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Effect of α-Amylase, Papain, and Spermfluid® treatments on viscosity and semen parameters of dromedary camel ejaculates

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ABSTRACT

Ejaculates from five clinically healthy dromedary camels (*Camelus dromedarius*) were used to evaluate the effects of different enzymatic treatments (Amylase, Papain, Spermfluid®) on liquefaction and seminal parameters. After collection, ejaculates were divided into 5 aliquots: (1) kept undiluted (control); or diluted 1:1 with: (2) Tris-Citrate-Fructose (TCF), (3) TCF containing Amylase, (4) TCF containing Papain or (5) Spermfluid® containing Bromelain. At 120 min after dilution, each aliquot was evaluated, at 20-min intervals, for viscosity, motility, viability and agglutination. Only the aliquots diluted with TCF containing Papain underwent complete liquefaction. Sperm motility decreased significantly during the observation times, except for the samples diluted with Spermfluid® ($P = 0.005$). Diluted samples showed different levels of agglutination, with the lowest being observed in the control and the highest in the Papain-treated samples. The viscosity of dromedary camel ejaculates could be effectively reduced by using the proteolytic enzyme Papain.

1. Introduction

The main physical characteristic of camelid ejaculate is its high seminal plasma viscosity (Tibary and Anouassi, 1997). After natural mating, the ejaculated coagulum acts as a reservoir within the uterus, slowly releasing spermatozoa during the time span between mating and ovulation (Brown, 2000) but, after semen collection, such viscosity is of primary concern because it limits the capacity to process the ejaculate (pipetting, preparation of slides, evaluation of concentration and motility) and prevents the blending of semen with extenders.

Liquefaction of camelid ejaculates enables accurate evaluation of sperm parameters and would enhance contact between sperm cell membranes and nutritive/protective compounds, particularly during equilibration and cryopreservation, thus improving post-thawing semen parameters. For these reasons, ejaculate liquefaction is of fundamental importance for the application of a wide range of Assisted Reproductive Technologies (ARTs), including Artificial Insemination.

The reduction of camelid ejaculate viscosity has been previously investigated. Niasari-Naslaji et al. (2007) found that mechanical stirring should eliminate the viscosity of Bactrian camel semen, whereas Wani et al. (2008) reported that dromedary camel ejaculates diluted 1:1 undergo spontaneous liquefaction within 1.5 h of collection. However, the latter authors also reported that such liquefaction is highly variable in time and often not satisfactory (Wani et al., 2008).

Other authors have evaluated the use of enzymes (trypsin, collagenase, hyaluronidase) but their results suggest limited application because of induced spermatozoa damage (Bravo et al., 1999, 2000; Ghoneim et al., 2010; Giuliano et al., 2010). Recently, Kershaw-Young et al. (2013) found that the proteolytic enzyme Papain (from *Carica papaya*) successfully reduces the viscosity of alpaca ejaculates. Another proteolytic enzyme, Bromelain, is currently being used to enhance the liquefaction of human ejaculates and facilitate its evaluation and processing (WHO, 2010). Both enzymes, Papain and Bromelain, are Cysteine endopeptidases that hydrolyze proteins, particularly at bonds involving the amino acids Arg, Lys, Glu, His, Gly, and Tyr and their action could be controlled using N-(trans-Epoxysuccinyl)-L-leucine 4-guanidinobutylamide (E64) (Kershaw-Young et al., 2013).

In addition to Bromelain, previous studies on human ejaculates have suggested that α-Amylase could be effective at reducing hyperviscosity (Dougherty et al., 1978; Mendeluk et al., 2000). El-Bahrawy (2010) evaluated different doses of α-Amylase for processing and cryopreserving dromedary camel ejaculates and obtained significant results using 5 μL/mL; however, the liquefaction of the ejaculates was subjectively evaluated and the α-Amylase efficacy was claimed mainly on the basis of the post-thawing sperm motility results.

To date, there are no reports regarding the efficacy of the above proteolytic enzymes on dromedary camel semen; therefore, the aim of the

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present study was to compare the efficacy of α-Amylase, Papain, and a commercial formulation containing Bromelain, at reducing the viscosity of dromedary camel ejaculates; their short-term effects on seminal parameters were also evaluated.

2. Materials and methods

2.1. Animals

The study was carried out at the Arid Lands Institute experimental station (Medenine, Tunisia) from 31 January to 19 March 2014. Five healthy dromedary bulls aged between 5 and 8 years were used. Animals were housed in single boxes with sand as bedding; they were watered every 2 days, and fed daily with oat hay and concentrate supplement (barley, wheat bran, olive cake, minerals and vitamins).

2.2. Collection and selection of ejaculates

Ejaculates were collected using a restrained female as a teaser and a bovine artificial vagina; mating sessions were carried out following the timings proposed by Padalino et al. (2015) and afterward used in Monaco et al. (2015): Maximal latency time: 15 min; Maximal mating time: 45 min; Maximal time between two copulations: 30 min; Maximal time standing on/over the female: 30 min; Maximal time walking around: 6 min. Within 5 min of collection, each ejaculate was transported to the laboratory, kept in a water bath at 37 °C and immediately evaluated for volume, viscosity, presence of sperms and sperm motility. Ejaculates were selected according to the following parameters: volume ≥ 4 mL, viscosity >25 mm and sperm motility ≥2. Any azoospermic, oligospermic and asthenospermic ejaculates were discarded.

The viscosity was assessed using the thread test as previously described by Kershaw-Young et al. (2013): 50 μL of the sample were drawn with a pipette and 25 μL of semen were pipetted onto a warm glass slide. The pipette was then raised vertically, forming a thread and the length at which this thread snapped (measured in mm) was recorded as the measurement of viscosity.

Two measurements were taken by two operators and where there was more than 10% discrepancy between the two operators the procedure was repeated. The mean of the two operators' values was considered as the sample viscosity.

Sperm motility was evaluated as previously described by Monaco et al. (2015); briefly, 15 μL of semen were placed on a pre-warmed slide; after 5 min at 37°, sperm movements were observed at 200× magnification. The motility of the sperms, whether oscillatory or progressive, was scored by two observers using a 0 (immotile)–5 (highly motile) scale (Crichton et al., 2015).

2.3. Enzymatic treatments and semen evaluation

Tris (3.03%), fructose (1.7%), citric acid (1.2%) extender (pH 7.5) was prepared (Wani et al., 2008). The selected ejaculates were split into five aliquots (0.8–1.0 mL) and treated as follows: (1) Control (undiluted); (2) diluted 1:1 with warm Tris-Citrate-Fructose Buffer (TCF); (3): diluted 1:1 with warm TCF-Amylase (α-Amylase final concentration: 5 μL/mL); (4) diluted 1:1 with warm TCF-Papain (Sigma-Aldrich, USA) (Papain final concentration: 1.7 U/mL, corresponding to 0.1 mg as used by Kershaw-Young et al. (2013); (5) diluted 1:1 with Spermfluid® (Gynemed, GmbH & Co., Germany) containing Bromelain (final concentration 5 U/mL). All aliquots (control and diluted) were kept in the water bath at 37 °C. After dilution, samples were evaluated at 0, 20, 40, 60, 90 and 120 min (T0–T120) for viscosity, sperm motility, viability and agglutination. Viscosity and motility were evaluated as previously described, whereas sperm viability was assessed by Eosin–Nigrosin staining. The viscosity percentage was calculated according to Kershaw-Young et al. (2013) using the following formula: (Viscosity/Viscosity at T0) × 100. Sperm head-to-head agglutination was evaluated by visual observation under a microscope at 200× magnification; a score from 0 to 4 was assigned according to the following findings: 0-absence of agglutination; 1—low degree of agglutination (<10% agglutinated sperm); 2—intermediate level of agglutination (10–30%); 3—high degree of agglutination (>30–50%) and 4—very high degree of agglutination (>50%) (Kojima et al., 2001).

2.4. Statistical analysis

Seminal parameters (viscosity, percentage of viscosity, motility, viability, agglutination) were subjected to Two Way Analysis of Variance using the GLM procedure (SAS, version 9 1999). Treatments (Control, Tris-Citrate-Fructose (TCF), TCF + Papain, TCF + Amylase, Spermfluid®), Time (T0–T120), and their interaction (Tr × Ti) were considered as fixed effects. Duncan's multiple range test was used as a Post Hoc test. Data were expressed as mean and standard error.

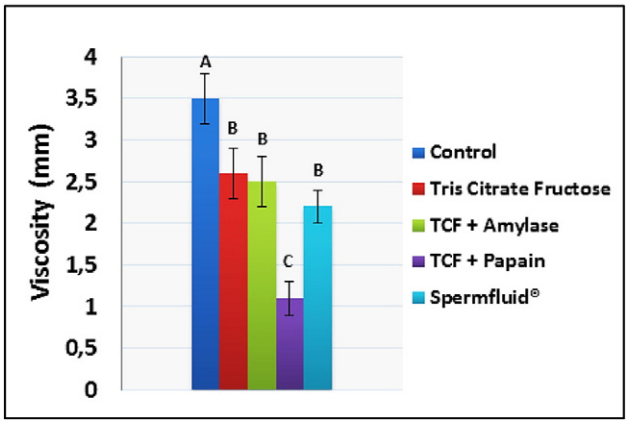
3. Results

Twelve ejaculates were selected and used for the experiment. The statistical model showed that treatments, time, and their interaction had a highly significant effect on the following parameters: Viscosity ($P < 0.0001$), Viscosity Percentage ($P < 0.0001$), Motility ($P < 0.0001$) and Agglutination ($P < 0.0001$). Sperm viability was not affected by the fixed effects ($P < 0.4354$).

Mean sample viscosity decreased significantly in the control as well as in the diluted samples (Graph 1). Viscosity percentage results showed that liquefaction rates were almost similar for control and diluted samples, while complete liquefaction was observed only in the TCF-plus-Papain-diluted samples, after 90 min of incubation (Graph 2). The F-test showed that the Mean Viscosity and Viscosity percentage variations were both due to the effects of Treatments ($P < 0.0001$) and Time ($P < 0.001$).

Sperm motility decreased significantly in the control and diluted samples, except for samples diluted with Spermfluid®. The lowest motility values were observed in undiluted samples and the highest value was recorded for samples diluted in Spermfluid® (Graph 3); significant differences were due to the interaction between Treatment and Time ($P = 0.0046$).

Sperm head-to-head agglutination increased in all samples except for the control; the highest values were recorded in Papain-treated samples (Graph 4). Significant variations were due to the Treatment × Time Interaction ($P < 0.001$).



Graph 1. Effect of different enzymatic treatments on mean viscosity (120 min) of dromedary camel ejaculates Different letters (A,B,C) indicate significant differences ($P < 0.05$).