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Review article

An update on semen collection, preservation and artificial insemination in the dromedary camel (*Camelus dromedarius*)

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ABSTRACT

Artificial insemination (AI) in domestic animals is an important tool to maximise the use of genetically superior males and thereby insure rapid genetic progress. However, the application of AI in camelids has been hindered by the difficulties involved in collecting, as well as handling the semen due to the viscous nature of the seminal plasma. This review describes the challenges of semen collection and discusses the role of seminal plasma as well as the reasons for the viscosity and how to liquefy it so that ejaculates can be more accurately evaluated. It also reports on the use of various extenders used for liquid storage of fresh and chilled semen and how pregnancy rates are affected by numbers of spermatozoa inseminated, site of insemination and timing of insemination in relation to GnRH injection given to induce ovulation. In addition, this paper reviews the latest research in cryopreservation of camel semen and addresses the various problems involved and possible improvements that can be made so that pregnancy rates can be increased with frozen semen.

1. Introduction

Artificial insemination is routinely used in many domesticated species such as the cow, sheep and horse as it offers the opportunity to increase the overall productivity of genetically superior males, thereby increasing the rate of genetic progress. It also eliminates the need to transport valuable animals between farms and helps prevent the spread of venereal diseases as contact between the male and female is prevented. In addition, once the semen has been collected it can be preserved indefinitely by deep freezing and storing in liquid nitrogen at −196 °C. Among other advantages, this extends the reproductive lifespan of the male even after his death.

Various studies have compared different extenders for preserving camel semen at room temperature, 4 °C or in liquid nitrogen (LN2), and recorded sperm motility, morphology and viability (Deen et al., 2003; Niasari-Naslaji et al., 2007; Sieme et al., 1990; Wani et al., 2008; Zhao et al., 1996); however only a few insemination trials have been published, most of which involve Bactrian camels (Chen et al., 1990; see reviews Bravo et al., 2000a; Skidmore et al., 2013; Zhao et al., 1994). In addition, the ejaculate generally has a low sperm concentration, low sperm motility and is highly viscous in nature making handling it difficult (Bravo et al., 2000a; Deen and Sahani, 2000). This review discusses methods of semen collection, handling and research performed on semen preservation and

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AI. While focussed on the dromedary camel, it draws upon findings in other camelid species (the Bactrian, llamas and alpacas) that may be relevant to the subject of this review.

2. Semen collection

Semen collection in camelids presents many challenges partly due to their mating behaviour in sternal recumbency, which makes it physically difficult for the operator, and the lengthy copulation period that can take from 5–10 min (Musa et al., 1992). Semen can be collected from camels by either electro-ejaculation or AV. Electro-ejaculation, can be achieved using a standard bovine ejaculator with the male secured in sternal recubency and turned on his side as described by Tingari et al. (1986). The procedure requires heavy sedation (Detomidine hydrochloride 80 μg/kg BW, i.m.; Al-Qarawi et al., 2002) or even general anesthesia and therefore can present a risk to valuable animals.

Alternatively the semen can be collected by AV which has already been described in many previous reviews (Tibary and Anouassi, 1997a; Bravo et al., 2000a; Skidmore et al., 2013). This technique uses a modified bull AV, which has a foam insert, or a constriction to provide a narrowing, to simulate the cervical rings which are essential for stimulating ejaculation in camels. The volume of semen collected can vary between 2–10 ml but is sometimes contaminated with sand due to the copulatory behaviour of the male in sternal recumbency. More recently, a camel dummy has been designed with an internal AV in an attempt to reduce the risk to, and physical efforts of, the operator and in the hope of providing cleaner samples (Ziapour et al., 2014). Ziapour et al. (2014) compared ejaculates collected from four bulls using the dummy with six bulls collected by AV and found no differences in duration of semen collection or semen parameters such as volume, motility, osmolarity and concentration. In addition, they found that the number of specimens contaminated with visible particles was higher in the samples collected by AV (72.7%) than those collected by using the dummy (0%).

3. Seminal plasma

Evaluation of the semen presents a major challenge due to its thick viscous consistency which immobilizes the sperm. The viscosity is attributed to secretions by the bulbourethral glands and the prostate, (as camelids do not have seminal vesicles), which make up the seminal plasma and which aid coitus (Kershaw- Young and Maxwell, 2012; Tibary and Anouassi et al., 1997b). This seminal plasma contains proteins that are important for sperm function and fertility (Kershaw- Young and Maxwell, 2012). Kershaw-Young and Maxwell (2011) investigated the effect of seminal plasma on alpaca sperm function by incubating ejaculated semen (that had been processed by centrifugation to remove seminal plasma) and epididymal spermatozoa separately in 0, 10, 25, 50 and 100% seminal plasma for up to 6 h and assessing motility, acrosome integrity and DNA integrity. Both epididymal and ejaculated sperm maintained motility longer when incubated in the presence of 10% seminal plasma compared with 0, 25, 50 or 100% seminal plasma ($P < 0.001$) and the mean percentage of epididymal sperm with intact acrosomes was less ($P < 0.001$) in samples incubated with 0% seminal plasma (39.4%) compared with 10% (75.3%) or 100% (77.4%) seminal plasma within 1 h of incubation. The mean viability of ejaculated sperm was reduced in the presence of 100% (12.7%) compared with 10% (36.2%) seminal plasma within 1 h of incubation. It was concluded that the presence of at least 10% seminal plasma is necessary to maintain motility, acrosome integrity and viability of spermatozoa *in vitro* in alpacas.

The viscous seminal plasma is evenly distributed throughout the ejaculate making it difficult to assess parameters such as sperm motility until the semen is liquefied. In addition, there is considerable variation in semen quality between males or even between ejaculates from the same male (Tibary and Anouassi et al., 1997b). Viscosity is usually measured using the thread technique, *i.e.* measuring the strand formed between a pipette tip and a semen sample placed on a glass slide (Fig. 1; Tibary and Anouassi et al., 1997b), and measures between 4–8 cm. It is possible that the viscosity plays a role in the lubrication of the vagina for intromission and could also function to ensure the semen remains in the female genital tract following mating, thus preventing sperm loss. Since ovulation occurs 28–48 h after mating (Anouassi et al., 1992; Vaughan and Tibary, 2006) this viscous seminal plasma could ensure that the spermatozoa are released slowly as the semen liquefies to optimize the time taken for them to reach and fertilize the oocyte in the oviduct (Deen et al., 2003).

The viscosity of camelid semen was initially thought to be caused by glycosaminoglycans (GAGs) as the concentrations of GAGs was 15 times higher in alpaca seminal plasma than in the ram (Kershaw-Young et al., 2012). However, enzymes that degrade GAGs do not completely reduce alpaca seminal plasma viscosity suggesting that GAGs are not the cause of viscosity in this case (Kershaw-Young et al., 2013). In contrast, other enzymes such as collagenase, fibrinolysin and trypsin will partially reduce the viscosity of alpaca seminal plasma (Bravo et al., 2000b), whereas the proteases papain (final conc 0.1 mg/ml) and proteinase K completely eliminate the viscosity within 20–40 min of treatment (Kershaw- Young and Maxwell, 2012). However, agglutination of sperm heads was higher in papain- treated samples than in untreated controls (Monaco et al., 2016), thus, these enzymatic treatments might decrease the viscosity of the semen but they can also have deleterious effects on the spermatozoa (Bravo et al., 2000b; Ghoneim et al., 2010).

Collectively, these results indicate that proteins are responsible for the viscosity of camelid semen and recent studies have shown that it is mostly related to mucin 5B which is five times more abundant in seminal plasma samples with high viscosity compared with those with low viscosity (Kershaw- Young and Maxwell, 2012). Mucin 5B is a member of the mucin protein family and is defined as a large gel-forming protein that is secreted by glandular epithelial cells. It has also been found in human seminal plasma (Russo et al., 2006) and the gel fraction of boar semen (Boursnell et al., 1970).

As stated previously, evaluation and processing of camelid ejaculates requires liquefaction in order to release entrapped