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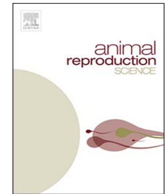

Physical and kinematic properties of cryopreserved camel sperm after elimination of semen viscosity by different techniques

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ABSTRACT

This investigation aimed to determine the influence of using different techniques for liquefaction of semen on post-thaw physical and dynamic characteristics of camel spermatozoa. A total of 144 ejaculates were collected from 3 adult camels, *Camelus dromedarius*, twice-weekly over 3 consecutive breeding seasons. A raw aliquot of each ejaculate was evaluated for physical and morphological properties, whereas the remaining portion was diluted (1:3) with glycerolated Tris lactose egg yolk extender, and was further subjected to one of the following liquefaction treatments: control (untreated), 5 μl/ml α-amylase, 0.1 mg/ml papain, 5 u/ml bromelain, or 40-kHz nominal ultrasound frequency. The post-thaw objective assessment of cryopreserved spermatozoa, in all groups, was performed by a computer-assisted sperm analysis (CASA) system. The results revealed that all liquefaction treatments improved ($P < 0.05$) post-thaw motility, viability and sperm motion criteria. However, an adverse effect ($P < 0.05$) was observed in acrosome integrity, sperm cell membrane integrity and percent of normal sperm in all enzymatically-treated specimens compared to both control and ultrasound-treated semen. These results elucidate the efficiency of utilizing ultrasound technology for viscosity elimination of camel semen. In addition, developing enzymatic semen liquefaction techniques is imperious to benefit from when applying assisted reproductive technologies, particularly AI and IVF, in camels.

1. Introduction

The application of assisted reproductive technologies in modern livestock industry, particularly artificial insemination (AI), *invitro* fertilization (IVF) and embryo transfer (ET), virtually contributes in accelerating genetic improvement in different domestic species. However, the successful utilization of these techniques relies on the biophysical characteristics of obtained ejaculates and its capacity for processing with minimum loss of sperm fertilization potential (Mostafa et al., 2014).

Dromedary camel semen is characterized by its hyper-viscous rheological nature due to the presence of a gel fraction in the ejaculate (Skidmore et al., 2013). Resembling human semen, the seminal plasma viscous material is distributed throughout the ejaculate (Kershaw-Young and Maxwell, 2012), and plays a vital role in maintaining sperm vitality and fertilization potential within the female reproductive tract after natural mating (Brown, 2000; Deen et al., 2005; Vaughan and Tibary, 2006). However, this semen feature is considered the major constrain for development of ARTs in camels (El-Bahrawy, 2010; Skidmore et al., 2013). Camel semen

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viscosity limits handling of semen and prohibits homogeneity in sperm concentration after dilution (Adler et al., 1997) and during straws packing (El-Bahrawy, 2010; Shekher et al., 2012). It also impedes the homogenous mixing of semen with the nutrients and cryoprotective components in the diluent (Deen et al., 2003). Preeminently, it hampers qualitative sperm physical and morphological assessment in raw/diluted ejaculates (El-Zanaty et al., 2004; Deen, 2008).

During the last decade, different techniques have been used to improve semen rheological properties and enhance sperm motility in the camelidae family. Some investigators applied mechanical liquefaction; i.e. vortexing (Vaughan et al., 2003), stirring (Mosaferi et al., 2005; Niasari-Naslaji et al., 2007), needling and pipetting (Morton et al., 2008), and centrifugation (El-Bahrawy, 2010). Most recently, ultrasound exposure was successfully utilized to eliminate viscosity and improve short-term chilled preservation of dromedary semen (Rateb, 2016). Other authors supplemented the semen diluent with different enzymes to eliminate viscosity. These comprise using hydrogen peroxide decomposition catalysts (Medan et al., 2008), long-chain carbohydrates break-down catalysts (El-Bahrawy, 2010; Monaco et al., 2016), and proteolytic enzymes (Morton et al., 2008; Shekher et al., 2012; Kershaw-Young et al., 2013; Kershaw-Young et al., 2016; Mal et al., 2016; Monaco et al., 2016). Worthwhile, semen assessment in all previous investigations was conducted subjectively by conventional semen evaluation techniques.

Recent advances in utilizing computer-assisted semen analysis (CASA) proved its ability for accurate estimation of sperm function and semen heterogeneity compared to traditional semen evaluation (Holt et al., 2007). CASA warrants detection of subtle changes in sperm motion, velocity and morphology; which confer decisive discrimination between treatments in semen processing studies (Amann and Katz, 2004). Therefore, artificial insemination centers, notably for humans, adopt CASA to increase objectivity in determination of sperm motility (Kathiravan et al., 2011). Accordingly, the current investigation aimed to determine the efficiency of applying different techniques for liquefaction of dromedary camel semen on CASA-derived physical, morphological and motion characteristics of cryopreserved spermatozoa.

2. Materials and methods

2.1. Animals and management

The current investigation was implemented over 3 consecutive breeding seasons. Three adult bull camels (*Camelus dromedarius*), aged 15–22 years, with an average body weight of 650 ± 50.0 kg and body condition score 2.5 ± 0.5 (Faye et al., 2011) were used. The camels were housed individually in pens belonging to Artificial Insemination Lab., Mariout Research Station (Latitude 31° 00′ N; Longitude 29° 47′ E), Alexandria, Egypt. Throughout the 3 breeding seasons, the camels were kept under the same management condition and were fed a concentrate mixture composed of (50% corn, 47% barley, 2% minerals, 1% salt) at the rate of 4 kg/animal/day. Furthermore, Egyptian clover, *Trifolium alexandrinum*, hay was offered *ad libitum*, and fresh water was available once daily in mid-day. Prior to the onset of each breeding season, the camels were subjected to clinical examination and were found free of disease or reproductive disorders. All experimental procedures were conducted following the EU Directive for protection of experimental animals (2010/63/EU).

2.2. Semen extender preparation

All reagents were obtained from Sigma (Sigma-Aldrich) unless otherwise stated. A glycerolated Tris-lactose egg yolk extender was freshly prepared for dilution of semen (El-Bahrawy et al., 2006). Briefly, the extender was composed of Tris buffer (3.25%, w/v), lactose (5.5%, w/v), citric acid (1.67%, w/v), glucose (1%, w/v), and was further supplemented with egg-yolk (20%, v/v) and glycerol (3%, v/v) to reach a final pH of 7.4 and 0.351 osmol/kg osmotic pressure. The glycerolated diluent was added to the semen specimens in a one-step dilution technique.

2.3. Semen collection

A total of 144 ejaculates were collected from the camels over 3 consecutive breeding seasons (January − March) by an artificial vagina (AV) on a teaser female. Semen collection was performed at 7:00 a.m. twice-weekly throughout each breeding season (Bahrawy et al., 2012). The AV was inlayered with a disposable polyethylene sheath, and double-jacket collection tubes were used to maintain the ejaculates at 37 °C during the collection sessions (Bahrawy et al., 2012; Rateb, 2016). Ejaculates showing contamination with urine, feces, dirt, abnormal color or odor, were discarded.

2.4. Experimental design

Immediately after collection each ejaculate was transported to the laboratory, in a portable water path adjusted at 37 °C, for further physical and morphological assessment (El-Bahrawy et al., 2006). Upon examination, only ejaculates exhibited initial total motility exceeding 90% (oscillatory and/or progressive) were processed. Mean values of physical and morphological properties of raw (neat) semen throughout the 3 breeding seasons are illustrated in Table 1. Moreover, semen viscosity was determined by the thread formation technique as previously described (Kershaw-Young et al., 2013).

Meanwhile, the other portion of split semen was diluted (1:3) with Tris lactose egg yolk extender, and was further subjected to one of the following treatments for viscosity elimination: control (untreated), 5 μl/ml α-amylase (TERMAMYL SUPRA, Novo Nordisk, Denmark) (El-Bahrawy, 2010), 0.1 mg/ml papain (Sigma-Aldrich, USA), 5 u/ml bromelain (Gynemed, GmbH & Co., Germany)

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