Semen Characteristics and Artificial Insemination in the Bactrian Camel
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X.X. Zhao

Department of Veterinary Medicine, and Department of Animal Science and Technology, Gansu Agricultural University, Lanzhou, Gansu, China.

Summary
Artificial insemination is an important technique to ensure rapid genetic improvement in Bactrian camels and recent new research on the semen characters and artificial insemination is reviewed in this paper. A detailed account of procedures for semen collection, processing and insemination are also included. As there are many unique features of reproduction in these animals, the application of advanced breeding techniques that are routinely used in other domestic species has been slow, and in some cases, are not even applicable. A fuller understanding of the endocrine changes and mechanisms accompanying reproductive processes such as puberty, breeding season and rutting behavior is therefore necessary before we can improve our results from artificial insemination in these animals on the farm, or on studs. In addition, development of techniques to freeze semen from these species is very important and has obvious advantages for breeding, including crossbreeding between species to improve products such as fiber, and to assist in preservation of some of the more endangered wild camels.

Introduction
Artificial insemination (AI) has been the most powerful tool for livestock improvement ever available to the breeder; however, this technique has not been well developed as a routine method for breeding camelids compared with its fast and universal application in other farm animals. This review will summarize our current understanding of semen characters and AI in the Bactrian camel [1-6].

Semen Collection
During the breeding season all anticipated semen donors for AI programs are examined to ensure they are in good condition. Sexual behavior such as libido and the number of females each male mounts is recorded daily, as well as their acceptance of different kinds of artificial vaginas (AV) and dummies. Some incidental irregularities of semen characters may be explained by deviations from normal mating behavior of a donor bull. Only males with excellent reproductive performance that produce high quality semen and have good production potential should be selected for AI. Attempts to collect semen can then be carried out on alternate days, or if necessary daily, but with at least one rest day each week. Semen can be collected reasonably easily using an AV similar to that used for bulls with an inner rubber lining. The male Bactrian camel is not very sensitive to temperature, pressure and lubrication inside the AV, but since copulation can be a lengthy process, taking anything from 3 - 25 minutes, and since the temperature during the winter rutting-season in China is low (as low as -20°C), the temperature inside the AV should be 40 - 41°C. The water-jacketed semen collection flask should also be kept warm at 37°C to avoid cold shock for the sperm. When collecting semen, the male should tease a receptive female in order to establish olfactory contact. The female is then restrained in a recumbent position, so that she cannot stand up when mounted, and the male is lead up from behind. When the male sits down on the female and makes a few thrusts, the operator grasps the male's sheath and directs his penis into the AV, and holds it there by manual pressure at the base of scrotum (Fig. 1). The
male will make several thrusts, interspersed by periods of rest, until ejaculation is completed and the whole process of semen collection, from intromission of the penis into the AV to the completion of ejaculation, can take an average of 4.95 ± 1.32 minutes although it may occasionally take a lot longer (20 - 25 min).

Figure 1. Semen collection from a male Bactrian camel using an artificial vagina. - To view this image in full size go to the IVIS website at www.ivis.org.

Once collected, all further evaluation and handling of the semen should be carried out at room temperature and care must be taken to ensure it does not cool to below 20°C without adding medium or extender. Each ejaculate must be carefully examined and pass a test for semen quality as well as meet rigid motility standards before it can be used for AI. The quality assessment includes firstly, observing the general appearance of the semen, then measuring the volume, pH, motility and concentration as well as examining the morphology of the sperm. Semen collected from mature males trained to serve the artificial vagina should have at least 70% motile spermatozoa, but only the number of progressively motile spermatozoa is included in the calculation for the AI dose [7].

**Semen Characteristics**

- The semen is whitish or creamy in color, is odourless, but has a little gelatinous material and a pH of 7.37 ± 0.06 [5].
- In Bactrian camels the volume of the ejaculate can be highly variable between individuals, but the average volume produced is 4.35 ± 1.86 ml (range 1.0 - 12.5 ml) with 70% of males producing below 7.0 ml.
- The average total number of spermatozoa per ejaculate is 24.32 ± 1.04 x 10⁹ cells, giving a mean concentration of 5.59 ± 1.20 (range 2.2 - 12.5) x 10⁹ sperm/ml.
- On average the total number of motile sperm per ejaculate is 23.10 x 10⁹ sperm with generally 70 - 90% being progressively motile, 5.01% ± 1.52 on average being dead and 4.9% (2.2 - 6.5%) being abnormal.
- The length of the sperm is 51.05 ± 4.54 µm, the length of the head is 7.68 ± 1.64 µm [5].
- The seminal plasma of the Bactrian camel has been analyzed and the concentrations of enzymes and elements are shown in Table 1 [5,8].

**Semen Preservation**

The commonly used extenders for the Bactrian camel semen are glucose, sucrose and lactose based media. In previous studies split semen samples were diluted in different extenders as indicated below. For 100 ml of each extender prepare as shown:

1. **SYG-1** used for Bactrian camel (86.5 ml of 12% Sucrose, 10% egg yolk and 3.5% glycerol) [2],
2. **SYG-2** (73 ml of 12% sucrose, 20 ml egg yolk, penicillin 1000iu/ml and streptomycin 1000 µg/ml) and 7% glycerol (v/v), added after initial equilibration,
3. **Tris-Bull extender** (Tris 30.28g, Fructose 17.0 g, citric acid monohydrate 12.50 g, distilled water added up to 100 ml, 20%(v/v) egg yolk, penicillin 1000iu/ml and streptomycin 1000 µg/ml) and 7% (v/v) glycerol [13],
4. **SCDE** (sodium citratedihydrate 23.2 g, egg yolk 200 ml, glycerol 70 ml, penicillin 1000iu/ml, streptomycin 1000 µg/ml), distilled water added up to 1000 ml) [13],
5. **Stallion extenders** (82 ml of 11% sucrose, 13% egg yolk, 5% glycerol, penicillin 1000iu/ml and streptomycin 1000 µg/ml) [13],
6. **Porcine extender** (77 ml of 5% glucose, 20% egg yolk, 3% glycerol, penicillin 1000iu/ml and streptomycin 1000 µg/ml) [13],
7. **Ram extender** (Tris 4.361 g, citric acid monohydrate 2.388 g, glycerol 6 ml, penicillin 1000iu/ml,
streptomycin 1000 µg/ml), distilled water added up to 100 ml [14].

8. Buck extender (Tris 4.345 g, citric acid monohydrate 2.606 g, egg yolk 3.0 ml, glycerol 6 ml, penicillin 1000 iu/ml and streptomycin 1000 µg/ml), distilled water added to 100 ml) [14].

<table>
<thead>
<tr>
<th>Components</th>
<th>Mean</th>
<th>Components</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride (mEq/l)</td>
<td>173.2±59.11</td>
<td>Copper (mg/dl)</td>
<td>0.131±0.06</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>12.65±4.7</td>
<td>Zinc (mg/dl)</td>
<td>0.826±0.527</td>
</tr>
<tr>
<td>Inorganic phosphate (mg/dl)</td>
<td>12.61±1.2</td>
<td>Iron (mg/dl)</td>
<td>2.8±0.815</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>12.15±9.59</td>
<td>Manganese (mg/dl)</td>
<td>0.054±0.048</td>
</tr>
<tr>
<td>Total lipids (mg/dl)</td>
<td>23.52±11.40</td>
<td>Selenium (mg/dl)</td>
<td>0.121±0.065</td>
</tr>
<tr>
<td>Total nitrogen (g/dl)</td>
<td>2.66±0.04</td>
<td>Fluorine (mg/dl)</td>
<td>1.424±0.079</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>1.31±0.06</td>
<td>Phosphorous (mg/dl)</td>
<td>1.96±1.588</td>
</tr>
<tr>
<td>Dry matter (g/dl)</td>
<td>3.34±1.05</td>
<td>Sulfur (mg/dl)</td>
<td>8.020±5.769</td>
</tr>
<tr>
<td>Fructose (mg/dl)</td>
<td>37.9±0.9</td>
<td>Lactic acid (mg/dl)</td>
<td>11.00±3.02</td>
</tr>
<tr>
<td>Lactic dehydrogenase (u/dl)</td>
<td>8.13±1.62</td>
<td>Potassium (mEq/l)</td>
<td>9.03±8.4</td>
</tr>
<tr>
<td>Alkaline phosphates (u/dl)</td>
<td>25.13±11.13</td>
<td>Sodium (mEq/l)</td>
<td>163.77±13.18</td>
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</tbody>
</table>

Preliminary findings, after diluting the semen in the above extenders, indicated that SYG-2 extenders gave the best results. Extender SYG-2 supplemented with GnRH (50 µg/ml), LRH-A3) is nowadays routinely used as the extender for AI of camels in China [8-11].

When semen is collected into the collection flask it is around 38-40°C and although it is protected against the cold winter temperatures by the water-jacketed collection vessel, it is usually brought into the semen processing room as quickly as possible and placed in a water bath or in incubator at 35 - 37°C. Then the raw semen is mixed with the extender in a ratio of 1:3 or 1:4 (semen: extender SYG-2 without glycerol) or, 1:10 or 1:15 (semen: extender SYG-2, supplemented with GnRH analogue but without glycerol). The diluted semen is gradually cooled to 20°C within two hours to protect against rapid cooling, and then directly placed in a fridge at 4°C for a further four hours before glycerol, also at 4°C, is added slowly in a dropwise fashion. (The volume of glycerol added is 7% of the total volume of the mixture of extender and semen). Exactly 10 minutes after adding the glycerol, each ampoule is filled with approximately 2.0 ml of diluted semen. The actual volume depends upon the number of spermatozoa as at least 3.6 x 10^8 sperm are required per ampoule. This collection may represent more than one ejaculate from a camel. For example, two ejaculates collected in the same morning may be combined after cooling and processed as one collection [10-11]. Our experiments show that it is not necessary for camel sperm to equilibrate in the presence of glycerol; equilibration for at least four hours at refrigerator temperature in glycerol-free extender is desirable then the glycerol should be added just before freezing. Freezing should be accomplished within 6 to 10 hours of collection of the semen. The ampoules of semen are then frozen using a hand-controlled freezing device. The temperature is lowered by controlling the distance between the ampoules and the level of nitrogen vapour so that it takes 6 minutes to cool from 4°C to -196°C. A wire frame with copper netting is placed in a casket containing liquid nitrogen. The ampoules are put on the netting, which can then be adjusted to three levels above the nitrogen surface. The sequence is illustrated in Table 2 [1,3,10].
### Table 2. Freezing sequence of ampoule semen.

<table>
<thead>
<tr>
<th>Freezing steps</th>
<th>Distance from netting to liquid nitrogen surface (cm)</th>
<th>Duration (min)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>3</td>
<td>-5</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>-75</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>-175</td>
</tr>
<tr>
<td>4</td>
<td>in liquid nitrogen</td>
<td>3</td>
<td>-196</td>
</tr>
</tbody>
</table>

After each batch of semen is frozen, one ampoule is taken and thawed, by placing it in a water bath at 50 - 55°C for 1 - 2 minutes and then sperm motility is evaluated. Providing it is above 30% the rest of the batch can be packed, labelled and stored in liquid nitrogen until needed for insemination. When required for insemination, semen is thawed as described above, drawn into a pre-warmed syringe and inseminated immediately.

**Insemination**

Successful insemination of the Bactrian camel depends upon many factors, of which the most important ones include: the quality of the semen used, a female being reproductively healthy and in oestrus, proper storage and handling of the semen and use of proper insemination procedures. Either rectal palpation or teasing is used to detect females at the correct stage of their follicular wave cycle to be inseminated. If rectal palpation is used, the ovarian follicles should be no less than 12 mm in diameter and if the females are teased by males, they should be inseminated five days after first showing sexual receptivity. Females are inseminated in a recumbent position in a comfortable, clean place as an atmosphere of calmness is required. The animals are restrained with ropes as shown in Fig. 2, but no further sedation is required. The rectum is evacuated of feces and the perineal regions are cleaned thoroughly taking care to ensure no feces are wiped between the vulva lips. Insemination is carried out using a porcine insemination catheter that is connected to a pre-warmed syringe containing the thawed semen. Then with a gloved hand in the rectum holding the cervix, the operator directs the inseminating catheter through the vagina (Fig. 3) and into the external os of the cervix so that the semen is deposited at the anterior end of the vagina or in the body of the cervix.

**Fertility Traits with Frozen-Thawed Semen**

The following studies were performed to evaluate the fertility results for frozen-thawed camel semen. Pregnancies were diagnosed by measuring progesterone (using an RIA assay) and confirmed by recording the calvings. The treatment protocols were as follows:

1. Double insemination: Females were inseminated twice 24 hours apart with two ampoules of frozen semen. Each ampoule contained 0.5 ml native semen (diluted to 2.0 ml with extender SYG-2) with $2.975 \times 10^9$ motile spermatozoa and $1.48 \times 10^9$ progressively motile spermatozoa. The first insemination was used for inducing ovulation and the second for pregnancy. Fertility was 95.77% (68/71).
2. hCG injection/single insemination: 1000 iu hCG was injected intravenously (i.v.) into each female to
induce ovulation, and two ampoules of frozen semen were inseminated 24 hours later. All ten
inseminated camels conceived (100%).
3. LH injection/single AI: 200 iu LH was injected i.v. into each female, and two ampoules of frozen
semen were inseminated 24 hours later. Again all ten inseminated camels conceived (100%).
4. Single AI/double dosage: Females were inseminated once with four ampoules of frozen semen. All ten
inseminated camels became pregnant (100%).
5. Single AI/single dosage: Females were inseminated with two ampoules of frozen semen. Four of five
inseminated camels were pregnant (80%).
6. Single AI with 10 times diluted semen with GnRH supplemented extender SYG-2. Each ampoule
contained 2.0 ml diluted semen and 100 µg GnRH analogue. Two ampoules were inseminated and
therefore the total dosage of GnRH inseminated intravaginally into each camel was 200 µg, the total
sperm number was 5.6 x 10^8 with 2.9 x 10^8 being progressively motile spermatozoa. All of the 45
inseminated camels were diagnosed pregnant (100%).
7. Single AI with 15 times diluted semen with GnRH supplemented extender SYG-2: The dosage of
GnRH and the volume of semen inseminated were the same as in Group 6, but the total number of
spermatozoa was reduced to 3.6 x 10^8. Forty-eight out of forty-nine inseminated camels were
confirmed pregnant (98%).

Blood samples were taken from each camel on day 15 after insemination and plasma progesterone was
estimated by direct radioimmunoassay using the commercial radioimmunoassay kits provided by Beifang
Immunological Agent Institute. The minimal detectable concentration was 61 pg/ml, intra- and inter-assay
coefficients of variation were 7.68% (n = 8) and 15.21% (n = 4), respectively. Progesterone levels were below
1.0 ng/ml before insemination, but following mating and in females that conceived, levels rose to 1.6 ng/ml on
day 4, 1.70 ng/ml on day 12, 5.54 ng/ml on day 14 and then kept rising gradually. Progesterone concentrations
declayed to basal levels on day 14 after mating if the ova were not fertilized, therefore any camel with a
plasma progesterone concentration of more than 1 ng/ml on day 15 after insemination was assumed to be
pregnant [9-12] and this was later confirmed with the birth of a calf. Results showed that ovulation in the
Bactrian camel can be induced by artificial insemination of diluted semen, but at least 1 ml of semen is
necessary to induce ovulation [4], otherwise using an extender supplemented with GnRH, or injecting
hormones such as GnRH (200 - 250 µg), hCG (1000 - 1500 iu) or LH (200 - 300 iu) i.v. must be used to
induce ovulation. It should be possible to use semen from one male for 450 inseminations [6]. The use of
frozen semen is effective in improving fertility in the camel. However, the main constraints on the planning
and execution of AI in Bactrian camel rearing in desert regions include: the long distances to be covered, lack
of transportation, poorly managed small holdings and a lack of knowledge regarding AI techniques among
farmers. However, these successful results have increased the farmers' trust for the program, which has given
them the opportunity to improve the quality of their animals. Nevertheless, further progress cannot occur
without the continuous effort and the trust of the farmers' participation in the AI program and considerable
work is necessary to ensure the real application of frozen semen in AI of Bactrian camels.

Conclusion
Opportunities to improve reproductive efficiency in Bactrian camel are limited, not only by the long gestation
period and the short breeding season, but also by the continuing use of traditional systems of reproductive
management in most breeding herds. These age-old methods make it difficult to ensure that an optimum
number of females are pregnant at the end of season and they may also lead to widespread venereal infections
consequently decreasing fertility. The AI technique can be employed to overcome some problems, especially
to impregnate as many females as possible at the start of the breeding season, thereby giving them the best
chance to conceive again as soon as possible after parturition. In addition, the ability to successfully freeze
semen and transport it to several different countries would lead to the genetic improvement of Camelidae
stock worldwide.

References
1. Chen BX, Zhao XX, Huang YM. Freezing semen and AI in the Bactrian camel (Camelus bactrianus). In:
Proceedings of the UCDEC Worshop "Is it possible to improve the reproduction performance of the camel"

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