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Artificial insemination in dromedary camels☆

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ABSTRACT

Artificial insemination (AI) is an important technique in all domestic species to ensure rapid genetic progress. The use of AI has been reported in camelids although insemination trials are rare. This could be because of the difficulties involved in collecting as well as handling the semen due to the gelatinous nature of the seminal plasma. In addition, as all camelids are induced ovulators, the females need to be induced to ovulate before being inseminated.

This paper discusses the different methods for collection of camel semen and describes how the semen concentration and morphology are analyzed. It also examines the use of different buffers for liquid storage of fresh and chilled semen, the ideal number of live sperm to inseminate and whether pregnancy rates are improved if the animal is inseminated at the tip of the uterine horn verses in the uterine body. Various methods to induce ovulation in the female camels are also described as well as the timing of insemination in relation to ovulation. Results show that collection of semen is best achieved using an artificial vagina, and the highest pregnancy rates are obtained if a minimum of 150×10^6 live spermatozoa (diluted in Green Buffer, lactose (11%), or I.N.R.A. 96) are inseminated into the body of the uterus 24 h after the GnRH injection, given to the female camel to induce ovulation.

Deep freezing of camel semen is proving to be a great challenge but the use of various freezing protocols, different diluents and different packaging methods (straws verses pellets) will be discussed. Preliminary results indicate that Green and Clear Buffer for Camel Semen is the best diluent to use for freezing dromedary semen and that freezing in pellets rather than straws result in higher post-thaw motility. Preservation of semen by deep-freezing is very important in camelids as it prevents the need to transport animals between farms and it extends the reproductive life span of the male, therefore further work needs to be carried out to improve the fertility of frozen/thawed camel spermatozoa.

1. Introduction

Artificial insemination (AI) offers many advantages to commercial animal production and is used routinely these days in several domestic animals such as the cow, sheep and horse (Adams, 1982). The method offers the opportunity to increase the overall productivity of a particular male thereby increasing the overall rate of genetic progress.

The use of AI as a breeding technique has been reported in camelidae since the 1960s with the first camelid offspring from AI being reported in a Bactrian camel in 1961 (Elliot, 1961). However, it has only been during the last 25 years that this technique has started to be used more frequently as interest grows in trying to improve genetic traits such as milk, meat and wool production as well as racing ability in the Middle East. A major advantage of artificial insemination is that it can be used to increase the overall reproductive efficiency of the species however, some essential prerequisites are required before an

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artificial insemination programme can begin. Firstly the male camel has to be trained to the use of an artificial vagina (AV) so that semen can be collected, and then it has to be diluted in a suitable extender to maximize the use of each ejaculate. Secondly, as camels are induced ovulators, ovulating only when mated, ovulation has to be induced in each female camel that is to be inseminated.

In the dromedary camel several authors have studied semen preservation and insemination (Anouassi et al., 1992; Bravo et al., 2000; Skidmore and Billah, 2006a), but the majority of studies report low post-thaw motilities and few, if any, pregnancies with AI of chilled or frozen semen (Deen et al., 2003). This could be because of the difficulties involved in collecting semen from male camels and that the ejaculates are of low volume, low sperm concentration and are highly viscous. The spermatozoa are entrapped within this viscous seminal plasma which makes the handling, diluting and cryopreservation difficult (Bravo et al., 2000; Deen and Sahani, 2000).

This paper discusses methods for collection and insemination of semen, evaluates the optimum extender for dilution and number of spermatozoa to inseminate and discusses methods of short and long term preservation of semen.

2. Advantages of artificial insemination

Artificial insemination has many advantages as it allows more efficient use of genetically superior males by inseminating more than one female with a single ejaculate, thereby reducing the number of females the male has to mate, and increasing his number of offspring. Secondly, the use of AI would eliminate the need to transport the male or female animal to the stud, as it is much easier to transport semen, and this would reduce the costs and risks of transporting valuable animals. It would also reduce the risk of disease and infection, as all contact between male and female is prevented, and the semen can be treated with antibiotics before insemination. Thirdly, it helps eliminate behavioural problems as often a male camel will refuse to mate a particular female, or get aggressive towards her, so AI would eliminate the risks of injury. In addition, AI allows for the preservation of semen, either for 24 h by chilling, which enables the semen to be transported between farms/countries for insemination in other breeding herds, or for many years by deep freezing, which could extend the reproductive lifespan of the camel even beyond its death. Finally, AI can also help manage cross breeding programmes between different species of camelidae as it eliminates problems associated with size, behaviour and living in different habitats (Skidmore et al., 1999).

3. Collection of semen

Semen collection in camels presents many difficulties mainly due to the nature of their copulatory behaviour i.e. mating in sternal recumbancy, lengthy ejaculation throughout copulation (from 5 to 20 min) and the highly viscous nature of the semen itself. The main techniques used these days for collecting semen from camels are the AV or, more rarely, electro-ejaculation.

For collection by AV a modified bull vagina (30 cm long, 5 cm internal diameter) has given the best results (Bravo et al., 2000). However, if the semen makes contact with the rubber liner this can have an adverse effect on sperm motility, thus a shortened AV is more popular allowing the semen to pass directly into the collection flask. Alternatively, an additional disposable plastic inner liner may be inserted to avoid contact with the rubber material although the authors have found that this is not accepted well by the males. The AV is prepared by filling with water at 55–60 °C to give an internal temperature of 38–40 °C and pressure inside the AV, to stimulate ejaculation, is obtained by blowing air between the inner liner and outer rigid wall. A clear, glass water-jacketed (35–37 °C) semen vessel is attached to the apex of the internal latex rubber liner to keep the semen warm during the lengthy ejaculation process and enable visualization of ejaculation. Observation of natural matings suggested that the highly mobile urethral process of the camel penis may need to gain entry to the cervix to stimulate ejaculation during the extended copulatory process. For this reason, a foam imitation cervix of about 8 cm in length is placed inside the AV and the entrance of the AV is lubricated with KY jelly before use.

To collect the semen a sexually receptive female is first teased by the male to make olfactory contact and get him aroused before the bull is lead up behind the sitting female. As soon as the bull has sat down on the female and makes a few thrusts, the operator grasps the bull's sheath and directs his penis into the AV, holding it there by manual pressure at the base of the scrotum. The bull makes several thrusts, interspersed by periods of rest, until ejaculation is complete and during this time care must be taken to ensure the penis stays inside the AV otherwise the ejaculate will become contaminated with sand and dirt introduced by the penis. The ejaculate is usually expected in fractions and this whole process can take between 5 and 10 min, although it may occasionally last for 20 min or longer (Rai et al., 1988). It is very difficult to ascertain when ejaculation has been completed so the collection procedure is usually continued until the male stands up. As the collection procedure can be rather prolonged it can be advantageous to add about 1–2 ml of extender to the collection vessel before the collection.

Electro-ejaculation may be employed if collection by AV cannot be achieved, using a standard bovine ejaculator (12 V and 180 mA; Standard Precision Electronics, Denver, CO) although it is very stressful for the animal and does not yield a representative sample of semen. The bull is secured in sternal recumbancy and then turned on his side as described by Tingari et al. (1986). The animal is restrained physically with ropes and chemically by sedation with Domosedan (detomidine hydrochloride, 30–35 $\mu g\,kg^{-1}$ bodyweight (bwt) i.v. or 70–80 $\mu g\,kg^{-1}$ bwt i.m.) which is superior to other sedatives such as xylazine and acepromazine for obtaining semen by electro-ejaculation (Jochle et al., 1990). Electro-ejaculation can be achieved by using the rectal probe lubricated with a copious amount of jelly, to ensure good contact with the mucosa, and applying electrical impulses of increasing intensity until ejaculation occurs. Two sets of stimulation are generally used, each of 10–15 pulses of 3–4 s duration,

with a rest of 2–3 min between the two series of impulses. The collection is made into a flask held at the prepucial orifice with occasional milking of the prepuce to expel all the semen. The individual responses to the electrical impulses can be very variable and failure to obtain an ejaculate or only get a few sperm cells contaminated with urine and cellular debris is common, hence this method is not recommended.

4. Semen evaluation

Once collected the semen has to be evaluated and the following parameters noted:

Volume: The volume of the ejaculate can be measured directly from the graduated collection tube and it can vary from 2 to 10 ml as there is great variation between males and even between ejaculates from the same male.

Colour: The colour of the ejaculate can vary from a greyish translucent colour, if the ejaculate is predominantly the gelatinous seminal plasma fraction and not very concentrated, to a creamy white colour as the concentration of spermatozoa increases.

Viscosity: One of the main characteristics of camelid semen is its high viscosity which makes handling and estimation of the spermatozoa parameters difficult. This viscosity is usually attributed to the presence of mucopolysaccharides from secretions of the bulbourethral gland or the prostate, but the degree of viscosity depends on the individual male and on the quantity of the gel fraction in the ejaculate. According to some authors the semen will partially liquefy if stored at 25–37 °C for 10–20 min (Abdel-Raouf and El-Naggar, 1976; Musa et al., 1993) but other studies show it can take up to 8 h (Tibary and Anouassi, 1997a). Evaluation of the viscosity can be estimated by measuring the strand formed between the glass slide and a pipette.

Sperm concentration: After an aliquot of the semen is diluted 1:100 or 1:200 in formal citrate the concentration can be measured using a haematocytometer. There are few reports on the normal range of sperm concentrations in camels but it is suggested to be between 200 and 300×10^6 ml^{-1} (Anouassi et al., 1992).

Motility: Motility is best estimated by placing a drop of diluted semen (1:1 in a suitable extender) on a pre-warmed slide and examining it under the microscope. The initial motility can be very low depending on the viscosity of the semen but increases as the semen liquefies, thus a true evaluation can only be made after the semen has liquefied. Other methods of determining sperm motility such as computerized semen motility analyzers have been used (Al-Qarawi et al., 2002) but tend to be difficult in camelids due to the gelatinous nature of the semen.

Sperm viability: Sperm viability is assessed by exclusion of dyes such as Eosin-nigrosin, Eosin-fast green or Propidium iodide. Stained smears are made by placing 1–2 drops of Eosin-nigrosin stain and semen on one end of a slide, then the drops are mixed and smeared forming a thin film. Live sperm have an intact membrane and exclude the stain, while the stain penetrates dead sperm with non-intact membranes. Live sperm are pink and dead sperm purple.

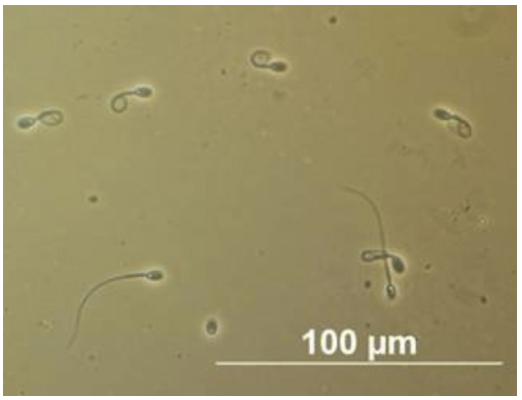

Fig. 1. Dromedary camel spermatozoa after the HOS test. Coiled sperm – intact plasma membrane. Straight sperm – non-intact plasma membrane.

Sperm morphology: Assessment of sperm morphology is made using smears prepared and stained on a microscope slide. A minimum of 100 spermatozoa are then examined under high power magnification (1000×) and assessed as follows: normal, abnormal heads (large, small, tapering, pyriform, vacuolated, double head), abnormal midpiece, (distended or irregular, abnormally thick/thin), abnormal tails (short, multiple, broken, coiled, absent, bent, presence of cytoplasmic droplets).

Sperm plasma membrane integrity (PMI): Sperm PMI is assessed using the hypo-osmotic swelling test (HOS). Neat or diluted semen is diluted (1:4) with a hypo-osmotic solution (Fructose-sodium citrate, 60 mOsm/kg) and incubated (35 °C for 45 min). Sperm with intact plasma membranes will display coiled tails and those with non-intact membranes will have straight tails (Fig. 1).

Sperm acrosome membrane integrity: Sperm acrosome integrity is measured by either Giemsa or FITC-PNA stain. Smears are made with neat or diluted semen, air dried, stained with Giemsa (10%, v:v in ddH_2O for 40 min) rinsed and dried. For FITC-PNA staining smears are made, fixed with ethanol (96% for 30 s) and stained with FITC-PNA (10 μg/mL; 37 °C for 30 min). Slides are then rinsed in PBS and assessed (Morton et al., 2008).

5. Short term semen preservation

Several extenders have been used for dilution of freshly collected camel semen (Sieme et al., 1990) such as skimmed milk-glucose extender (Kenney et al., 1975), Dimitropolous 11, (Rasbech, 1984), Laiciphos (Cassou, 1959), Androhep (Waberski et al., 1989), glucose–EDTA (Martin and Klug, 1979), sodium–citrate-egg yolk (Kupper, 1954) and lactose-egg yolk (Anouassi et al., 1992), and Green buffer-egg yolk (I.M.V. L'Aigle, France; Skidmore et al., 2000). Most of these extenders contain an energy source (glucose or fructose), a protein for cold shock protection (lipoprotein from egg yolk or casein from milk), a buffering system and antibiotics. Once collected the semen is diluted in a ratio of 1:1–3:1 (extender:semen) depending on the concentration of the ejaculate, with warmed (30–35 °C) extender added slowly to the semen. It is better to then allow the semen to liquefy before insemination to aid better mixing

of the semen with the extender and to allow more accurate assessments of concentration and motility.

Sieme et al. (1990) assessed sperm motility to be better in Laiciphos (60%) and Androhep (68%) compared with glucose–EDTA (42%) but no pregnancy results were reported, whereas Anouassi et al. (1992) achieved pregnancy rates of 50% (5/10) with an extender containing 11% lactose and 20% egg yolk. In a subsequent study the best pregnancy rates were achieved with Laciphos + 20% egg yolk (53%; 7/13), although two subsequently aborted, and Green Buffer + 20% egg yolk (47%; 10/21) compared with other extenders such as skimmed milk extender (0%; 0/6; Skidmore et al., 2000). More recently, further studies have compared the extender INRA-96 (I.M.V.) with Green Buffer. Results showed that whereas motility was higher after dilution in Green Buffer (67%) compared with INRA-96 (59%), membrane integrity was higher after dilution in INRA-96 (65%) compared with Green Buffer (56%). However, sperm viability and acrosome integrity were similar for both buffers and pregnancy rates were unaffected by diluent, Green Buffer (34%) and INRA-96 (34%; Morton et al., 2010a).

6. Liquid storage of camel semen

Dilution of semen in the above extenders and keeping at room temperature or 37 °C is only suitable if the semen is to be inseminated within an hour or so of collection. For longer preservation in the liquid form (up to 48 h) semen needs to be cooled slowly to 4–5 °C. Slow cooling can be achieved by putting the tube of extended semen into a beaker of water at room temperature and placing in the refrigerator which allows it to cool to 5 °C over 1 h. Alternatively the semen can be cooled in an equine Equitainer (Hamilton Thorn, Danvers, MA, USA) where the cans used to cool the equitainer are placed in a freezer for at least 24 h prior to use. The semen is then sealed in a plastic universal, wrapped in two thermal blast bags at room temperature and placed within a plastic cup inside the equitainer before closing the lid. The advantage of using an equitainer is that semen can be shipped between farms, or even countries, providing it can be inseminated within 24 h and has a motility of at least 35–40%.

Niasari-Naslaji et al. (2007) investigated the effect of different cooling rates and compared an average slow cooling rate of 0.14 °C/min with a faster cooling rate of 55 °C/min on the viability of chilled semen. Sperm motility was assessed when the specimens reached 4 °C and after 12 and 24 h of storage at 4 °C. The results indicated that the progressive forward motility of sperm cooled at the faster rate was higher (47%) compared with that cooled at the slower rate (31%) after 24 h of storage.

Several authors have investigated the efficacy of various extenders for cooling camel semen but only a few have carried out insemination trials. Deen et al. (2004) diluted split ejaculates with Tris based and Biociphos extenders, cooled them to 4 °C and examined sperm motility at 24, 48, 72 and 96 h. They found that none of the samples diluted in Biociphos exhibited any motility after 24 h, whereas 40% of spermatozoa diluted in Tris were motile after 24 h; however this was reduced to 10% by 96 h. Wani et al. (2008) also used a split-sample technique to investigate the efficacy of five different extenders for storing camel semen at 4 °C for 24–48 h. Semen samples were diluted in tris–tes + egg yolk, tris–lactose + egg yolk, citrate + egg yolk, sucrose + egg yolk, tris fructose + egg yolk before being stored in a refrigerator at 4 °C and examined 24 and 48 h later. The results showed that the highest proportion of motile and viable sperm was achieved with tris–lactose + egg yolk (80–90%) followed by tris–tes + egg yolk and sucrose + egg yolk, but there was a gradual decline in motility in all extenders to <20% by 48 h. Another tris-based diluent (SHOTOR; comprising 214.6 mM tris, 64.2 mM citric acid, 66.6 mM glucose, 49.9 mM fructose) was compared with lactose (10%), sucrose (10%) and Green Buffer for the short-term preservation of Bactrian camel semen at 4 °C for 24 h (Niasari-Naslaji et al., 2006). These authors found that the forward progressive motility of spermatozoa diluted in SHOTOR (65%) and Green Buffer (60%) diluents was superior to those diluted in Lactose (31%) and sucrose (28%). None of these studies, however, reported insemination results.

Other studies have reported pregnancy rates of 25–30% after insemination of chilled semen diluted in Green buffer + 20% egg yolk (see review Bravo et al., 2000) but, this is much less than the 50–55% pregnancy rate reported in camels inseminated with fresh, diluted semen. In a more recent investigation by Morton et al. (2010a) the authors compared Green buffer with INRA-96 for chilling camel semen, and found that whereas motility, membrane integrity and acrosome integrity were similar, sperm viability was higher after chilling in INRA compared with Green Buffer. In comparison with the earlier study their pregnancy rates were higher after AI with semen chilled in INRA (17.6%) than with Green Buffer (0%).

Supplementation with catalase: Although the lifespan of spermatozoa has been prolonged by chilling to 4 °C, fertility results are dramatically reduced after as little as 24 h. Various additives have therefore been added to semen extenders to try and improve sperm longevity and fertility. For example, catalase enzyme, which is necessary for decomposition of the toxic product hydrogen peroxide produced during the course of anaerobic metabolism of spermatozoa (Salisbury and Lodge, 1963), has been added to semen extenders for pigs to try and overcome this increase in production of hydrogen peroxide (Roca et al., 2005). Medan et al. (2008) studied the effects of adding different concentrations of catalase enzyme (0, 250, 500 and 1000 IU/ml) to cooled dromedary camel semen, extended in tris–fructose–egg yolk extender, on semen quality, during storage at 5 °C for 5 days. They showed that the addition of catalase enzyme at concentrations of 500 IU/ml significantly increased sperm motility (from 53.2 to 62.7%) and significantly decreased the percentages of dead spermatozoa (from 22.4 to 16.5%), sperm abnormalities (from 13.4 to 7.9%) and acrosomal damage (from 7.5 to 4.5%). Over the period of 5 days, however, sperm motility decreased from 62.7 to 30.1% and the percentages of dead sperm and abnormalities increased from 16.5 to 42.2% and 7.9 to 26.7% respectively. Moreover the conception rates of female camels inseminated with (i) whole fresh, (ii) extended, cooled, catalase-free and (iii) extended, cooled semen

supplemented with 500 IU/ml catalase enzyme were 46.2, 22.2 and 37.5% respectively which would indicate that the addition of 500 IU/ml catalase enzyme to semen extender can be used to prolong camel spermatozoa survival during storage at 5 °C.

7. Deep freezing of camel semen

The best preservation technique for any semen is deep freezing and storing in liquid nitrogen. This means the semen can be preserved almost indefinitely, can be used even after the male's death and can more easily be transported within and between countries with less time constraints on getting it inseminated before the camel ovulates as is the case with chilled semen. One of the major determinates of the success of AI with frozen semen is the freezing protocol used, of which two important factors are the packaging method and the diluent. The main difference between diluents used for liquid storage of semen and freezing semen is the addition of a cryoprotectant to the freezing extender. A cryoprotectant, such as glycerol, is necessary for the spermatozoa to survive at such low temperatures and the final concentration of glycerol and method of addition are critical to the survival of spermatozoa. In addition, semen can be packaged as pellets, in plastic straws of various volumes, (0.25, 0.5, 4 ml) or in ampoules, and as the packaging method affects both the freezing and thawing rates this will also affect the survival and fertilizing ability of the spermatozoa.

Although various authors have compared different extenders for freezing camel semen and recorded the sperm motility, morphology and viability (Deen et al., 2003; Niasari-Naslaji et al., 2007; Sieme et al., 1990; Zhao et al., 1996), only a few insemination trials have been published, most of which involve Bactrian camels (Chen et al., 1990). In a previous study Sieme et al. (1990) adapted a variety of extenders, used for other species, for use in Bactrian camels. They used a split-sample technique to compare the different freezing methods used for bull, ram, dog, stallion and boar semen preservation and recorded post-thaw morphology, motility and viability of spermatozoa. Their results indicated that the best extender and freezing method for Bactrian semen was a modification of the techniques used for boar semen by Westendorf et al. (1975). This technique uses two extenders, a cooling extender (11% lactose + 20% egg yolk), which is added immediately after collection, and a freezing extender (cooling extender + 6% glycerol + 1.5% OEP-Equex paste), which contains the cryoprotectant glycerol and an emulsifying agent (OEP paste) to help stabilize sperm plasma membranes.

The method they used for diluting and freezing the semen was as follows:*Dilution and cooling of straws:*

- Diluted semen at 25–30 °C (1:1, v:v) with cooling diluent (11% lactose + 20% egg yolk)
- Cooled to 15 °C in 2.5 h.
- A second dilution with cooling diluent to give a sperm concentration of 150×10^6 ml^{-1}
- Cooled to 5 °C in 1.5 h.
- A further dilution to sperm concentration of 100×10^6 ml^{-1} with the above cooling diluent containing 6% glycerol and 1.5% OEP (Equex) giving a final glycerol concentration of approximately 2%.
- Filled 0.25 ml/4 ml straws (Fa. Makrotub, Landshut, Germany) with diluted semen.

Freezing of straws:

- from 58 °C to −120 °C by suspending in liquid nitrogen vapour for 20 min;
- then plunged into liquid nitrogen (−120 °C to −196 °C).

Thawing semen.
Carried out in a water bath

- 0.25 ml straws: 40 °C for 10 s,
- 4 ml straws 50 °C for 40 s.

Using this technique they achieved post-thaw motility rates of 70% but, 48% were morphologically abnormal. No result was recorded regarding the 0.25 vs 4 ml straw packaging method and the semen was not inseminated.

Similar studies have been carried out by Chen et al. (1990) comparing two sucrose-based (SYG) extenders: SYG-2 (12% sucrose + 10% egg yolk and 3.5% glycerol) and SYG -3 (12% sucrose + 20% egg yolk + 7% glycerol) and a packaging method using ampoules.

Their method used for diluting and freezing the semen was as follows:
Dilution and cooling of straws.

- diluted semen (1.5 ml) with a sperm count of over 10^8 was injected into an ampoule.
- It was equilibrated at 37 °C, 20 °C, 10 °C for 10 min each respectively and then stored at 4 °C for 4 h in the refrigerator.
- Only semen exhibiting a motility of over 60% was used for freezing.

Freezing of ampoules:

- The freezing took place on a wire grid placed above liquid nitrogen in 4 steps
- Step (1) 3 cm for 3 min (corresponding to −5 °C)
- Step (2) 2 cm for 2 min (−75 °C)
- Step (3) 1 cm for 1 min (−175 °C)
- Step (4) plunged into liquid nitrogen (−196 °C).

Thawing of semen.

The frozen ampoule of semen was thawed in a 45–55 °C water bath for 30 s–1 min and sperm motility recorded. Only semen with motility above 30% was used for insemination.

Their results indicated that better post-thaw motility, viability, and acrosome integrity were obtained using the SYG -3 extender and after insemination of each female camel twice, at an interval of 24 h, with frozen-thawed semen, they achieved amazing pregnancy rates of 93% (29/31).

In subsequent studies Niasari-Naslaji et al. (2007) conducted experiments comparing the effects of different concentrations of glycerol (4, 6, 8%) on the post-thaw viability of frozen Bactrian sperm. Semen was diluted (1:1) in SHOTOR diluent and cooled to 4 °C over a period of 1 h. An equal volume of cooled freezing media (SHOTOR diluent containing 8, 12, or 16% glycerol) was added over the next 30 min giving final concentrations of 4, 6 and 8% glycerol. The semen was loaded into 0.5 ml straws, placed on a rack standing 4 cm above the surface of liquid nitrogen for 20 min and then plunged into liquid nitrogen. The results indicated that post-thaw viability was better using a final concentration of 6% glycerol compared with 4 or 8%. SHOTOR diluent +6% glycerol was then compared with freezing semen in a combination of Green and Clear Buffers (I.M.V.) using the same method as above, and results showed that progressive forward motility of frozen-thawed spermatozoa was greater using SHOTOR diluent (29.9%) compared with Green and Clear buffers (4.2%).

More recently, studies carried out by Morton et al. (unpublished data) have compared Green/Clear Buffers with lactose, Tris–lactose and Tris–tes diluents for cryopreservation of dromedary camel semen. The semen was diluted (1:1) with the appropriate diluent + 20% egg yolk, cooled to 4 °C over 2 h before being further diluted 1:1 with appropriate freezing diluent + 20% egg yolk + 12% glycerol (final concentration = 6%). It was then loaded into 0.5 ml straws and frozen in a programmable freezer. The semen was thawed in a 37 °C water bath for 1 min and sperm motility, membrane integrity and viability were recorded. The results indicated that the Green/Clear buffers were the best combination to use but, although 50% of spermatozoa were motile after cooling, this was reduced to 35% immediately post-thaw and to 0% 3 h post thaw.

In a subsequent study which compared packaging in 0.5 ml straws to freezing in pellets the semen was diluted and frozen using Green/Clear Buffers as above and then loaded into 0.5 ml straws and loaded into a programmable freezer or frozen as 250 μl pellets on dry ice. The semen was again thawed in a water bath at 37 °C for 1 min (straws) or until the semen had melted (pellets) and the sperm motility, membrane integrity and viability were recorded. The results showed that pellet frozen sperm showed higher 0 h and 3 h post-thaw motility, sperm membrane integrity and sperm viability compared with sperm frozen in straws. However there was no difference in sperm acrosome integrity (Morton et al., 2010b).

8. Induction of ovulation in female camels

As mentioned previously camelids are induced ovulators but when preparing animals for AI mating them to an intact male is not an option. Alternative methods to induce ovulation in dromedaries have been investigated and whereas mating to a vasectomized male, intramuscular injection of seminal plasma or injection with LH-like gonadotrophic hormones (such as GnRH or hCG) will induce ovulation (Chen et al., 1985; Marie and Anouassi, 1987; Musa et al., 1993), manual stimulation of the cervix or intrauterine injection of water or cloprostenol will not (Anouassi et al., 1992; Sheldrick et al., 1992). However, mating to a vasectomized male or injecting with seminal plasma is impractical due to the difficulty of collecting semen and the risk of spreading veneral diseases, which leaves hormonal treatment at the optimal time in the follicular cycle as the best alternative. Previous studies have shown that a single injection of either 20 μg of the GnRH analogue (Buserelin), or 3000 IU of hCG will induce ovulation as long as the camel is treated at the correct stage of the follicular cycle. The results showed that an ovulation rate of approximately 80% was achieved when the dominant follicle measures between 1.0 and 1.9 cm in diameter but this was dramatically reduced to <20% if the follicle diameter increased to 2.0–2.9 cm and no follicle >3.0 cm ovulated (Skidmore et al., 1996). More recent studies indicate that the best time to inseminate is when the mature follicle measures between 1.2 and 1.8 cm in diameter (Skidmore and Billah, 2006a; Tibary and Anouassi, 1997b).

9. Method and optimum number of spermatozoa to inseminate

Cervical insemination. Fibroscopic evaluation of the camel's cervix before and after mating has shown that semen is deposited partly intra-uterine and partly intracervical (Tibary and Anouassi, 1997c). Therefore in AI the semen is generally deposited directly into the uterus, just cranial to the cervix, by means of a manually guided bovine insemination catheter passed through the relatively short, straight camel's cervix. Pregnancy rates of 50% have been achieved after insemination of 300×10^6 live spermatozoa (Bravo et al., 2000) or as few as 100×10^6 (Anouassi et al., 1992) directly into the uterine body. However with a short, open cervix that occurs during oestrous there can be a considerable loss of spermatozoa, due to backflow of semen through the cervix, when it is deposited just into the body of the uterus. Therefore subsequent studies investigated whether better results may be obtained if the semen is deposited at the tip of the horn rather than the body of the uterus.

Deep Uterine insemination. In other species it has now been proposed by several authors that semen be deposited at the tip of the uterine horn based on the fact that the major preovulatory sperm reservoir maybe at the uterotubal junction (UTJ) rather than the body of the uterus or cervical canal (Hunter, 1988). The advantage of deep uterine insemination is that the semen is deposited nearer the UTJ and thus should further reduce the number of spermatozoa needed for successful fertilization (Lopez-Gatius, 2000). The complex anatomy of certain domestic animals, however, has impeded the development of procedures for non-surgical insemination into uterine horns. For example, in sows the corkscrew cervical canal and the length and coiled nature of the uterine horns makes passing a catheter difficult (Martinez et al., 2001). Insemination of camels however is easier as the cervix is shorter and straighter and the uterus less coiled, therefore it is simpler to pass a catheter through the cervix and guide it up the uterine horn *per rectum*.

Another study was therefore carried out to investigate more closely the optimum number of spermatozoa needed and preferred site of insemination (Skidmore and Billah,

Table 1
Pregnancy rates following uterine body or deep insemination of 150, 80 or 40 million spermatozoa.

Method of insemination	Number of spermatozoa inseminated $\times 10^6$ Pregnancy rate %; (no. pregnant/no. inseminated)		
	150	80	40
Deep uterine	43[a] (6/14)	40 (6/15)	7 (1/14)
Uterine body	53[a] (8/15)	7[b] (1/14)	0[c] (0/14)

Columns with same superscript are not significantly different ([aa]$p = 0.8$); whereas rows with different superscripts are significantly different ([ab]$p = 0.014$; [ac]$p = 0.002$).

2006a). A total of 40, 80 or 150 $\times$ 10^6 motile spermatozoa were deposited either just through the cervix into the uterine body or at the tip of the uterine horn ipsilateral with the ovary containing the dominant follicle and the results are shown in Table 1. These results indicate that although a pregnancy rate of 53% or 43% could be achieved after insemination of 150 $\times$ 10^6 motile spermatozoa into the body of the uterus or at the tip of the horn respectively, a pregnancy rate of 40% could also be achieved when a reduced number of only 80 $\times$ 10^6 spermatozoa were deposited at the tip of the uterine horn. However, as only one camel inseminated with 40 $\times$ 10^6 spermatozoa at the tip of the horn conceived it would suggest that perhaps 80 $\times$ 10^6 is the minimum number of sperm needed to establish a pregnancy by deep intrauterine insemination.

10. Timing of insemination in relation to ovulation

One of the most important aspects of insemination in camelidae is its timing in relation to ovulation. The interval from mating to ovulation is not precisely known in camels although based on clinical observations most authors have suggested it is between 32 and 40 h after mating in dromedaries (Marie and Anouassi, 1986, 1987; Tibary and Anouassi, 1996) and 30–36 h in Bactrian camels (Xu et al., 1985). However, in a more recent study where the ovaries of dromedary camels were scanned at regular 2 h intervals from 20 to 36 h after induction of ovulation with 20 μg GnRH (i.v.), the majority of camels ovulated between 26 and 30 h after injection (Skidmore and Adams, unpublished data). This more rapid ovulatory response could be because the GnRH injected intravenously is absorbed more quickly by the pituitary thus causing a more rapid LH preovulatory peak.

To investigate the most appropriate time to inseminate the camels a study was carried out where females were inseminated with 150 $\times$ 10^6 live spermatozoa either at the same time or 24 h after the GnRH injection. The results indicated that whereas 53% of camels conceived if they were inseminated 24 h after GnRH injection, only 36% conceived if they were inseminated at the same time as the GnRH injection (Skidmore and Billah, 2006b). This would suggest that to maximize the chances of conception after AI the camel should be inseminated 24 h after GnRH injection.

Table 2
Results of hybrid inseminations.

Hybrid cross Female $\times$ male	Number of inseminations	Number of conceptions	Result
C $\times$ G	50	2	- aborted d260 - still born d 365
C $\times$ L	48	1	- resorbed d 30
L $\times$ C	30	7	- 3 resorbed d 25–38 - 5 LIVE d 328– 336
G $\times$ C	80	8	- 7 resorbed/aborted d 25–365 - 1 LIVE d 344.

C = camel, L = llama, G = Guanaco.

11. Use of artificial insemination to produce hybrids between New and Old World camelids

Many centuries past Old and New World camelids were one species that originated in North America and then around 30 million years ago they split into two genera *Camelus* and *Lama* (Harrison, 1979; Romer, 1966). Today camels and llamas have some remarkable similarities and some striking differences as a result of their adaptation to different environments. For example, they are all induced ovulators, have a bicornuate uterus in which the left horn is larger that the right and have a diffuse, epitheleochorial placenta. They differ in that the New World camelids are smaller, cloven hoofed and have a dense, fine-wool coat suited to living at high altitudes in the Andes, whereas the camels are larger, have a single broad footpad, less dense hairy coat and are adapted to living in desert environments.

The four species of New World camelids, (llama, alpaca, guanaco, vicuna) can interbreed, and the two species of Old World camelids (Bactrian and dromedary camels) can also hybridize and produce fertile offspring (Gray, 1972), but New and Old World camelids cannot interbreed naturally due to the disparity in size and because they live in different habitats. However, with the use of AI the first

Fig. 2. Rama the Cama; the first hybrid between New and Old World camelids. Born February 1998 at the Camel Reproduction Centre in Dubai, UAE.

hybrids between female guanacos/llamas and male camels have been produced (Skidmore et al., 1999). In these studies semen was collected from male camels and inseminated into female guanacos/llamas, and llama semen was collected and inseminated into female camels; the results are shown in Table 2. The hybrid offspring, Camas, have the woolly coat, nose and nostrils of the New World camelids, whereas the ears and tail are midway in length between camels and llamas and the footpad intermediate between the single footpad of the camel and cloven feet of the llama. Size wise they are larger and stronger than llamas but not as big as camels, and there is no sign of a hump (Fig. 2), therefore they would make useful pack animals in colder climates like the Andes.

12. Conclusions

Opportunities to increase the reproductive efficiency of camels are limited by the long gestation period, short breeding season and the continued use of traditional methods of reproductive management in most breeding herds. These traditional methods make it difficult to ensure an optimum number of females are pregnant at the end of the season and can lead to widespread venereal infections with a consequent decrease in fertility. Artificial insemination is a straightforward procedure in camels and can be used to overcome some of these problems. It also allows more efficient use of your genetically superior males, leading to better quality offspring. Although the results to date may seem rather low to be commercially attractive, AI would also be of use for the international movement of genetics once methods for the cooling and deep freezing of camel spermatozoa are successfully achieved. This would lead to the genetic improvement of Camelidae stock worldwide.

Conflict of interest statement

I confirm that there has been no conflict of interest, financial or otherwise, with any other person or institution whilst carrying out the work in this manuscript.

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