Old World camel reproduction: nature, current technologies and future prospects

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Abstract:

With global warming now an established reality, the world's deserts are advancing. Only the camel has good prospects of survival as a suitable livestock for projects of sustainable agriculture and animal production under these harsh conditions. In times of ecological crisis the camel is suitable for farming in those dry land areas. However the reproductive nature of camels presents a challenge. Dromedary camels are known for their unique sexual behaviour during the rutting season. They also have natural constraints in the area of reproduction, such as the long period of arrival at puberty for males and females, their restricted breeding season, difficulties in induced ovulation, long gestation periods and inter-calving intervals, and the high incidence of early embryonic death. Developing our understanding of ovarian function and semen nature, and the application of assisted reproductive technologies (ARTs) will enable enhancements in reproduction and genetic improvement in camels. In recent years research has examined ways of overcoming these constrains and increasing the productivity of camels. This paper presents the developments in camel reproductive technologies over the past 20 years, outlining the current state of assisted reproductive technologies and describing future prospects. Among these methods are artificial insemination, *in vitro* fertilization, embryo transfer, sexing, gamete and embryo micromanipulation, genome resource banking, and cloning.

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1. Camels are the best productive livestock under harsh conditions

In the past, the primary use of the camel was for the transportation of goods and passengers in desert and semi-desert areas, while wool, milk, skin and meat were by-products. (Williamson and Payne, (1987)). Whereas, Qureshi (1986) reported that using low-cost diets comparable with other animals give 1.5 kg daily weight gain for males and about 1.0 kg daily for females which mean that camel is an efficient meat producer. Recently, Kadim *et al.*, (2008) reported that the dromedary camel is a good source of meat especially in areas where the climate adversely affects the performance of other meat animals

On the other hand, cattle kept in desert produce milk only during the rainy season, for approximately two months (Kohler-Rollefson, (1994)). Recently, Khanvilkar *et al.*, (2009) described camels as a desert-friendly animal, mentioning that it is an important component of desert ecosystems. The majority of the population of the desert depends upon the camel for meat, milk, hides and also for transportation in the desert. From time immemorial it has been recognized as "the ship of the desert".

2. Camels are suitable animals in conditions of ecological crisis

The camel is an important species uniquely adapted to hot and arid environments (Schwartz, (1992)), and as such contributes significantly to the food security of nomadic pastoral households. This unique adaptability makes the species ideal for

exploitation under the arid and semi arid land conditions. Yagil *et al.*, (1994) reported that camels can lactate under severe drought conditions even when dehydrated and when other milk-bearing animals perish. Pastoralists, those whose livelihoods depend on livestock, have a sophisticated survival system of seasonal adjustment to rainfall, shifting their livestock and, in the case of nomads, their communities, according to the availability of water and forage. However, this fragile relationship of people, livestock and environment is being upset by climate change. Global warming has increased the frequency and severity of drought in marginal areas, such as the semi-arid and arid/desert areas. Recent droughts have resulted in all households losing livestock, especially donkeys, cattle, sheep and goats, which are less hardy than camels. This has increased the prevalence of hunger. Most pastoralists realize that, in order to survive the new reality of more frequent drought, they need more camels. Camels are hardier, because they graze on shrubs and trees that other livestock do not. A camel can also go much further from the water source to access forage; a camel can go for 10 days without water, whereas cattle, sheep and goats need to be watered every 2-3 days. For these reasons, Faye *et al.*, (2008) recommended increasing the distribution of camel populations in areas in the world affected by climatic changes. The camel has always been known as an indispensable bulwark against the frequent droughts and devastating famines that regularly afflict desert peoples (Ahmad *et al.*, (2010)).

3. Present situation and nature of camel reproduction

3.1. The reproductive nature of male camels

Male reproduction, sexual behaviour and seasonality

Camels are known as seasonal breeder animals. Dromedary seasonal sexual behaviour is very variable due to the wide geographical distribution of camels, but generally it is related with the period of low humidity, low temperature and increased rainfall (Gombe and Oduor-Okelo, (1977) and Yagil and Etzion, (1980)). For this reason, recently Grigg *et al.*, (2009) studied the adaptive hypothermia in bull dromedary camels during rut and the availability of this study in increasing reproductive success

Camels are known to exhibit with unique sexual behaviours during the rutting season. However, males were sexually quiescent throughout the rest of the year. Male aggressiveness, and a predisposition to attack other males as well as people, is observed. This behaviour is accompanied with loud vocal gargling and protrusion of a mobile soft palate from the mouth especially, poll gland secretions, flehmen posture, masturbation in the presence of an estrous female or the presence of other males. (El-Bhrawi, (2005))

Semen characteristics

Mosaferi *et al.*, (2005) reported that the volume of Bactrian camel semen ejaculate varied from 1.2 to 26 (mean = 8.2 ± 0.7 ml). The majority of semen samples (83.6%) were milky in colour and dense in consistency. The mean concentration of spermatozoa was 414.8±25.04 X 10^6 cells/ml. The percentage of forward progressive motility of spermatozoa was 62.4 ± 1.57%. The percentage of live spermatozoa was 85.6 ± 1.15%. In India, Hafez and Hafez, (2001) reported for dromedary and Bactrian camels that semen ejaculate volume was 1-12 ml, and total sperm per ejaculate 600 x 10^6 sperm/ml. However, Zeidan *et al,.* (2001) also reported that

semen characteristics for 6-11 years old dromedary male camel during the rutting season was 83.33±0.71% for sperm motility, 10.33±0.4% for dead spermatozoa, 6.83±0.41% for sperm abnormalities and sperm concentration was 431.67±9.11 x 10^6/ml. These results varied significantly with age and type of season. Al-Qarawi and El-Belely, (2004) reported sperm concentration 12±1.3 X10^6/ml., motility 68.2 ± 6.7%, live spermatozoa 73.2 ± 8.3% and abnormalities 3.3 ± 0.6%.

3.2. The reproductive nature of female camels

Ovarian activity

Among different domestic animal species, the camel exhibits a unique reproductive pattern. The she-camel is considered to be an induced ovulator where ovulation is induced mainly by coitus, and follicular growth occurs in regular waves during the breeding season (Musa *et al.*, (1992)). Although the right and left ovaries are of equal function, 99% of pregnancies occur in the left uterine horn (Tibary and Annouassi, (1997)). Sexual activity in female camels has been reported to start as early as 2-3 years of age, but they are not bred until they are 4 years old. The ovarian follicular wave pattern was found to vary considerable between camels and can be divided into three phases: the growth phase of 10.5 ±0.5 days, a mature phase of 7.6±0.8 day and a regression phase of 11.9±0.8 day (Skidmore *et al.*, (1996); Skidmore, (2004) and Skidmore, (2010)).

The main triggers for sexual receptivity and ovarian activities in camels are low climatic temperature, rainfall and better grazing conditions. Outside the breeding season, Skidmore, (2004) reported that mating activity ceases and the ovary becomes inactive or only has a few small follicles. However, reports concerning the beginning and the length of the breeding season are rather conflicting. On the other hand, Vyas *et. al.*, (2004) reported that the she-camel is sexually active throughout the year and during the non-breeding season of the male. El-Harairy *et al.*, (2010) reported in their study that environmental temperature, relative humidity and daylight length seemed to play the major role in the regulation of seasonal ovarian activity in the female dromedary camels. Most recently, Albomohsen *et al.*, (2011) made correlations between metabolic changes in blood serum and biochemical composition of follicular oocyte quality during the peak breeding season

Pregnancy and parturition in camel

The pregnancy period of a female dromedary camel is approximately 12-13 months. The female generally produces one calf at a time. Khanvilkar *et al.*, (2011) described in details the normal signs of camels parturition: Generally parturition occurs in sitting position, swelling of vulva, restlessness, frequent urination; the camel finds a corner or a dark place and cleans it with the help of fore legs. The fore limbs of the young one appear first followed by the head. The navel cord generally breaks by itself when the camel licks her young and the placenta is expelled soon after parturition. The labour pains continue for 5 to 10 hours. The she-camel remains in a recumbent position for a few minutes after parturition. The camel calf stands on its own within 6-8 hrs after birth.

Natural constraints for camels reproduction

Skidmore, (2003) noted that the reproductive efficiency of camels is low due to the short breeding season, the difficulty of collecting semen, the gelatinous nature of the produced semen, the late age of puberty for both males and females, the restricted breeding season, difficulties in induced ovulation, long gestation periods and inter-calving intervals, and the high incidence of early embryonic death.

Studying reproduction and the possibility of genetic improvement in camels depends on developments in camel reproductive technologies and a discussion of the current state of the assisted reproductive technologies (ARTs) and their future prospects. Among these methods are artificial insemination, *in vitro* fertilization, embryo transfer, sexing, gamete and embryo micromanipulation, genome resource banking, and cloning.

4. Current technologies and present reproduction approaches in camel reproduction

Importance of the application of assisted reproductive technologies in camels

The interest in applying modern reproductive technologies in old world camelids has increased dramatically over the last decade. This is due in part to their unique reproductive aspects and the possibility of utilizing their productive potential for commercial application (Faye, (2008)).

Tibary *et al.*, (2005) and Skidmore, (2005) reported that several achievements have been made in recent years in reproductive biotechnologies in domestic animals. Camelids are not as well studied, but could benefit from the introduction of reproductive biotechnologies.

Semen collection

Semen collection from the male camelidae is a very important step for semen evaluation and processing. Several methods of semen collection for different species were modified to adapt to the male camel with respect to the nature of the camelidae copulating behaviour (the sitting position and long lasting mating duration). Tibary and Anouassi, (1997) reported that failure of ejaculation (complete failure, incomplete ejaculation, ejaculation of gel only) is very common and constitutes up to 60% of all attempts of collection. Also, further observations by Deen *et al.*, (2003) showed that male camels ejaculated semen into the artificial vagina in 74.6% of collection attempts. Deen *et al.*, (2001) reported that an average of 24.4% of total collection trials, either have no ejaculates or have ejaculates with azoo / oligospermia. It was concluded that steps must be taken to create and develop methods of training and precoital stimulation practices for the optimization of semen collection in camels. Tajik and Hassan-Nejad Lamsoo, (2008) used camel semen aspirated from epididymal sperm obtained from slaughter houses for further use in ARTs.

Electrical ejaculation

Early studies used the electro-ejaculator to collect camel semen, as reported by Elliott, (1961). Tingari *et al.*, (1986) used the electro-ejaculator; the method uses a lubricated rectal probe inserted into the anus and a 12-volt current is applied for

stimulation of semen ejaculation. Two electric shocks were applied, each of 10-15 pulse of 3-4 seconds duration, with 2-3 minutes rest in-between. Jöchle *et al.*, (1990) reported that the use of the electro-ejaculator is always accompanied with sedation. Musa *et al.*, (1992) recommended a procedure to induce ejaculation by rectal massage of the ampullae before starting collection of semen with the electro-ejaculator. On the other hand, Tibary and Anouassi, (1997) refused the use of the electro-ejaculator for semen collection in camelids because they found that it was very stressful, does not yield a representative sample of semen and does not allow evaluation of sexual behaviour. In addition the technique may be ethically unacceptable in some societies.

Artificial vagina with teaser female

Camel semen was early collected by the use of an artificial vagina using a female teaser by Khan and Kohli, (1973). Tibary and Anouassi, (1997) recommended that great care should be taken to keep the penis within the artificial vagina during collection otherwise semen samples will be contaminated by sand or dirt introduced by the penis. Skidmore, (2004) also reported that care must be taken to avoid the contact of the ejaculate with the inner rubber liner of the artificial vagina, since this has been shown to adversely affect sperm motility, by using a disposable plastic inner liner. Contrariwise, Arthur and Tigani, (1990) reported that collection of semen in camels was impossible by an artificial vagina, due to copulation at ground level and the relatively long copulation time. Deen and Sahani, (2006) reported that collection of semen from camels is considered to be a difficult task because of a long copulation duration, copulation at ground level, refusal to serve the artificial vagina, incomplete ejaculation and sand contamination.

Modified methods for semen collection

Several semen collection techniques have been modified and adopted for camels with varying degrees of success. Homeida *et al.*, (2001) described a safer and more efficient semen collection technique. Semen is collected from underneath the male camel using an under-ground room with a square loop hole in the room to collect semen with a modified bovine artificial vagina through the loop hole using a teaser female. Another modified technique was developed by El-Hassanien, (2003) in which a female camel dummy similar in shape and size to the teaser female is used in a sitting mating position, where the artificial vagina is placed in the end of the dummy. Semen is collected from underneath the dummy, as a well-equipped lab for immediate semen analysis is found under the dummy. It has been noted that using such a dummy overcame most of the camel semen collection problems especially female wounding and the restlessness of the male, and above all it provides safety for the operator and gives high quality semen.

Semen processing extenders for camel semen preservation

In China, for Bactrian camels, the use of semen extender gave the best results specially when supplemented with GnRH (50 µg/ml). This extender is used routinely nowadays as the extender for AI of Bactrian camels, with the highest reported conception rate reaching 100 % (Li and Zhao, (1998) and Zhao, (2000)). As for dromedaries, Skidmore, (2004) noted that the best results on conception rates have been achieved with three commercial extenders, Green buffer (IMV, L'Aigle, France) with 20% (V:V) egg yolk, Laiciphos (IMV) and Androhep or an extender containing

11% (w:v) lactose and 20% (V:V) egg yolk, as compared with other extenders. (El-Bahrawy *et al.,* (2006); Wani *et al.,* (2008); and Niasari *et al.,* (2006)) tried different additives for the improvement of chilled and frozen camel semen for the preservation of the plasma membrane of camel sperms.

Few studies have yet been conducted to determine the minimal number of spermatozoa used for insemination in camelidae. Chinese researchers suggested the use of 400 million sperm in Bactrian camel (Zhao *et al.,* (1994); Zhao *et al.,* (1996)), while for dromedary semen, Skidmore and Billah, (2006) studied the site of insemination as well as insemination doses using 150, 80 and 40 × 10^6 spermatozoa into the uterine body resulted in conception rates of 53, 7 and 0%, respectively, whereas insemination at the tip of the uterine horn resulted in conception rates of 43, 40 and 7%, respectively.

Semen viscosity

The mucoid nature of camelid semen is considered one of the major problems that delay the application of artificial insemination and semen processing (Skidmore, (2003)). Deen *et al.,* (2005) correlated between the high failure of fertilization and the viscous form of camel semen, which may play a role as a sperm reservoir and protect the viability of spermatozoa in the female genital tract by entrapping sperm. Ejaculate viscosity also makes it difficult to handle semen, to determine sperm concentration and motility as well as to dilute semen in extender (El-Zanaty *et al.,* (2004)) thus causing technical problems during filling straws. The high viscosity in camelid semen results in oscillatory movement of sperms (Agarwal *et al.,* (1995); Bravo *et al.,* (2000)). This is due to entrapment of spermatozoa in mucus, and sperm can develop motility only after its liquefaction. El-Bahrawy and El-Hassanein, (2009) indicated that some mucolytic agents may totally eliminate viscosity in camel semen with obvious improvement of sperm forward motility as compared with untreated semen. However, these mucolytic agents may have a deleterious effect on acrosomal integrity after equilibration. El-Bahrawy, (2011) reported that 5, 10, 15 µl/ml of alpha-amylase concentrations were very effective in overcoming seminal plasma viscosity, enhancing the post-thaw forward motility of camel sperm with no significant detectable effect on both acrosomal integrity and abnormalities.

Artificial insemination, hormonal treatments and estrous synchronization

Durrant, (2009) noted that artificial insemination (AI) is the least invasive assisted reproductive technology, and is therefore of great interest to breeders of companion animals, non-domestic, and endangered species. The first offspring from AI in Camelidae was reported in Bactrian camel inseminated with frozen semen collected by electro-ejaculator (Elliott, 1961). Artificial insemination is claimed to have been highly successful in Bactrian camels, but in dromedary camels results have been less encouraging. Deen *et al.,* (2005) reported that artificially inseminated camels resulted in pregnancy rates of 50-60% using fresh diluted semen within 30 minutes of collection. However, they added that conception rate decreased dramatically to 25-30% if semen was not used for 24 hrs. On the other hand, Skidmore, (2003) mentioned that no pregnancy could be established when frozen-thawed semen was used. Likewise, pregnancies were almost nil when diluted-chilled/ frozen-thawed semen was used compared to 40 % when AI was performed using whole semen (Deen *et al.,* (2003)).

For the successful development of an artificial insemination program, several hormonal protocols were used, using PMSG, hCG, LH, GnRH, FSH and progesterone in different combination protocols. Although all these trials are good steps in achieving the purpose, all of them are still missing a complete regime for induction and synchronization of estrus, synchronize ovulation, super ovulation in the she-camel, during the breeding and the non-breeding season.(Deen and Sahani, (2006); Nikjou *et al.*, (2008)). Skidmore *et al.*, (2009a) studied various treatments intended to synchronies follicular wave cycles in dromedary camels by removing the existing follicle of unknown size and replacing it with a follicle capable of ovulating at a known time

In vitro fertilization, *in vitro* maturation and embryo transfer

Embryo transfer (ET) is a more complex and costly procedure than AI, requiring much more specialized equipment and training. Therefore (ET) is recommended for regions where other livestock improvements have already been introduced. In the late nineties, Tinson and Singh, (1998) reported that in camels, ET programs can be used as field programs without necessarily involving high costs. Nowshari *et al.*, (2005) succeeded in achieving a healthily born calf; this offspring was introduced as the first camelid produced following transfer of a frozen-thawed embryo. Nowadays embryo transfer is in use in the Arab world (Khatir and Anouassi, (2006)), for example in the production of racing camels.

Embryo cryopreservation and storage allows the conservation of the full genetic complement of the sire and dam and thus has enormous potential for protecting and managing species and population integrity and heterozygosity. However, the success of applying this technology to wildlife will be dictated by the uniqueness of the embryo of each species (Pukazhenthi and Wildt, (2004)).

In camelids, both ovarian slicing and follicular aspiration have been used for oocyte collection in camels (Abdoon, (2001); Nili *et al.*, (2004)). In dromedaries, aspiration with a needle attached to a syringe or to a vacuum pump resulted in oocyte recovery rates of 31–33%, whereas 94% of the oocytes were recovered by follicle dissection (Nowshari, (2005)). Aspiration produced a recovery rate of 49%, with an average of seven oocytes per female (Khatir *et al.*, (2004)). Wani, (2009a) optimized several protocols for *in vitro* maturation of dromedary camels' oocytes. He also succeeded (Wani, (2009b)) in achieving *in vitro* embryo development using epididymal spermatozoa; further and continuous studies were conducted for different methods for *in vitro* and *in vivo* development (Khatir *et al.*, (2009)) as well as the development of camel embryo cryopreservation (Skidmore *et al.*, (2009b)).

Cloning of camels

Al-Jassim, (2007) reported that cloning is generally inefficient with a small number of viable embryos, so has limited potential for large-scale agriculture. However, it is useful because it offers an opportunity for careful copying of a genetically desirable animal and the making of many more with exactly the same genes. It could be seen, if used at all in the Arab world, as a last chance for endangered species that might otherwise be lost forever. Khatir and Anouassi (2008), embarked on a preliminary assessment of somatic cell nuclear transfer in dromedary camels. Finally, Wani *et al*, (2010) demonstrated the use of somatic cell nuclear transfer to produce the first cloned camelid, a dromedary camel (*Camelus dromedarius*) belonging to the family

Camelidae; a healthy calf, named Injaz, born from the pregnancy by an embryo reconstructed with cumulus cells.

5. FUTURE PERSPECTIVES

Intra-cytoplasmic sperm injection (ICSI)

Improvements in intra-cytoplasmic sperm injection (ICSI) techniques in some domesticated animals could be useful for conservation programs. The importance of intra-cytoplasmic sperm injection (ICSI) to camel reproduction or rare animal preservation appears limitless, as even non-viable sperm in cattle has resulted in the birth of live calves (Goto *et al.*, (1991)). There are other studies and reviews detailing the use of ICSI in horses (Squires, (2005)), cattle (Rho *et al.*, (2004)), sheep (Tibary *et al.*, (2005)), goats (Keskintepe *et al.*, (1997)).

Sexed semen

In 2001, Tinson *et al.*, reported the importance of production of pre-sexed racing camel offspring using embryo biopsy. Sex pre-selection of offspring through the use of sexed spermatozoa has great potential; unbalanced sex ratios, especially excessive male births, can play havoc with small population management of wildlife (Pukazhenthi and Wildt, (2004)). Producing predominantly female offspring is advantageous in accelerating the re-population rate, especially in species that are notorious for slow reproduction (Andrabi and Maxwell., (2007)). Thus, recent advances in sexing mammalian sperm on the basis of differences in DNA content in X-compared with Y-chromosome bearing sperm deserve consideration for wildlife conservation (Pukazhenthi and Wildt, (2004)).

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