Zygote

cambridge.org/zyg

DNA fragmentation and morphometric studies in sperm of stallions supplemented with maca (*Lepidium meyenii*)

Emanuele D'Anza[1], Sara Albarella[1], Giacomo Galdiero[1], Simona Tafuri[1], Chiara Del Prete[1], Natascia Cocchia[1], Francesca Ciani[1], Vincenzo Mastellone[1], Maria Pia Pasolini[1], Domenico Carotenuto[2], Maria Selvaggi[3], Francesca Ciotola[1] and Vincenzo Peretti[1]

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy; [2]UNMSM, Universidad Nacional Mayor San Marcos, San Borja, Lima, Peru and [3]Department of Agricultural and Environmental Science, University of Bari Aldo Moro, Bari, Italy

Short Communication

Cite this article: D'Anza E *et al*. (2021). DNA fragmentation and morphometric studies in sperm of stallions supplemented with maca (*Lepidium meyenii*). *Zygote*. page 1 of 6. doi: 10.1017/S0967199420000751

Received: 12 May 2020
Revised: 2 October 2020
Accepted: 21 November 2020

Keywords
Fertility; Food supplements; Horse; Seminal parameter; Spermatozoa

Author for correspondence:
Sara Albarella. Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Via Delpino 1, CAP 80137, Naples, Italy. Tel: +39 081 2536502.
E-mail: sara.albarella@unina.it

Summary

The reproductive performances of livestock play an essential role in the economic management of the farm. The improvement of semen quantity and quality through the use of food supplements that lack substances which are forbidden in animal feeding, or that may have detrimental effects, is an important goal. Maca (*Lepidium meyenii*) is a plant that has been used for centuries in the Andes for nutrition and fertility enhancement in humans and animals. The aim of this study was to evaluate the effects of food supplementation of stallions with maca during the breeding season on spermatozoa parameters such as DNA fragmentation and shape, which are two predictive indexes of spermatozoa functionality. For this purpose, ejaculate volume, semen gel-free volume, sperm concentration and motility, total sperm count, sperm DNA fragmentation and sperm head parameters (length, width, perimeter, area, shape factor, roughness) were measured in four stallions. Maca food supplementation in stallions during breeding reduced the percentage of spermatozoa with fragmented DNA, increased significantly sperm concentration and exerted an elongation of the spermatozoa head, a condition that is believed to improve spermatozoa functionality, suggesting that food supplementation of maca could be useful in horse breeding during the breeding season.

Introduction

Reproduction and fertility are main concerns in horse breeding (Albarella *et al.*, 2018) and the discovery of new plant-based food supplements that are safe, economically valid and able to improve these parameters is an aim of the horse industry. As antioxidants have a positive effect on semen storage and maintenance of the functionality of spermatozoa (Del Prete *et al.*, 2019) close attention has been paid to plants that are rich in them, such as maca (*Lepidium meyenii*). This is a Peruvian plant, the hypocotyl of which has been used for centuries in the Andes for nutrition and fertility enhancement in humans and animals (Gonzales, 2012; Tafuri *et al.*, 2019a). As regard semen characteristics it has been proven that the main effects of an oral supplementation with maca are: increased sperm count and motility, improved DNA fragmentation index, a better quality of semen after storage at 5°C up to 72 h (Clément *et al.*, 2012; Del Prete *et al.*, 2018a). All these beneficial effects are probably due to the antioxidant–oxidant balance induced by macamides and the lipid-extractable fraction of maca with an unknown mechanism of action (Melnikovova *et al.*, 2015; Tafuri *et al.*, 2019b).

Although pregnancy rates is the preferable endpoint to evaluate the fertilizing capacity of a semen in human and animals, a lot of techniques have been used to assess the quality of fresh and cooled semen (Casey *et al.*, 1997; Pauciullo *et al.*, 2012; Yániz *et al.*, 2015; Agarwal *et al.*, 2016; Del Prete *et al.*, 2018b). The aim of this study was to assess the effects of maca food supplementation during the breeding season on stallions spermatozoa. To this end, ejaculates of four Italian thoroughbred stallions were evaluated using conventional semen analyses, DNA fragmentation index and spermatozoa morphometric measures. The latter two methods have never been used to evaluate maca's effects on *Equus caballus* sperm, even though they provide useful data. Sperm is a specialized cell in which chromatin is the main constituent, and its integrity is essential for successful fertilization and normal embryo development. Sperm DNA fragmentation (SDF) is the index used to evaluate chromatin integrity and it is inversely related to fertility. Unlike conventional semen analyses for quality assessment, such as concentration, motility and morphology, SDF allows the evaluation of sperm genetic integrity; moreover, its rate is not necessarily linked to other sperm parameters. In fact, it has been observed that

© The Author(s), 2021. Published by Cambridge University Press.

CAMBRIDGE UNIVERSITY PRESS

Downloaded from https://www.cambridge.org/core. 03 Mar 2021 at 12:13:30, subject to the Cambridge Core terms of use.

infertile men with normal semen may show a poor SDF index (Agarwal *et al.*, 2016). Although structures such as the acrosome and flagellum are of great importance for spermatozoa functionality, the shape of the head, too, strongly influences their motility and fertilization ability. In a variety of mammal species it has been shown that sperm head morphometry is correlated with fertility (Hirai *et al.*, 2001; Ostermeier *et al.*, 2001; Vicente-Fiel *et al.*, 2014; Waheed *et al.*, 2015). Malo *et al.* (2006) affirmed that spermatozoa with more elongated heads may reach a higher swimming speed because they are more hydrodynamic. According to Yániz *et al.* (2015) morphometric analyses could be a useful predictive tool for semen fertility and storage, once the technique to perform them is standardized. Length, width, area and perimeter are the morphometric measures mainly used to objectively characterize sperm head shape.

The spreading of artificial insemination (AI), mainly performed with cryopreserved semen, has given a strong boost to genetic selection and improvement in livestock breeding. A good outcome of the semen cryopreservation process requires a starting semen of good quality and quantity. Therefore to confirm that nutraceutical substances such as maca (*Lepidium meyenii*) are able to improve, if only transiently, ejaculate quality and may have important economic consequences not only for equine reproduction, but also for reproduction of other livestock species.

Materials and methods

Experimental design

The study was planned so that maca administration was performed for one full horse spermatogenic cycle of 57 days (spermatocytogenesis, meiosis and spermiogenesis) (Johnson *et al.*, 1997), and its effects were controlled for the next two cycles. Oral maca administration was performed for 60 days starting in April 2016, and sampling continued for 5 months after the end of treatment (October 2016). The first semen collection was planned 1 week before the beginning of maca administration, and its parameters were used as baseline control (T0); the subsequent collections were planned for 15 (T1), 35 (T2), 60 (T3), 75 (T4), 90 (T5) and 180 (T6) days after the first maca administration, for 28 samples. The experiment was carried out in accordance with the code of ethics (D.lgs. 26-04/03/2014) and the Ethics Committee of the Department of Veterinary Medicine and Animal Productions at the University of Naples Federico II, Italy (protocol no. 0003909), approved the protocol and procedures.

Animals

Eight healthy Italian thoroughbred stallions (four treated and four controls), aged between 9 and 16 years, were selected for this study. All stallions were evaluated cytogenetically to exclude chromosome abnormalities (Macrì *et al.*, 2014) that could affect sperm production (Albarella *et al.*, 2013) according to the protocols described in Ciotola *et al.* (2012). All stallions were housed at a farm located in Teggiano (Salerno, Italy) under the same breeding conditions and used for AI. The animals were fed twice daily with hay and concentrates, and they had water provided *ad libitum*.

Source and supplementation of maca

Yellow maca hypocotyls used for this experiment were harvested in the Junín district, in the Andean highlands of Peru (4100 m above sea level), and exposed for 2 months to extreme temperature cycles, strong light conditions and atmospheric pressures typical of a high-altitude environment (>3500 m), therefore reproducing traditional open-field drying. Hypocotyls were then selected, washed, milled to a powder with a particle size of 0.8 mm and packaged to be used.

Each stallion received a daily dosage of 4 g of maca/100 kg body weight. The dose was chosen according to that found to show beneficial effects on spermatogenesis in humans and rats (Zheng *et al.*, 2000; Gonzales *et al.*, 2001; Cicero *et al.*, 2002). *Lepidium meyenii* Walp. improves sexual behaviour in male rats independent of its action on spontaneous locomotor activity (Cicero *et al.*, 2002; Gonzales *et al.*, 2004).

Total glucosinolates content of dry extract from maca powder used for this work was 6.67% of which 3.33% was benzyl glucosinolate, 0.34% was *m*-methoxybenzyl glucosinolate and 3% was 3-oxo-2-(2-entenyl)cyclopentane octanoic acid.

Ejaculate and semen processing

Immediately after collection, the total amount of ejaculate (semen and gel) was established using a graduated laboratory bottle (Sigma, Italy), the gel fraction was removed using a nylon semen filter (Minitube, Germany), semen was filtered through a semen filter pouch (Minitube, Germany) and the quantity was measured. Sperm motility was visually assessed under a phase contrast microscope (Nikon Eclipse 80i) at ×100 and ×200 magnification. Sperm count was determined using a biophotometer (Eppendorf), total sperm count (TSC) was calculated based on Jasko (1992) and Juhász *et al.* (2000).

For morphometric evaluation, fresh semen samples were washed by centrifugation in physiological saline (0.9% NaCl) at 1000 *g* for 5 min, and then re-extended to a concentration of 100×10^6 cells/ml. Amounts measuring 10 µl of the sperm suspension were fixed on slides and stained with a modified haematoxylin standard protocol: 10 min in Mayer's haematoxylin (code no. 05-M06002, Bio-Optica, Milano, Italy), after removing the excess of stain with water the slides were immersed in distilled water for 2 min and then for 5 min in eosin Y, 1% solution (code no. BP2419, Fisher Scientific, Geel, Belgium). Slides were then immersed in distilled water for 5 min and rinsed twice. Serial passages in ethanol (50% to absolute) were performed. Then slides were treated with Xilolo and mounted with Eukitt (code no. CL04.0503.0500, Chem-Lab NV, Zedelgem, Belgium).

Morphometric analysis

At least 200 spermatozoa (50 per slide) from each ejaculate were observed in a bright field under a Nikon Eclipse 80i microscope (×100 magnification), captured with a digital camera (Nikon DS-Ri1) and analyzed with Nis Elements Imaging Software 4.00.02 (Nikon). Morphometric parameters of the head measured for each spermatozoon were length (L), width (W), perimeter (P), area (A), shape factor (SF) and roughness (R).

Sperm DNA fragmentation analysis

Sperm chromatin fragmentation was evaluated for each semen sample only on treated stallions using a Halomax kit for *Equus caballus* (Halotech® DNA) according to the user manual. Slides were observed in a bright field under a Nikon Eclipse 80i microscope (×20 magnification), and 300 spermatozoa from each semen sample were captured using a digital camera (Nikon DS-Ri1) and analyzed with Nis Elements Imaging Software 4.00.02 (Nikon).

Downloaded from https://www.cambridge.org/core. 03 Mar 2021 at 12:13:30, subject to the Cambridge Core terms of use.

Statistical analysis

For statistical analysis, data for each sample were grouped into four time periods: T0 + T1, T2 + T3, T4 + T5 and T6, corresponding to before the maca effect (P1), starting maca effects (P2), maca effect (P3) and resting period (P4), respectively.

The effects of time of maca supplementation on quality, SDF data and morphometric semen parameters were analyzed by a repeated measurements procedures using mixed-effects models including the random animal effect (SAS PROC MIXED 8.02; SAS Institute, Cary, NC). The level of significance was fixed at $P < 0.05$.

Results

Semen quantity and quality

Table 1 shows the data on semen quantity and quality parameters of the T0 group in all the stallions used in this study. According to the data reported the groups were similar as regard semen parameters. Table 2 shows mean values (±SD) of all the parameters for semen quality and quantity in the four periods. Total volume of ejaculate increased gradually from P1 to P3 and then it decreases in P4 in treated stallions while it remained similar to P3 in control group. Semen gel-free volume and TSC increased from P1 to P2, then slightly decreased in P3 and increased again during the resting period (P4) in both groups. TSC shows statistically significant differences in the treated group when comparing P1 with P4 and between the groups only in the P4 period ($P < 0.05$). Sperm concentration showed an increase during the whole period under examination in both groups, however statistically significant differences were observed only when comparing P1 and P4 ($P < 0.05$) and when comparing the treated group with control one, starting from the P2 period. In particular, in P4, the difference was highly significant ($P < 0.01$). For sperm motility there were no statistically significant differences during the whole time period analyzed and between the two groups, while SDF gradually decreased from P2 to P4, showing a statistically significant difference ($P < 0.05$).

Morphometric analysis

Mean values (±SD) of all the parameters for semen morphometry of the four periods are shown in Table 3.

Values of spermatozoa L, W, P and A increased significantly from P1 to P3 ($P < 0.01$) and then they decreased in P4 ($P < 0.01$). This trend was observed in both groups, but was greater in the treated subjects than in the control.

SF showed a statistically significant difference in P1 to P2 and P3 ($P < 0.05$) and in P2 and P3 to P4 ($P < 0.01$). R showed a statistically significant difference in P1, P2 and P3 versus P4 ($P < 0.05$).

Discussion

The mean values obtained in this study for quantity and quality parameters conventionally used to evaluate stallion semen (gel-free semen volume, sperm concentration, TSC and sperm motility) confirm results already reported in the literature: maca food supplementation improves semen in horses (Del Prete *et al.*, 2018a; Tafuri *et al.*, 2019b).

SDF progressively decreased from P2 to P4, which could indicate that active substances in maca prevented spermatozoa from DNA damage due to ageing after the second meiotic division has settled, and during the period they stand in the genital tract. In particular, the decrease in percentage of spermatozoa with fragmented DNA may indicate that DNA spermatozoa is more fragile during the first stages of gametogenesis. Therefore, it is conceivable that maca components are more effective in DNA damage prevention if they are present in the seminal tract from the first stages of the differentiation of the gametes. Unfortunately, it was not possible to carry out this same test on control horses, therefore this hypothesis needs be confirmed in the future with appropriate experimental tests.

According to Waheed *et al.* (2015), stallion spermatozoa L and W increases from spring to summer and then decrease in autumn in a not statistically significant manner. The same trend could be observed in the eight stallions studied in this work, however the differences observed in this case were statistically significant and were greater in the subjects treated with maca. All this was then reflected on the measurements relating to P and A (Table 3).

Among all the morphometric parameters measured, L was the one that mainly increased (Table 3), indicating an elongation of the sperm head, which is a shape that can positively affect sperm fluidodynamic behaviour, making it move more efficiently. In fact, a recent study of Iberian reed deer (Ramón *et al.*, 2013) showed that spermatozoa with rapid and linear movements were strongly correlated with spermatozoa having a small and elongated head; both subpopulations occur more frequent in high-fertility males. Sperm morphometry has also been successfully used in sperm competition studies. Sperm competition has been associated with an increase in total sperm dimensions and in sperm head elongation, and both aspects have been related to an improved sperm migratory efficiency (Sánchez *et al.*, 2013). When spermatozoa increase in size, all sperm components increase in size simultaneously (Tourmente *et al.*, 2011), and are able to produce more energy and swim faster (Sánchez *et al.*, 2013). TSC, motility and sperm L, W, P and A, even when analyzed separately for the four stallions, improved when associated with maca food integration. Figure 1 shows how improvements in the quantity and quality parameters of sperm were different in the four stallions indicating a marked individual response to this food supplement. However, in all the animals the highest values were in P3, corresponding to the maca effect period.

In this study, the use of maca as a food supplement to improve stallion sperm parameters has been evaluated using not only classical quality and quantity sperm parameters, but also DNA fragmentation and morphometric measures. Data obtained for sperm quantity and quality, on the four analyzed stallions and compared with that from a control group of stallions managed in the same conditions of the treated group, suggested that maca administration improved stallion semen quality during a stressful period such as the reproductive season. This product may improve spermatozoa morphometric measures and, in particular, would cause lengthening of the head of the spermatozoon. Future trials could be aimed to increase the number of the stallions and to assess pregnancy rate of mares sired with stallions supplemented orally with maca, or artificially inseminated with their cryopreserved semen, this would confirm its usefulness as a dietary supplement in horse reproduction.

Downloaded from https://www.cambridge.org/core. 03 Mar 2021 at 12:13:30, subject to the Cambridge Core terms of use.

Table 1. Stallions semen quality and quantity parameters at T0 collection time

	Treated				Control			
Parameter	1	2	3	4	5	6	7	8
Ejaculate volume (ml)	25	25	47	50	30	27	50	55
Gel-free semen volume (ml)	10	15	25	24	20	14	25	20
Sperm concentration ($\times 10^6$/ml)	164	98	133	155	160	120	100	143
TSC ($\times 10^9$)	1.64	1.47	3.32	3.72	3.20	1.68	2.50	2.86
Motility (%)	90	30	70	75	80	60	80	70
SDF (%)	8.38	12.10	7.40	7.90	ND	ND	ND	ND

Table 2. Mean values (±SD) of stallions' semen quantity and quality parameters in the four periods (P1, before maca effect; P2, starting maca effect; P3, maca effect; P4, resting period)

Parameter	Group	P1	P2	P3	P4
Ejaculate volume (ml)	Tratt	36.75 ± 10.67	45.42 ± 17.50	60.88 ± 29.09^{e}	47.5 ± 23.63
	Contr	38.32 ± 2.89	49.69 ± 8.05	48.84 ± 3.67^{f}	48.09 ± 1.05
Semen gel-free volume (ml)	Tratt	18.50 ± 5.25	26.50 ± 3.03	25.13 ± 3.72	35.00 ± 5.25
	Contr	21.68 ± 2.88	32.03 ± 7.21	29.15 ± 2.44	36.67 ± 1.55
Sperm conc. ($\times 10^6$/ml)	Tratt	137.50 ± 45.80^{a}	183.75 ± 26.44^{e}	178.88 ± 32.39^{e}	279.00 ± 45.80^{b,E}
	Contr	126.66 ± 5.78	153.30 ± 20.82^{f}	155.82 ± 29.21^{f}	181.67 ± 32.50^{F}
TSC ($\times 10^9$)	Tratt	2.54 ± 1.97^{a}	5.34 ± 1.14	5.27 ± 1.39	8.83 ± 1.97^{b,e}
	Contr	2.71 ± 0.23	4.81 ± 0.38	4.55 ± 0.98	6.69 ± 1.47^{f}
Motility (%)	Tratt	66.25 ± 11.70	70.00 ± 6.75	78.75 ± 8.27	67.50 ± 11.70
	Contr	73.33 ± 11.57	63.34 ± 12.60	72.50 ± 14.07	70.01 ± 17.31
SDF (%)	Tratt	8.18 ± 2.13	11.57 ± 4.11^{a}	8.57 ± 3.15	5.64 ± 2.99^{b}

In the row: $^{a,b}P < 0.05$ (significant differences for different letters).
In the column: $^{e,f}P < 0.05$; $^{E,F}P < 0.01$ (significant differences for different letters).

Table 3. Mean values (±SD) of the parameters for stallions' semen morphometry in the four periods

Parameter	Group	P1	P2	P3	P4
Length (L)	Tratt	5.73 ± 0.01^{A}	5.87 ± 0.01^{B}	6.17 ± 0.01^{CE}	5.95 ± 0.01^{DE}
	Contr	5.73 ± 0.01	5.85 ± 0.01	5.94 ± 0.01^{F}	5.62 ± 0.01^{F}
Width (W)	Tratt	3.08 ± 0.01^{A}	3.09 ± 0.01^{AE}	3.17 ± 0.01^{BE}	3.01 ± 0.01^{CE}
	Contr	3.07 ± 0.01	2.99 ± 0.01^{F}	3.07 ± 0.01^{F}	2.79 ± 0.01^{F}
Perimeter (P)	Tratt	14.32 ± 0.07^{AD}	14.88 ± 0.04^{A}	15.26 ± 0.05^{CE}	14.40 ± 0.07^{De}
	Contr	14.33 ± 0.04	14.66 ± 0.04	14.26 ± 0.04^{F}	13.99 ± 0.04^{f}
Area (A)	Tratt	12.13 ± 0.05^{A}	12.45 ± 0.03^{Be}	13.23 ± 0.04^{CDe}	13.03 ± 0.05^{DE}
	Contr	12.14 ± 0.05	12.92 ± 0.05^{f}	12.95 ± 0.05^{f}	12.30 ± 0.05^{F}
Shape factor (SF)	Tratt	0.79 ± 0.01^{a}	0.77 ± 0.01^{Abe}	0.77 ± 0.01^{AbE}	0.81 ± 0.01^{B}
	Contr	0.79 ± 0.01	0.80 ± 0.01^{f}	0.82 ± 0.01^{F}	0.82 ± 0.01
Roughness (R)	Tratt	1.02 ± 0.01^{A}	1.03 ± 0.01^{A}	1.03 ± 0.01^{AE}	1.01 ± 0.01^{BE}
	Contr	1.02 ± 0.01	1.03 ± 0.01	1.01 ± 0.01^{F}	1.02 ± 0.01^{F}

In the row: $^{a,b}P < 0.05$; $^{A,B,C,D}P < 0.01$.
In the column: $^{e,f}P < 0.05$; $^{E,F}P < 0.01$.

Downloaded from https://www.cambridge.org/core. 03 Mar 2021 at 12:13:30, subject to the Cambridge Core terms of use.

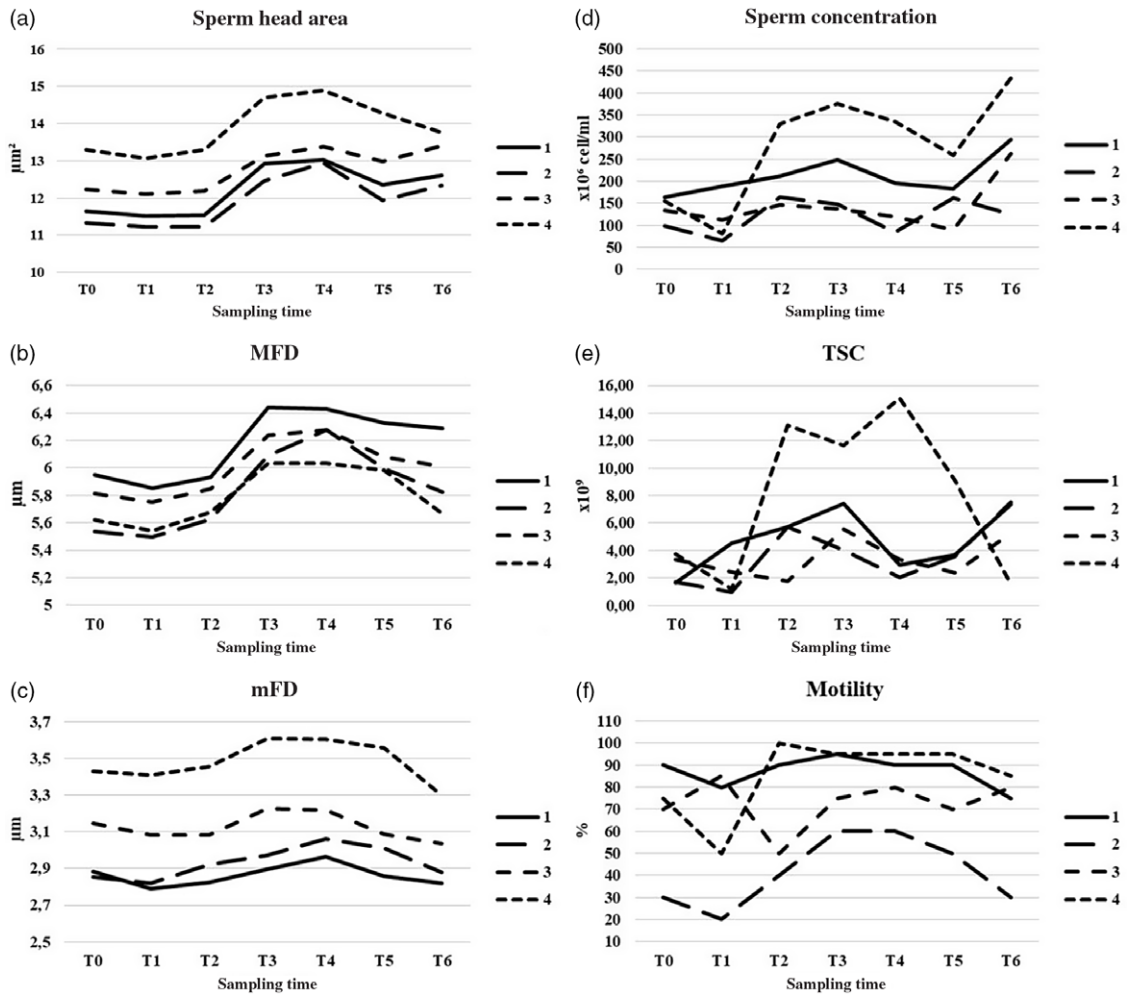

Figure 1. Spermatozoa morphometry and quality parameters of the four stallions treated with maca at each sampling time. (a) Sperm head area (μm^2). (b) MFD, maximum Feret's diameter (µm). (c) mFD, minimum Feret's diameter (µm). (d) Sperm concentration ($\times 10^6$). (e) TSC total sperm count ($\times 10^9$). (f) Motility (%). T0, 1 week before maca administration; T1, Day 15 of maca administration (m.a.); T2, Day 35 of m.a.; T3 = Day 60 of m.a.; T4, Day 75 of m.a.; T5, Day 90 of m.a.; T6, Day 180 of m.a.

Financial support. This work was supported by the Department of Veterinary Medicine and Animal Production University of Naples Federico II (grant number 37-25/06/2015).

Conflict of interest. None of the authors has any conflict of interest to declare.

Ethical statement. The experiment was carried out in accordance with the code of ethics (D.lgs. 26 – 04/03/2014) and the Ethics Committee of the Department of Veterinary Medicine and Animal Productions at the University of Naples Federico II, Italy (protocol no. 0003909), approved the protocol and procedures.

References

Agarwal A, Majzoub A, Esteves SC, Ko E, Ramasamy R and Zini A (2016). Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. *Transl Androl Urol* **5**, 935–50.

Albarella S, Ciotola F, Coletta A, Genualdo V, Iannuzzi L and Peretti V (2013). A new translocation t(1p;18) in an Italian Mediterranean river buffalo (*Bubalus bubalis*, 2n = 50) bull: cytogenetic, fertility and inheritance studies. *Cytogenet Genome Res* **139**, 17–21.

Albarella S, De Lorenzi L, Catone G, Magi GE, Petrucci L, Vullo C, D'Anza E, Parma P, Raudsepp T, Ciotola F and Peretti V (2018). Diagnosis of XX/XY blood cell chimerism at a low percentage in horses. *J Equine Vet Sci* **69**, 129–35.

Casey PJ, Gravance CG, Davis RO, Chabot DD and Liu IK (1997). Morphometric differences in sperm head dimensions of fertile and subfertile stallions. *Theriogenology* **47**, 575–82.

Cicero A, Piacente S, Plaza A, Sala E and Arletti RCP (2002). Hexanic maca extract improves rat sexual performance more effectively than methanolic and chloroformic maca extracts. *Andrologia* **34**, 177–9.

Ciotola F, Albarella S, Pasolini MP, Auletta L, Esposito L, Iannuzzi L and Peretti V (2012). Molecular and cytogenetic studies in a case of XX SRY-negative sex reversal in an Arabian horse. *Sex Dev* **6**, 104–7.

Clément C, Witschi U and Kreuzer M (2012). The potential influence of plant-based feed supplements of sperm quantity and quality in livestock: a review. *Anim Reprod Sci* **132**, 1–10.

Del Prete C, Tafuri S, Ciani F, Pasolini MP, Ciotola F, Albarella S, Carotenuto D, Peretti V and Cocchia N (2018a). Influences of dietary supplementation with *Lepidium meyenii* (maca) on stallion sperm production and on preservation of sperm quality during storage at 5°C. *Andrology* **6**, 351–61.

Del Prete C, Ciani F, Tafuri S, Pasolini MP, Della Valle G, Palumbo V, Abbondante L, Calamo A, Barbato V, Gualtieri R, Talevi R and Cocchia N (2018b). Effect of superoxide dismutase, catalase, and glutathione peroxidase supplementation in the extender on chilled semen of fertile and hypofertile dogs. *J Vet Sci* **19**, 667–75.

Del Prete C, Stout T, Montagnaro S, Pagnini U, Uccello M, Florio P, Ciani F, Tafuri S, Palumbo V, Pasolini MP, Cocchia N and Henning H (2019). Combined addition of superoxide dismutase, catalase and glutathione

Downloaded from https://www.cambridge.org/core. 03 Mar 2021 at 12:13:30, subject to the Cambridge Core terms of use.

peroxidase improves quality of cooled stored stallion semen. *Anim Reprod Sci* **210**, 106195.

Gonzales G, Cordova A, Gonzales C, Chung A, Vega K and Villena A (2001). *Lepidium meyenii* (maca) improved semen parameters in adult men. *Asian J Androl* **3**, 301–4.

Gonzales G, Gasco M, Cordova A, Chung A, Rubio J and Villegas L (2004). Effect of *Lepidium meyenii* (maca) on spermatogenesis in male rats acutely exposed to high altitude (4340 m). *J Endocrinol* **180**, 87–95.

Gonzales GF (2012). Ethnobiology and ethnopharmacology of *Lepidium meyenii* (Maca), a plant from the Peruvian highlands. *Evid Based Complement Alternat Med* eCAM vol. **2012**, 193496.

Hirai M, Boersma A, Hoeflich A, Wolf E, Foll J, Aumüller R and Braun J (2001). Objectively measured sperm motility and sperm head morphometry in boars (*Sus scrofa*): relation to fertility and seminal plasma growth factors. *J Androl* **22**, 104–10.

Jasko DJ (1992). Evaluation of stallion semen. *Vet Clin North Am Equine Pract* **8**, 129–148.

Johnson L, Blanchard T, Varner D and Scrutchfield W (1997). Factors affecting spermatogenesis in the stallion. *Theriogenology* **48**, 1199–216.

Juhász J, Nagy P, Kulcsár M and Huszenicza Gy (2000). Methods for semen and endocrinological evaluation of the stallion: a review. *Acta Vet Brno* **69**, 247–59.

Macrì F, Ciotola F, Rapisarda G, Lanteri G, Albarella S, Aiudi G, Liotta L and Marino F (2014). A rare case of simple syndactyly in a puppy. *J Small Anim Pract* **55**, 170–3.

Malo A, Gomendio M, Garde J, Lang-Lenton B, Soler A and Roldan E (2006). Sperm design and sperm function. *Biol Lett* **2**, 246–9.

Melnikovova I, Faith T, Kolarova M, Fernandez E and Milella L (2015). Effect of *Lepidium meyenii* Walp on semen paraemters and serum hormone levels in healthy adult men: a double-blind, randomized, placebo-controlled pilot study. *Evid Based Complemen Alternat Med eCAM* **2015**, 324369.

Ostermeier G, Sargeant G, Yandell B, Evenson D and Parrish J (2001). Relationship of bull fertility to sperm nuclear shape. *J Androl* **22**, 595–603.

Pauciullo A, Nicodemo D, Peretti V, Marino G, Iannuzzi A, Cosenza G, Di Meo GP, Ramunno L, Iannuzzi L, Rubes J and Di Berardino D (2012). X-Y aneuploidy rate in sperm of two 'minor' breeds of cattle (*Bos taurus*) by using dual color fluorescent *in situ* hybridization (FISH). *Theriogenology* **78**, 688–95.

Ramón M, Soler AJ, Ortiz JA, García-Alvarez O, Maroto-Morales A, Roldan ER and Garde JJ, (2013). Sperm population structure and male fertility: an intraspecific study. *Biol Reprod* **89**, 110–7.

Sánchez V, Bastir M and Roldan E (2013). Geometric morphometrics of rodent sperm head shape. *PLoS One* **8**, e80607.

Tafuri S, Cocchia N, Vassetti A, Carotenuto D, Esposito L, Maruccio L, Avallone L and Ciani F (2019a). *Lepidium meyenii* (maca) in male reproduction. *Nat Prod Res* **2019**, 1–10.

Tafuri S, Cocchia N, Carotenuto D, Vassetti A, Staropoli A, Mastellone V, Peretti V, Ciotola F, Albarella S, Del Prete C, Palumbo V, Esposito L, Vinale F and Ciani F (2019b). Chemical analysis of *Lepidium meyenii* (maca) and its effects on redox status and on reproductive biology in stallions. *Molecules* **23**, 24.

Tourmente M, Gomendio M and Roldan E (2011). Mass-Specific metabolic rate and sperm competition determine sperm size in marsupial mammals. *PLoS One* **6**, e21244.

Vicente-Fiel S, Palacín I, Santolaria J, Fantova E, Quintin-Casorran F and Sevilla-Mur E (2014). *In vitro* assessment of sperm quality from rams of high and low field fertility. *Anim Reprod Sci* **146**, 15–20.

Waheed M, Ghoneim I and Abdou M (2015). Morphometric characteristics of spermatozoa in the arabian horse with regard to season, age, sperm concentration, and fertility. *J Equine Vet Sci* **35**, 244–9.

Yániz J, Soler C and Santolaria P (2015). Computer assisted sperm morphometry in mammals: a review. *Anim Reprod Sci* **156**, 1–12.

Zheng B., He K, Kim C, Rogers L, Shao Y and Huang Z (2000). Effect of a lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. *Urology* **55**, 598–602.

Downloaded from https://www.cambridge.org/core. 03 Mar 2021 at 12:13:30, subject to the Cambridge Core terms of use.