EFFECT OF FICIN ENZYME ON SEMEN VISCOSITY IN DROMEDARY CAMEL

M. Keshavarz1, A. Niasari-Naslaji1, H. Zare2, S. Ziapour1, M. Mirtavoosi1, M. Omidi1, A. Kalantari3 and A.A. Moosavi-Movahedi2

1Department of Theriogenology, Faculty of Veterinary Medicine
2Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
3Camel Breeding Centre, Ministry of Agriculture, Torud, Semnan, Iran

ABSTRACT

The viscous nature of camel semen limits its processing, evaluation and preservation. Present study investigated the effect of Ficin enzyme on camel semen viscosity. Semen was collected using phantom and diluted with Shotor diluent. Following initial mechanical stirring (150 rpm for 5 min), semen sample was assigned into 2 groups (Ficin and Control), with three equal fractions for each group. Ficin was added at the concentration of 0.05 mg/ml after 5 minutes initial stirring (Time 0). All samples were stirred for another 10, 20 and 30 min. At each time point, semen viability was assessed and the rest of the fraction was centrifuged at 600g for 7 min at 25°C. Semen viscosity was eliminated in all enzyme treated fractions. In all control tubes, pellet could not be separated from supernatant after 10 and 20 min; whereas, after 30 min, pellets were separated from supernatant in 50% of the samples. Treated samples displaced better total motility 20 and 30 min after adding Ficin (P<0.05). Progressive motility was also higher in treated group compared to control 20 min after adding Ficin (P<0.05). No detrimental effects on semen viability parameters were noticed following the addition of enzyme. In conclusion, Ficin could reduce semen viscosity without compromising sperm viability parameters in dromedary camel.

Keywords: Camel semen, enzyme, ficin , protease, semen viscosity

High semen viscosity in the camelidae created difficulties in semen collection, processing and artificial insemination. The cause of this viscosity and its elimination has been a challenging subject in the world of camelid research. It was suggested that mucopolysaccharide secreted by bulbourethral gland may be responsible for camel semen viscosity (Perk, 1962). However, recent studies indicated that these chemical structures that is renamed as glycosaminoglycans (GAGs) may not be the main source of semen viscosity in camelid semen (Kershaw-Young et al, 2012; 2013). There is sufficient evidence to accept that proteins within seminal plasma such as mucin may be responsible for viscosity in camelid semen as the viscosity of semen can be reduced by adding protease such as papain to semen (Kershaw-Young et al, 2013; 2016). Apart from enzymatic approach to reduce semen viscosity, mechanical and ultrasonic approaches were also investigated. Stirring of camel semen with paper clips, on very low speed (150 RPM) for 15 minutes (Mosaferi et al, 2005), gentle pipetting of semen in a diluent (Morton et al, 2008) and passage of semen back and forth through a needle could also be used to reduce semen viscosity (Santiani et al, 2005). However, application of vortex was highly detrimental for alpaca sperm and could not reduce semen viscosity (Vaughan et al, 2003). More recently, it was proposed that 40 KHz ultrasound wave imposed to dromedary camel semen for 2 min, interspersed for 2 min and repeated 4 times could reduce semen viscosity without having deleterious effect on semen viability (Rateb, 2016). While all of these methods could be simple and fairly effective, they do not completely eliminate semen viscosity. Present study investigated the effect of another natural plant protease “Ficin” on semen viscosity and sperm viability parameters of dromedary camel. The name Ficin is used to describe the endoproteolytic activity in latex of the genus Ficus (Jones and Glazer, 1970), and is recognised as a cysteine protease in fig trees (Lienier, 1961).

Materials and Methods

Experimental location

This study was conducted at Dromedary Camel Breeding Centre, Ministry of Jehad-e-Agriculture, Torud, Semnan province, Iran (latitude:35°20′88″N; longitude:55°00′80″E; altitude:885 m) throughout breeding season (November to March).
**Experimental animals**

Dromedary camel bulls (n=5; 8-14 years old) were exposed to natural day length and ambient temperatures and kept at individual pen and received 5 Kg Alfalfa hay, 3 Kg straw, 1.5 Kg concentrate included 70% barley, 30% wheat bran and 5gr for each camel mineral mixture (Availafor, USA) for one month before starting the experiment.

**Semen collection**

Semen was collected using phantom (Total collection: 10 samples) as previously described (Ziapour et al, 2014). Artificial vagina was prepared according to the method described previously (Mosaferi et al, 2005).

**Semen evaluation**

Semen was held inside incubator (35°C) throughout assessment. Total and progressive forward motility, live percentage and plasma membrane integrity of sperm were determined according to methods explained by Ziapour et al (2014).

**Semen extender**

Shotor diluent was used as semen extender in this study. It includes tris (2.6 gr; hydroxymethyl-aminomethan, Merck, Germany), citric acid (1.35 gr; Sigma-Aldrich Inc., St Louis, USA), glucose (0.9 gr; Sigma-Aldrich Inc., St Louis, USA), and fructose (0.9 gr; Merck, Germany), penicillin G Sodium (1000 IU/ml; Pen Sodium®, Jaber-Ebne-Hayyan pharmaceutical company, Iran), streptomycin sulfate (1000 mg/ml; streptocin®, Jaber-Ebne-Hayyan pharmaceutical company, Iran). The osmolality and pH of media were 330 mOsm/kg and 7.2, respectively. Egg yolk (20%) was added to all diluents.

**Enzyme preparation**

Ficin was extracted and purified from Sabz cultivar latex according to the procedure outlined (Zare et al, 2013). Peak V of Ficin D with greatest proteolytic and autolytic activity was used in the present study and preserved at -20°C until it was diluted in phosphate buffer for experiment. Ficin was dissolved in phosphate buffer (1mg/ml; 0.01 M phosphate buffer: 0.608 gr NaH₂PO₄ and 0.866 gr Na₂HPO₄; pH=7.0) and leave at incubator (35°C) prior to use.

**Experimental Design**

Following semen collection, the specimen was incubated at 35°C and diluted with Shotor diluent at the ratio of 1:11. While specimens were inside incubator, process of mechanical stirring and enzyme addition took place. Initial mechanical stirring was carried out at 150 rpm for 5 min. Then each semen sample was assigned into 2 groups (Ficin and Control), with 3 equal fractions for each group. Ficin was added at the concentration of 0.05 mg/ml after 5 minutes initial stirring (Time 0). All samples were stirred for another 10, 20 and 30 min. At each time point, semen viability was assessed and the rest of the fraction was centrifuged at 600g for 7 min at 25°C. The round formation of pellet at the bottom of conical tube and the ability to separate the supernatant during decanting were considered as the elimination of semen viscosity. Pellet formed in an oblige shape at the door toward the bottom of conical tube and inability to separate it from supernatant during decanting was considered as viscous semen.

**Statistical analysis**

Data were subjected to Arcsin transformation and analysed using GLM with LS Means included in the model in SAS (SAS, 2014). Results were reported as Mean ± SEM.

**Results and Discussion**

Total motility was greater in enzyme treated than control group after 20 and 30 min stirring (P<0.05; Fig 1). There was a significant decrease in total motility after 30 min stirring in both experimental groups (P<0.05). Progressive forward motility (PFM) was higher in enzyme treated group (36.9 ± 3.81) than control (27.8±5.1) 20 min after stirring (P<0.05). There was a significant decrease in PFM after 30 min stirring in both experimental groups (P<0.05). Plasma membrane integrity (PMI) was higher in enzyme treated group than control group 10 min after stirring (P<0.05; Fig 1). There was no difference in live percentage of sperm between experimental groups (P>0.05). Semen viscosity was eliminated in all enzyme treated fractions from 10 min after stirring. In all control tubes, pellet could not be separated from supernatant after 10 and 20 min; whereas, after 30 min, pellets were separated from supernatant in 50% of the samples.

Present study investigated the effect of Ficin, as a natural proteolytic enzyme, on semen viscosity and viability parameters of sperm in dromedary camel. Accordingly, Ficin at the concentration of 0.05 mg/ml after 10 min exposure to semen could reduce semen viscosity without compromising semen viability parameters. Similarly, Papain, another protease, was used successfully to eliminate semen
viscosity in Camelidae (Kershwa-Young et al, 2013; 2016). Ficin is cysteine protease isolated from the latex of one of the commercially important plant Ficus species (Devaraj et al, 2008). The available information indicates that ficin apparently share many common properties with papain (Liener and Friedenson, 1970).

In the present study, we have demonstrated a new method to differentiate between viscous and non-viscous semen using enzyme treatment followed by centrifugation. Following enzyme treatment, round pellet forms at the bottom of conical tube in association with the ability to separate supernatant from pellet. However, centrifugation of controls did not form a clear round pellet at the bottom of the tube, nor it could be separated from the supernatant. Studies to remove Ficin and seminal plasma by centrifugation and re-dilution of semen in fresh diluent aiming at enhancing sperm viability and preservation are could be a subject for further investigation.

In conclusion, Ficin enzyme at the concentration of 0.05 mg/ml along with stirring and centrifugation could be used to eliminate semen viscosity, without detrimental effect on sperm viability in dromedary camel.

References


