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Introduction

Lepidium meyenii (Maca) is a Peruvian plant used as phytotherapeutic drug to increase physical and mental faculties in popular medicine. Since ancient times, Andean people have also used Maca to solve fertility problems and improve sexual performance. Several studies showed that Maca administration increases the concentration and the motility of spermatozoa in humans [1], rats [2] and bulls [3] and its effects on the reproductive system have been related to lipid fraction, mainly containing fatty acids and macamides [4].

Aim

The aim of this study was to evaluate the effects of Maca on behaviour, sperm quality, sperm morphometric parameters, chromatin integrity in the sperm head of 4 Italian trotter stallions.

Materials and Methods

Throughout the experimental period, the horses, aged between 11 and 17 years, were stabled in the same farm and managed under the same conditions. Semen collection at T0 was performed prior to the administration of the Maca, 20 gr/die in addition to feed for 60 days. Six more samples were taken at 15 (T1), 30 (T2), 45 (T3), 60 (T4), 90 (T5), 170 (T6) days after the first administration. Sperm sampling time, total ejaculation, filtered ejaculate, total motility and concentration were evaluated at each sperm collection following methods described in the literature [5]. Moreover, each sample was tested for chromatin fragmentation with Halomax equus-kit (Halotech® dna). Fresh semen slides were stained with hematoxylin-eosin for morphometric evaluation. 200 spermatozoa for stallion were studied using a Nikon NIS elements (100x magnification) and parameters such as major axes, minor axes, perimeters, area, shape factors and roughness, were collected. Data were statistically analyzed by ANOVA test using the IBM SPSS Statistics (Version 20), while the correlation between spermatozoa morphometric parameters, motility and data from the chromatin fragmentation test were studied by Pearson correlation test.

Quality Parameters	T0	T1	T2	T3	T4	T5	T6
Total Ejaculate vol. (ml)	36.75±13.62 ^a	40±12.25 ^a	38.75±11.79	57.5±18.48 ^a	65±37.64 ^a	56.75±22.63 ^a	47.5±23.63 ^a
Semen Gel-free vol. (ml)	18.5±7.23 ^b	19±4.69	28.5±12.23 ^b	32±5.60 ^b	28±12.57 ^b	22.25±3.30 ^b	35±17.80 ^b
Sperm concentration (x10 ⁶ /ml)	137.5±29.38 ^c	111.25±54.79 ^c	212.5±82.29 ^c	227.5±110.76 ^c	184±111.57 ^c	173.75±70.39 ^c	279±126.87 ^c
Total Sperm Count (TSC) (x10 ⁹)	2.54±1.15 ^d	2.23±1.62 ^d	6.58±4.75 ^d	7.15±3.31 ^d	5.86±6.12 ^d	4.69±2.99 ^d	8.83±4.37 ^d
Total Motility (%)	66.25±25.62 ^e	58.75±30.10 ^c	70±29.44 ^c	81.25±17.02 ^c	81.25±15.48 ^c	76.25±20.56 ^c	67.5±25.33

Tab. 1 Seminal Parameters expressed as mean ± SD

Morphometric Parameters	T0	T1	T2	T3	T4	T5	T6
Area (µm ²)	12.13 ± 1.33 ^a	11.99±1.26	12.07±1.34	13.3±1.54 ^a	13.56±1.4 ^a	12.89±1.5 ^a	13.03±1.17 ^a
Perimeter (µm)	14.32±1.62 ^b	14.13±1.47	14.65±2.74 ^b	15.87±2.85 ^b	15.41±2.13 ^b	15.10±1.8 ^b	14.40±0.74 ^b
Max Feret (µm)	5.73±0.37 ^c	5.66±0.36 ^c	5.77±0.36	6.20±0.39 ^c	6.25±0.37 ^c	6.09±0.37 ^c	5.95±0.42 ^c
Min Feret (µm)	3.08±0.32 ^d	3.02±0.34 ^d	3.07±0.34	3.17±0.38 ^d	3.21±0.37 ^d	3.12±0.36 ^d	3.01±0.29
Fragmentation (%)	8.95 ± 2.14 ^e	7.60 ± 2.63 ^e	12.02 ± 5.97 ^e	9.0 ± 3.27	8.65 ± 2.67	8.9 ± 5.07	5.7 ± 3.76

Tab. 2 Morphometric Parameters and percentage of Chromatin Fragmentation expressed as mean ± SD

Results

All stallions showed an improvement in sexual behavior, more evident in one subject. The maximum effect of the Maca was expected 60 days after the beginning of the treatment because the horse sperm cycle lasts about 60 days, therefore, in the period after T4. Considering the qualitative parameters at T4, a significant increase ($P < 0.01$) was observed in the total ejaculation volume, semen gel-free, total motility and concentration compared to the values at T0 (Tab.1). With regards to the morphometric parameters at T4, a significant increase in the sperm head area, perimeter, maximum diameter and minimum diameter ($P < 0.01$) was found. Regarding the chromatin fragmentation test no significant difference between the samples was evidenced, however the values of chromatin fragmentation of spermatozoa were inversely correlated with the sperm head area ($P < 0.01$).

Discussion

The findings of the current study suggests that the increase in morphometric parameter measurements might be related to the stability of the sperm DNA. In this preliminary study, in the stallions, the administration of Maca showed a positive effect on the quality of the sperm and on the morphometric parameters that, if the data are confirmed, could be used as indicators of sperm quality. In humans, in which the beneficial effects of Maca on reproductive performance have been studied for a long time [6], we could also consider morphometric parameters as a further tool for evaluating sperm function in the future. The uses could be multiple, from the natural insemination to the selection of the best sperm for in vitro reproduction techniques.

References

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