it appears that individual motility took a longer time to improve (Youssef *et al.*, 2003) with the addition of vitamins, which is not our case. Our experiment lasted only 6 wk in order to test the short-time effects of vitamin C and E supplementation on sperm motility, while other studies lasted for 12 wk (Youssef *et al.*, 2003) and 27 wk (Castellini *et al.*, 1999). Our findings are in agreement with those of El-Masry *et al.*

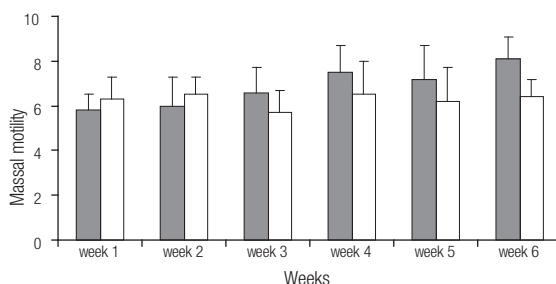


Figure 1: Variation of massal motility in fresh and raw semen of rabbit bucks for the treated ■ and control □ groups (means \pm standard deviation).

Table 1: Individual motility (IM) in the diluted semen stored at 4°C for 0, 2, and 4 h after collection in the treatment (T) and control (C) groups of bucks (means±standard deviation).

IM	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
IM ₀	T	3.1±0.6	3.2±0.4	3.1±0.8	3.2±0.8	3.5±0.7	3.7±0.4
	C	3.2±0.8	3.3±0.6	2.9±0.8	3.1±0.7	3.4±1.0	3.3±0.6
IM ₂	T	2.5±0.5	2.3±0.9	2.2±0.7	2.0±0.7	2.8±0.7	2.7±0.5
	C	2.5±0.7	3.0±0.4	2.0±0.7	2.0±0.8	2.5±1.3	2.5±0.5
IM ₄	T	1.8±0.9	1.6±0.8	1.5±0.9	1.6±0.9	2.2±0.6	2.4±0.8
	C	2.1±0.9	2.2±0.5	1.5±0.5	1.6±0.8	1.6±0.9	2.0±0.7

IM₀, IM₂, IM₄: individual motility at 0, 2 and 4 h after semen collection and storage at 4°C.

(1994), who did not find a positive effect of vitamin E (40 mg/kg) and selenium (0.7 mg/kg) on sperm motility and reproductive performance of bucks. Probably the short duration of our study was not enough to observe the overall improvement of semen quality reported by Castellini *et al.* (1999).

Determination of the vitamin dosages which may effectively improve the quality of semen parameters when administered daily through the drinking water is important for the implementation of simple protocols for use in rabbit breeding. According to Blesbois *et al.* (1993), an improvement of semen in fowl and consequently of their fertility can be achieved only when the level of vitamin E in plasma and cells is much higher (2 to 5 times) than the standard level (0.25 µg/mL).

Comparisons between breeds may vary from one study to another due to sampling differences or the effect of genotype interactions with other effects such as age and season (Zeidan *et al.*, 2004; Mechreky *et al.*, 2004). In addition, other experiments using New Zealand and California breed (Castellini *et al.*, 1999) showed the influence of breed on semen characteristics. Thus, we cannot rule out that the results on sperm motility obtained in this study may be influenced by the INAT breed.

CONCLUSIONS

In conclusion, the addition of vitamins C and E in drinking water for a relatively short period of time improved the massal motility in the fresh raw semen of INAT breed bucks, but not the individual motility of the diluted semen stored at +4°C up to 4 h after collection. Therefore, other works are necessary to further evaluate the effects of these vitamins on the semen quality and fertility of INAT breed bucks before precise recommendations can be provided to local breeders who wish to improve fertility in their rabbit breeding.

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