Vol 17 No 1, p 85-89

# EFFECT OF SOME EXTENDERS AND ENZYMES ON SEMEN VISCOSITY AND SPERM VIABILITY IN DROMEDARY CAMELS (*Camelus dromedarius)*

**I.M. Ghoneim, M.M. Al-Eknah, M.M. Waheed, A.K. Alhaider and A.M. Homeida[1]**
Department of Clinical Studies, College of Veterinary Medicine and Animal Resources,
[1]Camel Research Centre, King Faisal University, PO Box 1757, Al Ahsa 31982, Kingdom of Saudi Arabia

## ABSTRACT

The current work aimed to study the effect of 10 extenders/enzymes on the semen viscosity and viability of the dromedary camel. Eighty ejaculates were collected from 4 mature dromedary camels using an electro-ejaculator. Each semen sample was fractioned into 11 aliquots. One aliquot served as control and each one of the other 10 was diluted 1:1 with one of the extenders *viz.*; Androhep®, Green buffer®, Laciphos®, tris-fructose egg yolk, egg yolk sodium citrate dihydrate, trypsin (0.3% or 0.15%) or collagenase (1%, 2% or 4%). The effect of the extenders and enzymes on semen viscosity and sperm viability were studied. The initial viscosity of the collected semen samples ranged between highly viscous and mild viscous. Collagenase enzyme (2% and 4%) liquefied 100% of the semen samples within the first 15 minutes. The effect of tris-fructose egg yolk, Androhep®, Laciphose®, collagenase (1%, 2% and 4%) and Green buffer® on semen liquefaction was limited only to the first 15 minutes of incubation. The initial motility of semen samples ranged between 10 to 70%. Tris fructose egg yolk, Laciphose®, Green buffer® and collagenase (4%) stimulated the sperm motility significantly (P<0.01) within the first 15 minutes of incubation.

**Key words:** Dromedary, enzymes, extender, liquefaction, motility, semen, sperm

Low reproductive efficiency is one of the most important factors affecting profitable production of the camels (El-Wishy, 1987; Djelloli and Saint-Martin, 1992; Tibary and Anoussi, 1997; Skidmore, 2003). Application of assisted reproductive technologies such as artificial insemination, embryo transfer and *in-vitro* embryo production (Torner *et al,* 2003; Skidmore and Billah, 2006; Tibary *et al,* 2007; Wani, 2009) could offer an opportunity to improve the well known poor reproductive efficiency of the camel. Although, artificial insemination has been well developed in most farm animals, this technique has not developed well as a routine method for breeding in camels (Bravo *et al,* 2000b). One of the main physical characteristics of camelid semen is its high viscosity (Deen *et al*, 2003; Wani *et al,* 2008) which is the main constraint in handling and subsequent analysis of semen for artificial reproductive technologies. The composition and function of the viscous component of camelid seminal plasma is still unknown (Adams *et al,* 2009). Semen coagulum entrap the spermatozoa and impede the assessment of semen quality parameters, especially the motility (Deen *et al,* 2004), concentration and morphology which is considered as prerequisite to semen processing. There is a great need for development of reagent or technique to liquefy camelid semen without deleterious effect on the quality of spermatozoa. The objective of the present work was to study the relative effect of 10 extenders/enzymes on the camel semen viscosity and sperm viability.

## Materials and Methods

### *Experimental animals*

The study was conducted at the Camel Research Centre, King Faisal University. Four adult male dromedary camels aged 7 - 14 years and weighing 400 – 700 kg were used. The animals were in a healthy condition with sound history of fertility in the herd. Camels were maintained under standard conditions of feeding and management. They had no contact with the females during the period of the experiment (January to March).

### *Extenders and enzymes*

Three commercial (Androhep®, Green buffer® and Laciphos®) and 2 laboratory prepared extenders (Tris-fructose egg yolk and egg yolk sodium citrate dihydrate) besides trypsin and collagenase (Sigma Aldrich, St Louis, MO, USA) were tested in this

SEND REPRINT REQUEST TO M.M. WAHEED email: mmwaheed@hotmail.com

study. Androhep® (minitüb Tiefenbach, Germany), Green buffer® (I.M.V., L'Aigle France) and Laciphos® (I.M.V. International Corp., L'Aigle Cedex, France) were prepared as manufacturer instructions. Tris-fructose egg yolk and egg yolk citrate were prepared as described by Zeidan *et al* (2008). Two concentrations of trypsin (0.3% and 0.15%) and 3 concentrations of collagenase ( 1%, 2% and 4% ) were prepared in 1 ml 2.9% sodium citrate dihydrate. The semen extenders / enzymes were maintained at 30°C in water bath prior to collection of semen.

### *Semen collection*

Collection of semen was attempted twice a week for each animal using electro-ejaculation method as described by Tingari *et al* (1986). Prior to semen collection, camels were sedated with mixture of xylazine (0.15mg/kg) and ketamine (2.5mg/kg) administered, intravenously (White *et al,* 1987).

### *Semen processing and evaluation*

A total of 80 semen ejaculates from the 4 male camels (20 from each) were used in this experiment. Immediately after collection, semen samples were evaluated for volume and colour. Each ejaculate was divided into 11 equal aliquots. One aliquot served as control and each one of other aliquots was extended 1:1 in one of the 10 extenders/enzymes. Instantly after collection (0 hour) as well as after 15, 30, 45, and 60 minutes, extended and control semen samples were assessed for viscosity by pipetting the semen using 5 - 0 scale (5, 4, 3, 2, 1, 0 indicating highly viscous, moderate viscous, mild viscous, liquefied, fully liquefied semen, respectively). The percentage of progressively motile spermatozoa was subjectively described at 15, 30, 45 and 60 minutes in control and treated samples. The viability indices (Milovanov *et al,* 1964) were computed from the following equation:

$$VI= \Sigma [M \times (T-R/2)]$$

where; VI is the viability index, Σ is a sign for the sum total, M is the percentage of sperm motility, T is the time of next determination of motility and R is the time of previous determination of motility.

### *Statistical analysis*

Analysis of data was performed by t-test and analysis of variance (ANOVA) using a commercial software (Statistica for windows, 1993).

## Results

The colour of the semen samples varied from milky to creamy white. The average volume of the ejaculate was 6.48 ± 0.36 ml with a range of 2.5 to 13.0 ml. The initial viscosity of the collected semen samples ranged between highly viscous (5) and mild viscous (1). Table 1 shows the effect of different treatments on the liquefaction time. In control group, 55.0% (44 out of 80), 11.25% (9 out 80), 11.25% (9 out 80) and 0% (0 out of 80) were fully liquefied within 15, 30, 45 and 60 minutes, respectively. The 22.50% of samples (18 out of 80) was not liquefied within 60 minutes of incubation at 30°C. Hundred per cent of the semen samples (80 out of 80) treated with collagenase enzyme (2% and 4%) were completely liquefied within the first 15 minutes. Tris-fructose egg yolk, Androhep®, Laciphose® and collagenase (1%) had the same effect on the liquefaction time of the semen samples. The favourable effect of tris-fructose yolk, Androhep®, Laciphose®, collagenase (1%, 2% and 4%) and Green buffer® on liquefaction was limited only to the first 15 minutes of incubation. The highest per cent of non liquefied semen after 60

**Table 1.** Effect of different extenders and enzymes on liquefaction time of the incubated camel semen.

| Treatment | n | Complete liquefaction after | | | | Not liquefied |
|---|---|---|---|---|---|---|
| | | 15 min. | 30 min. | 45 min. | 60 min. | |
| Control | 80 | 44 (55.00%) | 9 (11.25%) | 9 (11.25%) | 0 (0%) | 18 (22.50%) |
| Citrate yolk | 80 | 44 (55.00%) | 18 (22.50%) | 9 (11.25%) | 0 (0%) | 9 (11.25%) |
| Tris-fructose yolk | 80 | 67 (83.75%) | 0 (0%) | 0 (0%) | 0 (0%) | 13 (16.25%) |
| Androhep® | 80 | 67 (83.75%) | 0 (0%) | 0 (0%) | 0 (0%) | 13 (16.25%) |
| Laciphose® | 80 | 67(83.75%) | 0 (0%) | 0 (0%) | 0 (0%) | 13 (16.25%) |
| Green buffer® | 80 | 60 (75.00%) | 0 (0%) | 0 (0%) | 0 (0%) | 20 (25.00%) |
| Trypsine 3% | 80 | 62 (77.50%) | 9 (11.25%) | 0 (0%) | 9 (11.25%) | 0 (0%) |
| Trypsine 0.15% | 80 | 67 (83.75%) | 0 (0%) | 0 (0%) | 13 (16.25%) | 0 (0%) |
| Collagenase 1% | 80 | 67 (83.75%) | 0 (0%) | 0 (0%) | 0 (0%) | 13 (16.25%) |
| Collagenase 2% | 80 | 80 (100.00%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Collagenase 4% | 80 | 80 (100.00%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |

minutes incubation was recorded in Green buffer® (25%, 20 out of 80). The 4% was considered as the best concentration (P<0.01) for induction of liquefaction of semen amongst the other (1% and 2%) concentrations. There was no significant difference between the effect of trypsin 0.15% and 0.3% on liquefaction. The initial motility of the semen samples ranged between 10 to 70%. The effect of various extenders and enzymes on motility and viability indices of incubated camel semen are presented in table 2. In control samples, the per cent of the motility decreased gradually by time and ceased completely by the end of the 60 minutes of incubation. Tris-fructose egg yolk, Laciphose®, Green buffer® and collagenase (4%) stimulated the sperm motility significantly (P<0.01) within the first 15 minutes of incubation. The beneficial effect of Green buffer® on the sperm motility sustained for one hour. On the other hand, the favourable effect of tris-fructose egg yolk, Laciphose® and collagenase (4%) on the motility did not persist. Androhep® deteriorated significantly (P<0.01), the sperm motility during the incubation time. The viability indices of the semen samples treated with citrate egg yolk, tris-fructose egg yolk, Green buffer®, and collagenase (1%, 2% and 4%) were significantly higher (P<0.01) than the control. Significant decrease of the viability indices of the semen samples treated with Androhep® and trypsin 0.3% was evident. Laciphose® and trypsin 0.15% had no significant effect on the viability indices. Semen treated with Green buffer® had the highest viability index (P<0.01).

## Discussion

The mean ejaculate volume 6.48 ± 0.36 ml with range of 2.5 to 13.0 ml was recorded in this study were close to those recorded by Khan and Kohli (1972) and Agarwal *et al* (2004) and less than the ejaculate volume collected by artificial vagina (Hemeida *et al,* 2001). The volume of semen recovered by electro-ejaculation is usually less than that collected by artificial vagina (Tingari *et al,* 1986; Skidmore, 2004). A wide range of the viscosity of the semen (5-1) were recorded in this study. In South American camelids, Tibary *et al* (1999) found varied degree of viscosity between males. The seminal traits also varied from animal to animal and week to week (Agarwal *et al,* 2004). The viscosity of the semen is usually attributed to the presence of mucopolysaccharides which came from the bulbourethral glands or prostate (Perk, 1962; Garnica *et al,* 1993; Hassan *et al,* 1995). The physiological significance of high viscosity of camelid semen is not clarified; it may act as a type of sperm reservoir or may be important for keeping sperm viability within the uterus (Mattner and Braden, 1969). In the present study, 22.50% of the semen samples didn't liquefy within 60 minutes. Wani *et al* (2008) could not obtain full liquefaction even after 3h of storage at 37°C. Bravo and Johnson (1994) recorded natural semen liquefaction within 23 hours after collection. The mechanism of coagulation and subsequent liquefaction of semen is not clear. Extension of camel semen with tris-fructose egg yolk or citrate egg yolk induced liquefaction within 1.5 h at 37°C (Wani *et al,* 2008). This couldn't be proved in our study and may be attributed either to the limited incubation period by one hour or to the incubation temperature of 30°C. As recorded in the present study, collagenase was found more effective than trypsine in eliminating llama and alpaca semen viscosity (Bravo *et al,* 2000a). In the present study, a wide range was recorded in the initial motility

**Table 2.** Effect of different extenders and enzymes on motility and viability indices of the incubated camel semen (mean±SEM).

| Extenders and additives | n | Sperm motility (%) during incubation at 30°C | | | | Viability indices |
|---|---|---|---|---|---|---|
| | | 15 minutes | 30 minutes | 45 minutes | 60 minutes | |
| Control | 80 | $29.00^{ad} \pm 1.88$ | $15.00^{a} \pm 2.01$ | $10.25^{a} \pm 1.74$ | $00.00^{a} \pm 0.00$ | $10.77^{ah} \pm 0.80$ |
| Citrate - yolk | 80 | $33.94^{ac} \pm 2.20$ | $19.38^{ac} \pm 2.68$ | $16.00^{bg} \pm 1.79$ | $10.00^{b} \pm 1.42$ | $13.14^{bjk} \pm 1.03$ |
| Tris – fructose yolk | 80 | $52.69^{e} \pm 2.56$ | $46.00^{d} \pm 3.23$ | $36.00^{c} \pm 2.09$ | $28.00^{c} \pm 1.65$ | $25.76^{c} \pm 1.73$ |
| Androhep® | 80 | $12.31^{f} \pm 1.57$ | $3.00^{e} \pm 0.45$ | $2.00^{d} \pm 0.45$ | $2.00^{d} \pm 0.45$ | $6.08^{d} \pm 0.33$ |
| Laciphose® | 80 | $33.00^{cg} \pm 2.23$ | $19.00^{ahi} \pm 1.96$ | $15.00^{bi} \pm 1.59$ | $00.00^{a} \pm 0.00$ | $12.46^{ab} \pm 0.96$ |
| Green buffer® | 80 | $54.94^{eh} \pm 2.28$ | $50.00^{dg} \pm 2.62$ | $49.13^{e} \pm 1.22$ | $43.06^{e} \pm 2.02$ | $31.29^{f} \pm 1.47$ |
| Trypsine 0.3% | 80 | $15.63^{bf} \pm 1.93$ | $15.00^{ah} \pm 1.69$ | $13.25^{ab} \pm 1.06$ | $4.50^{f} \pm 0.83$ | $8.13^{e} \pm 0.76$ |
| Trypsine 0.15% | 80 | $15.44^{bf} \pm 1.96$ | $21.25^{ci} \pm 2.26$ | $17.00^{ghi} \pm 2.30$ | $4.50^{f} \pm 0.83$ | $10.54^{ehik} \pm 1.20$ |
| Collagenase 1% | 80 | $27.00^{d} \pm 2.00$ | $29.75^{b} \pm 2.43$ | $26.50^{f} \pm 2.13$ | $10.50^{b} \pm 1.83$ | $14.85^{bg} \pm 1.45$ |
| Collagenase 2% | 80 | $35.00^{ag} \pm 2.80$ | $26.25^{b} \pm 1.84$ | $14.00^{ab} \pm 1.48$ | $7.50^{b} \pm 1.21$ | $16.05^{gj} \pm 1.24$ |
| Collagenase 4% | 80 | $51.25^{eh} \pm 2.53$ | $34.81^{j} \pm 2.39$ | $19.00^{g} \pm 1.93$ | $10.00^{b} \pm 1.18$ | $20.81^{c} \pm 1.38$ |

Means with different superscripts in the same column are significantly different at P<0.01

(10-70%). The same differences in motility were also reported previously (Deen *et al,* 2003). However, in other studies no sperm motility was reported in camel semen either fresh or diluted up to 12 hours of collection (Agarwal and Khanna, 1990; Khanna *et al,* 1990; Agarwal *et al*, 1995). In the persent study, tris-fructose egg yolk, Laciphose®, Green buffer® and collagenase (4%) showed a highly significant ($P<0.01$) beneficial effect on the sperm motility. In alpaca, tris diluent improved sperm motility (Morton *et al*, 2008). Good liquefaction and motility were recorded in camel semen diluted with tris based extenders (Wani *et al,* 2008). Watson (1979) pointed to the superior buffering qualities of tris over citrate and phosphate. Tris in addition to its better buffering capacity, it can readily diffuse into the sperm cells and serves as an intracellular buffer (Bartlett *et al*, 1962). Best results were achieved when the semen was diluted in Green buffer® (Bravo *et al,* 2000b; Skidmore and Billah, 2006), or a tris egg-yolk extender (Deen *et al,* 2003). Bravo *et al* (2000a) recorded that collagenase had little or no influence in decreasing sperm motility in llamas and alpacas. However, in Alpaca, Maxwell *et al* (2008) reported that collagenase was toxic at all concentrations. Tibary and Anouassi (1997) also reported that all enzymes had been seen to cause acrosomal damage in spermatozoa. Collagenase apparently had the least effect on killing sperm (Bravo *et al*, 2000a). In the present study, although the enzymes attained good result for liquefaction of semen. Tibary and Anouassi (1997) observed acrosomal damage in spermatozoa treated with these enzymes.

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