# Artificial insemination and ovulation induction in dromedary she-camel

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**Abstract:** Dromedary female camels are known as induced ovulators, which need different treatments for induction of ovulation. In this study, 24 adult female dromedary camels were randomly allocated into four equal groups. Animals of group (1) were introduced to the male for natural mating for two subsequent estrous cycles without any hormonal treatments. The second and third groups of animals were synchronized for ovulation using GnRH, and then animals of the second group were mated naturally while those in the third were artificially inseminated using frozen-thawed semen. In the forth group, estrous females were inseminated artificially after deposition of camel seminal plasma in the uterus for induction of ovulation. In the absence of an ultrasound device in this study, blind artificial insemination depended mainly on rectal palpation of the ovaries. Pregnancy rate was used as an indicator for successful induction of ovulation and fertilization. Raw semen samples (n=30) had 13.45 ± 1.7 ml. volume, 296.3 ± 41.7 X $10^6$/ml. sperm concentration, 64.16 ± 4.3 mass motility, 23.04 ± 2.3 % progressive forward motility, 96.6 ±0.57% intact acrosomes, 5.08± 0.4 % primary abnormalities and 8.08 ± 1.7 % secondary abnormalities. Raw semen samples (n=30) were collected from 6 adult males of 12 years old twice a week, pooled and processed for cryopreservation in 0.5 French straws, using Tris-lactose glycerolated extender with concentration of 75 x $10^6$ sperm/ml. Post-thawing progressive motility of 46.6± 9.2% using slow thawing procedure. The results indicated that during the breeding season, 6 (100%) animals conceived by using natural mating during two subsequent estrous cycles. On the other hand, estrus synchronization and ovulation induction protocols decreased conception rate to 5(83.3%) animals conceived when using natural mating and 3 animals conceived when using cryopreserved semen for insemination. No pregnancies were achieved after intrauterine deposition of seminal plasma for induction of ovulation in estrous females by using cryopreserved semen for insemination. In conclusion: to achieve high pregnancy rates using artificial insemination in camels, a good understanding of the mechanism of induced ovulation and hormonal treatments for ovulation induction must be employed accompanied by using high quality cryopreserved semen. [Nature and Science 2010;8(9):203-208]. (ISSN: 1545-0740).

**Key words**: artificial insemination/ ovulation/ camel/ semen/ cryopreservation.

## 1. Introduction

Artificial insemination (AI) is the least invasive assisted reproductive technology as it circumvents physical or behavioral impediments to natural mating and provides the genetic exchange between populations without transfer of live animals (Durrant , 2009). Opportunities to improve reproductive efficiency of dromedary camels are limited by different constrains as semen characteristics, long gestation, late sexual puberty and maturity, limited breeding season and the mechanism of estrous cycle and ovulation of she-camel (Deen, 2008). Recently, Adams *et al.*, (2009) reported for South American camelids that, to-date, artificial insemination efficiency is low, but progress has been made and viable offspring have been produced through the use of AI in domestic animals using both fresh and frozen semen. About 3–5 AI doses are obtained per ejaculate collected. The libido of male camel is low. Then the AI would reduce the number of females male had to mate. Although these numbers 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 (Skidmore and Billah, 2006). The dromedary camels are considered to be induced ovulators, mainly ovulation induction is initiated by coitus (Homeida *et al.*, 1988). However, mechanical stimulation of the cervix which triggers ovulation in species such as the cat and rabbit, does not induce ovulation in camels (Musa *et al.*, 1990, 1992). There are various stimuli that induce ovulation in family camelidea, but the most common mechanism is mating by an intact or vasectomized male (Fernandez-Baca, *et al.*, 1970), however, the mechanical stimulation only does not stimulate ovulation in all family camelidae.

Progress in semen preservation and related techniques in camelids has been slow in comparison to other livestock species (Bravo *et al.*, 2000). Artificial

insemination (AI) with frozen semen is not however well developed as a technique for breeding camelids, compared to its widespread application in other animals (Zhao *et al.,* 1994). Anouassi *et al.* (1992) reported a pregnancy rate of 50% in camels inseminated by fresh diluted semen within 30 minutes of collection, while, it decreased to 25% when using cold stored semen for 24 hours.

On the contrary, remarkable fertility has been reported for bactrians inseminated with frozen thawed semen (Zhao, *et al.,* 1990 and 1994). Mean pregnancy rate of 95% after insemination with frozen thawed semen was higher than the 65% obtained from natural mating.

Due to effective estrus detection requires time, skills and expenses, these have often been major constrains limiting the wide spread of AI. In addition, the nature of camel herds spreading widely in the desert which makes estrus monitoring very hard to be applied. Therefore this experiment was conducted as a primary attempt to study the ability of insemination of female camels either naturally or with cryopreserved semen doses under different estrus synchronization and ovulation induction regimes during the breeding season.

## 2. Material and Methods

### 2.1 Animals:

Six Dromedary male camels aged 12 years (average live body weight 550 kg) were used in the present study for semen collection, evaluation and cryopreservation. Twenty four adult females with known reproductive history, (8-12 years old and average body weight of 450 kg) were used during the breeding season (December 2003 to March 2004). All animals were fed daily at 9 a.m. on a pelleted concentrate feed mixture with a crude protein content of 14%. Egyptian clover hay as a roughage was also offered throughout the year. The animals were allowed to drink twice daily with the ability of free grazing periods from 4 to 6 hours daily.

The animals were allocated randomly into 4 equal groups. In group 1, animals were introduced to the male for natural mating for two subsequent estrous cycles without any hormonal treatment and served as a control group. The second and third groups of animals were synchronized for ovulation using GnRH (Receptal), and then animals of the second group were mated naturally while those in the third group were artificially inseminated using frozen-thawed semen. In the fourth group, the females were observed for estrus, and those exhibited signs of heat were captured and isolated for artificial insemination using thawed cryopreserved semen, via intra-uterine deposition of camel seminal plasma to induce ovulation (Ratto *et al*., 2005).

### 2.2 Semen collection and evaluation:

Semen was collected twice weekly at 8 p.m. using artificial vagina on camel dummy (El-Hassanein, 2003). Semen was immediately examined by naked eye for color and texture, volume was measured using graduated collecting glass tube. Motility was assessed with a phase-contrast microscope (Leica), with warm stage of $37^0$C. The motility was observed and graded at a power of 40X and an average of 5 fields was obtained to the nearest 5%. The intact acrosomes and primary and secondary abnormalities were examined in a sample of 10µl semen added to 200µl of 0.2 % glutaraldehyde solution using a phase contrast microscope at 800X according to Johnson *et al*. (1976). Concentration was determined using diluted semen samples in sodium citrate (2.9 %) containing 0.02% glutaraldehyde and the samples were counted using a heamocytometer counting slide.

### 2.3 Semen cryopreservation:

Tris-Lactose extender composed of 1:1 mixture of (Tris buffer) and lactose (11%) was used in this experiment. The extender was supplemented with a 3% glycerol, 20% egg yolk and 1% antibiotics in one step freezing method as descried by (El-Bahrawy *et al*., 2006).

Semen was packed in 0.5 ml French straws using an automatic mini-tübe filling and sealing machine (Type MPP133). After the completion of the equilibration period (4 hours), the straws racks were delivered into a programmable freezing unit adjusted at $-140^0$C using liquid nitrogen vapor, kept for 15 minutes at this temperature until frozed and finally were dipped in liquid nitrogen storing tanks. A mini-tübe thawing device was used for slow thawing for the straws at $37^0$C for 40 seconds.

### 2.4 Seminal plasma preparation:

After semen collection, semen was spinned out (18000 X g for 30 min.) and supernatant was aspirated in clean tubes, seminal plasma was stored frozen at $-20^0$C until used.

### 2.5 Estrous synchronization and ovulation induction:

Females of the second and third groups were synchronized for ovulation using 20 µg i.m injection of GnRH (Receptal®, Intervet International B.V., Boxmeer, Holland). Seven days later, each female received 500 µg $PGF_2\alpha$ (Cloprostinol®, Synchromate, Bremer Pharma, GMBH, Germany) to regress any corpora lutea which could have been formed either by

incidence of spontaneous ovulation or due to GnRH treatment (Monaco *et al*., 2009 and Skidmore *et al*., 2009). The artificially inseminated females were injected (i.m) with 5000 IU hCG (Pregnyle®, The Nile Co. for pharmaceuticals & chemical Industries, A.R.E) immediately after AI to induce ovulation (Skidmore *et al.,* 1996 and Bravo *et al.,* 2000).

**2.6 Artificial insemination procedures:**

Six semen straws of $75X10^6$ sperm (2 doses were used for insemination every 12 hours) with a minimum post-thawing motility of > 50% were used for AI using universal AI gun. Immediately after treatment of camels of group (3). Ten ml. of frozen male camel seminal plasma was deposited intra-uterine for ovulation induction immediately in estrous females of group 4. Insemination of camels was applied using rigid insemination catheter through the cervix and guide it into the tip of the uterine horn per rectum as the cervix is short and straight.

**2.7 Estrous detection and pregnancy diagnosis:**

The females were kept in an open yard with a male (always monitored to prevent mating). The female that showed signs of estrus was immediately isolated for artificial insemination. Pregnancy detection was based on rectal palpation two months after artificial insemination or natural mating.

**3. Result Analysis**

**3.1 Raw semen characteristics and semen doses.**

All the collected ejaculates were milky and highly viscous. The average results for ejaculate volume, sperm concentration per ml, the average mass percent, individual forward motility percent and the percentage of detached acrosomes in ejaculates, and primary and secondary abnormalities percent are presented in Table 1.

**Table 1. Raw camel semen characteristics:**

| Semen parameters | Mean ± SE |
|---|---|
| Volume (ml.) | 13.45 ± 1.7 |
| Concentration (sperm/ml) | 296.3 ± 41.7 X $10^6$ |
| Mass motility (%) | 64.16 ± 4.3 |
| Individual forward motility (%) | 23.04 ± 2.3 |
| Intact acrosomes (%) | 96.6 ± 0.57 |
| primary abnormalities | 5.08 ± 0.4 |
| Secondary abnormalities | 8.08 ± 1.7 |

**3.2 Artificial insemination and pregnancy diagnosis.**

Post-thaw motility for cryopreserved semen doses showed a motility of 46.6± 9.2, with a detached acrosome of 7.0± 2.1 %. In the absence of an ultrasound device in this study, blind artificial insemination depended mainly on rectal palpation of the ovaries and on results of pregnancy rates as an indication for successful induction of ovulation. The results presented in Table (2) showed that during the breeding season, 100% (6/6) of the animals conceived using natural mating for two subsequent estrous cycles (Control). Estrus synchronization and induction of ovulation protocol decreased the conception rate to 83.3% (5/6) group (2) and 50 % (3/6) when using cryopreserved semen for insemination (group 3). No pregnancies (0/6) were achieved in group 4 after intrauterine deposition of seminal plasma for induction of ovulation of estrous females using cryopreserved semen for insemination.

**4. Discussion**

The volume obtained value agrees with the results reported by Taha Ismali, 1988; El-Naggar, 1992; Hafez and Hafez, 2001). Mosaferi *et al.,* (2005) reported that ejaculate volume varied from 1.2 to 26 with a mean of 8.2 ml for bactrian camels. The motility percent was determined on basis of gross, but not forward motility. These results are within the ranges previously reported by (Musa *et al,* 1992; Billah and Skidmore, 1992 and Deen *et al.,* 2003). On the other hand, higher motility values were reported by Al-Qarawi and El-Belely (2004) as they reported 68.2 ± 6.7% sperm motility in dromedary camel. Knobil and Neill (1998) reported a range of 100 to 700 X $10^6$/ml., higher concentrations were reported by Hafez and Hafez (2001). Mosaferi *et al.,* (2005) reported a mean sperm concentration of 414.8 × $10^6$ /ml for bactrians, however, Al-Qarawi and El-Belely (2004) reported a mean sperm concentration

of 12 ±1.3 X$10^6$/ml. for dromedaries. Reacted acrosome was reported in similar values of 8.1 % and 7.5% (Merkt *et al.*, 1990; Musa *et al.*, 1992 and Willmen *et al.*, 1992). Zeidan *et al*. (2001) reported 6.83% abnormalities which is nearly similar to results presented in Table (1). However, Al-Qarawi and El-Belely, (2004) reported lower values for sperm abnormalities (3.3%).

The viscous nature of camel semen was always a constrain in AI. Deen *et al*. (2004) concluded that low sperm motility is due to coagulation of semen and entrapment of spermatozoa and a main reason of low post-thaw motility. In the present work, post-thaw motility seemed promising, being 46.6± 9.2 % for individual forward motility percent instead of 23.04 ± 2.3%. This is attributed to the elimination of the viscous nature of camel semen after processing (El-Bhrawi, 2005). Recently, Wani *et al*. (2008) reported that there is always slow liquefaction in the camel semen sample without an added extender as compared with diluted semen reporting that the best liquefaction was observed in Tris-lactose extender.

Results of pregnancy rates are still not encouraging. Low conceptions rates in the 3rd and 4th groups may be mainly due to ovulation failure in artificially inseminated animals are very important issue to be investigated. It was suggested that for the maximum fertility rate, AI should be practiced in combination with hormonal induction of ovulation. According to Chaudhary, (1995), induced ovulation makes artificial insemination more convenient and may offer good prospects to success for artificial insemination programs. Deen *et al*., (2003) found that impregnate female camels inseminated with fresh diluted semen, frozen thawed semen and fresh raw semen after hCG injection to induce ovulation resulted in 0/10, 1/13 and 4/10 pregnancies, respectively. However, with the short open cervix, during estrus there would be considerably greater loss of spermatozoa, due to backflow of semen from the cervix (Skidmore and Billah, 2006). Whenever semen is deposited into the body of the uterus or at the tip of the horn, a great variation in conception rate results were obtained when using ultrasound for follicle detection with a single intravenous injection of 20 mg GnRH, to induce ovulation. The results showed that, insemination doses of 150, 80 and 40 X $10^6$ spermatozoa deposited into the uterine body resulted in conception rates of 53, 7 and 0%, respectively. On the contrary, insemination at the tip of the uterine horn resulted in conception rates of 43, 40 and 7%, respectively.

Intrauterine deposition of seminal plasma (10 ml) in the she-camel failed to achieve ovulatory response and in turn no pregnancies were obtained. This could be the result of absence of a combination of stimuli, including a chemical factor in seminal fluid neuro-hormonal responses and the mechanical stimuli of coitus and pheromonal effects due to the presence of the male (El-Wishy, 1987; Marie and Anouassi, 1987).

Chen *et al*., (1985) noted that a small amount of semen is necessary to initiate ovulation. They also discussed the important effect of Gn-RH, LH or hCG with semen doses. In camels, there are certain prerequisites for AI. Firstly, they are induced ovulators and, therefore, AI involves induction of ovulation prior to insemination. Earlier studies showed that ovulation can be achieved by a single intravenous injection of 20 mg of the GnRH analogue, Buserelin. (Skidomre at al., 1996), and occurs between 28 and 36 h after the injection. Thus, semen was inseminated 24 h after treatment (Skidmore *et al*., 1996; Bravo *et al*, 2000).

More recently, Skidmore *et al*. (2009) studied the various treatments intended to synchronize follicular wave cycles in dromedary showing that two Gn-RH injections, 14 days apart, or two Gn-RH injections, 14 days apart and PG on day 7 after the first Gn-RH injection were the most effective methods to synchronize ovulation rate in dromedary camels at a fixed time interval of 14 days after treatment. It is very difficult to point out the main reason for failure of artificial insemination, but as a whole Deen, (2008) reported low fertility rates for natural mating noting the low fertility rates for this species.

**Table 2: Effect of different hormonal and insemination methods on pregnancy rate percent in she-camels:**

| Groups | No. of Animals | Treatment | No. of Pregnant animals | Pregnancy percent (%) |
|---|---|---|---|---|
| 1 | 6 | Using natural mating for 2 subsequent estrous cycles | 6 | 100 |
| 2 | 6 | Using estrus synchronization and induction of ovulation protocol with natural mating | 5 | 83 |
| 3 | 6 | Using estrus synchronization and induction of ovulation protocol with artificial insemination. | 3 | 50 |
| 4 | 6 | Using intrauterine deposition of seminal plasma for induction of ovulation using cryopreserved semen for insemination. | 0 | 0 |

## 5. Conclusion

Artificial insemination and induction of ovulation may be a promising process thus, taking in consideration the efficiency of the cryopreserved semen doses (post- motility) and site of insemination with good understanding of ovulation mechanisms either during natural mating or through hormonal treatments, specially the detection of the mechanical-hormonal relationship on ovulation.

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**Acknowledgment**

Sincere thanks and gratitude are due to Mr. Demian Youssef Gali, technician of AI laboratory in Maryout Station, Desert Research Center, for his efforts in applying of artificial insemination during this study.

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7/22/2010