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Assisted reproductive technologies in camel reproduction: A comprehensive review

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Abstract
Camels are uniquely adapted livestock species that play a vital role in the economy and survival of human populations inhabiting arid and semi-arid regions. Despite their importance, reproductive efficiency in camels remains relatively low due to distinctive anatomical and physiological characteristics such as seasonal breeding, induced ovulation, viscous semen, and prolonged copulation time. Assisted reproductive technologies (ARTs) provide effective tools to overcome these limitations and enhance genetic improvement, reproductive performance, and conservation of valuable camel germplasm. This review presents a detailed and comprehensive account of camel reproductive anatomy and physiology, followed by an in-depth discussion of major assisted reproductive technologies, including artificial insemination, semen collection and preservation, embryo transfer, cryopreservation, *in vitro* fertilisation, and intracytoplasmic sperm injection. Emphasis is placed on practical techniques, hormonal protocols, challenges encountered, and recent scientific advancements. The review aims to serve as a consolidated reference for students, researchers, and field veterinarians working in camel reproduction.

Keywords: Camel reproduction, assisted reproductive technologies, artificial insemination, embryo transfer, *in vitro* fertilisation, cryopreservation

1. Introduction
Camelids represent one of the most important livestock species in desert and semi-desert ecosystems, where conventional livestock species often fail to survive. Camels make significant contributions to food security, income generation, transportation, cultural practices, and sports, such as racing. Camel milk is gaining global recognition due to its therapeutic and nutritional properties, including high vitamin C content, low β-lactoglobulin concentration, hypoallergenic nature, and higher insulin levels compared to cow milk. These properties make camel milk particularly beneficial for children, diabetic patients, and individuals with milk allergies. Camel milk also helps in the treatment of autism disease found in children.

The global camel population is estimated to be around 41 million, of which approximately 95% are dromedary camels. India harbours a smaller but culturally significant camel population, mainly distributed in arid regions. Despite their socio-economic importance, camels are characterised by low reproductive efficiency, late sexual maturity, long calving intervals, and limited availability of elite breeding males and females.

Conventional natural mating alone is insufficient to meet the increasing demand for improved productivity and genetic advancement in camels. Therefore, assisted reproductive technologies (ARTs) have emerged as powerful tools to enhance reproductive efficiency, facilitate selective breeding, preserve superior genetics, and aid in conservation programs. This review discusses the basic reproductive biology of camels and provides a detailed overview of the principles, methodologies, achievements, and limitations of ARTs in camel reproduction. There are some challenges that have to be faced during ARTs in Camel reproduction.

2. Anatomy of the Female Camel Reproductive System
A thorough understanding of the anatomy of the female camel reproductive system is

essential for the successful application of assisted reproductive technologies. The reproductive tract consists of the ovaries, oviducts, uterus, cervix, vagina, and vulva, each showing specific adaptations.

2.1 Ovaries
The ovaries are paired organs located in the sub lumbar region, typically beneath the sixth and seventh lumbar vertebrae. They are suspended by the broad ligament and enclosed within a well-developed ovarian bursa. The size, shape, and weight of the ovaries vary with age, season, and reproductive status. During the non-breeding season, ovaries appear smooth with small inactive follicles, whereas during the breeding season, they become lobulated due to the presence of growing follicles or corpus luteum. Ovarian weight and diameter increase significantly during pregnancy, reflecting heightened endocrine activity.

2.2 Oviduct
The camel oviduct measures approximately 17-28 cm in length and is characterised by its long, flexuous nature. Unlike many domestic species, the oviduct opens directly into the uterine horn through a conical papilla. This anatomical feature is important for sperm transport, fertilisation, and early embryo movement toward the uterus.

2.3 Uterus
Camels possess a bipartite uterus that is T-shaped, with the left uterine horn being slightly longer and more frequently involved in pregnancy. The uterus is located in the abdominal cavity near the fifth to seventh lumbar vertebrae. The endometrium lacks caruncles, which distinguishes camels from true ruminants and influences placentation patterns.

2.4 Cervix
The cervix is relatively short and firm, containing three to six annular folds. Unlike cattle, the cervix does not show marked differentiation from the uterine body. Its consistency and patency vary with reproductive stage, becoming tighter during pregnancy and more relaxed during the follicular phase. These features are important considerations during artificial insemination and embryo transfer.

2.5 Vagina and Vulva
The vagina measures approximately 25-30 cm in length and is narrow and muscular, especially in young females. The vestibule is separated by a strong muscular band and the hymen. The vulva is small, located below the anus, and lacks a prominent clitoral fossa. These anatomical characteristics influence mating behaviour and reproductive intervention.

3. Reproductive Physiology of Camels
Camel reproductive physiology is unique and differs markedly from other domestic livestock species. These differences significantly influence breeding management and the application of assisted reproductive technologies.

Camels are seasonal breeders, with reproductive activity primarily observed during cooler months when environmental conditions and nutritional availability are favourable. Outside the breeding season, ovarian activity is minimal, characterised by small follicles and irregular follicular waves. (Musa & Abusinenia, 1978) [15]

3.1 Puberty
Female camels generally attain puberty between 2 and 3 years of age; however, breeding at this early age is not recommended. Females should reach at least 70% of their mature body weight before breeding to reduce the risk of abortion and poor reproductive performance. (Molash, 1990) Typically, first mating occurs at around 4 years of age, resulting in first calving at approximately 5 years.

3.2 Oestrous Behaviour
Oestrous behaviour in camels is subtle and variable. Common signs include restlessness, vulvar swelling, vaginal mucus discharge, frequent urination, straddling of hind legs, and receptivity to the male. Oestrus lasts on average 4-6 days but may range from 1 to 21 days. Behavioural oestrus may persist even after ovulation, complicating oestrus detection. The male raised his tail and showed the Flehmen reaction after sniffing her urine and vulva.

3.3 Oestrous Cycle and Ovulation
The oestrous cycle in camels averages 25-30 days and is marked by repeated follicular waves, absence of the luteal phase. Camels are induced ovulators, meaning ovulation occurs only after copulation or hormonal stimulation. Without ovulation, a functional corpus luteum does not form, so there is no true luteal phase. This unique feature requires precise hormonal control during artificial insemination and embryo transfer programs.

4. Assisted Reproductive Technologies in Camels
Assisted reproductive technologies have been progressively developed to address the inherent reproductive challenges in camels. These techniques aim to improve fertility, accelerate genetic gain, and conserve elite germplasm.

4.1 Artificial Insemination
Artificial insemination (AI) is one of the most widely studied and applied ARTs in camels. It enables the optimal utilisation of superior males, reduces the risk of venereal disease transmission, and facilitates controlled breeding programs. The first successful AI in a camel was reported in a Bactrian camel in 1961 (Elliot, 1961) [8]. AI also helps in managing the cross-breeding program between different species of Camelidae.

4.1.1 Semen Collection Techniques
Semen collection in camels presents unique challenges due to prolonged copulation time and the animal's sitting posture during mating. The commonly used methods include artificial vagina (AV), electroejaculation, and a camel dummy fitted with an internal AV. The artificial vagina method is mostly used by practitioners (Skidmore *et al.*, 2018) [23]. There are some problems encountered during collection, such as contamination of the semen sample (Skidmore, 2019) [19]; not all males will accept the AV and male dismount without completing copulation (El-Hassanein, 2017) [7]. The electro ejaculation can also collect semen. There are also problems faced during electro-ejaculation. Likewise, it consumes a lot of time, it contaminates the semen with urine and cellular debris (Tibary and Memom, 1999), it requires a lot of labour, it causes bleeding, injuries and fractures (El-Hassanein, 2003) [6], and it requires sedation or general anaesthesia. Among these, the camel dummy method is considered superior, as it

closely mimics natural mating, yields higher semen volume and motility, and minimises contamination and injury. It was designed to mount an AV inside it, such that the front edge of which is fitted in the same position as the vulva of the natural female (El-Hassanein, 2017) [7]. The only problem is that only one of six males will mount, and it requires training males to mount on a dummy.

Recent research has shown that the frequency of semen collection (once or twice per week) affects sexual behaviour, libido and semen characteristics. Collecting semen over 8 weeks during the camel breeding season shows that males collected once a week had greater libido ($p<0.05$) and greater sperm concentrations compared with males where ejaculates were collected twice weekly collection frequency caused a reduction($p<0.001$) in progressive motility. (Al Bulushi *et al.* 2018) [1]

4.1.2 Semen Characteristics and Handling

Camel semen is characterised by low ejaculate volume and high viscosity, which complicates evaluation, dilution, and preservation. The viscosity is primarily attributed to the presence of glycosaminoglycans and mucin proteins in seminal plasma. Mucin 5B is five times more abundant in seminal plasma samples with high viscosity compared with lower viscosity. (Kershaw-Young and Maxwell, 2012) [11]. Viscosity of alpaca semen is 15 times more abundant than that of ram semen (Kershaw-Young *et al.* 2012) [11]. Various enzymatic (α-amylase, papain), mechanical, and physical methods have been employed to reduce viscosity and improve sperm motility and handling efficiency. El-Bahrawy and El-Hussanein, 2009 [4] showed that α-amylase eliminated seminal viscosity and improved sperm motility post-dilution (46%) with Tris-lactose-based extender. Kershaw-Young and Maxwell 2012 [11] found that Proteases papain and proteinase K could eliminate the viscosity within 20-40 min of treatment. Mechanically by a more prolonged method of dilution can reduce viscosity. Rateb *et al.,* 2016 [18] stated that by ultrasound method reduces the viscosity of semen.

4.1.3 Semen Preservation

Short-term preservation of diluted semen at 4 °C has been successful for up to 24-48 hours. El-Bahrawy *et al.,* 2006 [5] showed for long-term preservation, cryopreservation using tris-based extenders supplemented with egg yolk and glycerol has shown promising results. Optimisation of freezing rates, thawing temperatures, and extender composition remains critical to improving post-thaw sperm viability. Tris-citrate-egg yolk-glycerol extender with 15µl/mL amylase enzyme significantly improved sperm post-thaw motility (61.6%) and decreased acrosomal damage (10.4%). Panahi *et al.,* 2017 [17] studied tris-based extender with plasma egg yolk of six different avian species and camel skim milk for chilled preservation of dromedary camel semen and found that 20% pigeon plasma and 20% camel skim milk could provide a suitable extender for chilled storage of dromedary camel semen. Rates used for cooling and thawing can also dramatically affect the successful result of frozen-thawed semen. Malo *et al.,* 2019 [13] investigated five different freezing rates achieved by placing the straws at either 1cm, 4cm or 7cm above LN for 15 min or 7cm for 5 min, followed by 4 cm for 3 min (7+4), or 4 cm for 5 min, followed by 1cm for 3 min (4+1). Motility was significantly higher for the faster freezing rate (1cm) at 0h post-thaw thaw although at 1h there were no differences in total motility between 1 cm and 4 cm. However, both were better than the slower freezing rate at 7cm or the 7+4 and 4+1 combinations, so it was concluded that a faster freezing rate was beneficial for camel sperm.

4.1.4 Insemination Protocols

Ovulation induction is mandatory before AI due to induced ovulation in camels. GnRH analogues or human chorionic gonadotropin are commonly used. Insemination is performed approximately 24 hours after ovulation induction, with semen deposited deep into the uterine horn ipsilateral to the ovary bearing the dominant follicle (Skidmore, 2019) [19]. Higher pregnancy rates are achieved with increased sperm concentration and precise semen deposition (Skidmore and Billah., 2006) [21]

4.2 Embryo Transfer

Embryo transfer (ET) is a powerful tool for increasing the number of genetically superior females and enhancing herd productivity.

4.2.1 Synchronisation and Superovulation

Successful ET requires synchronisation of donor and recipient oestrous cycles. Hormonal protocols involving progesterone and eCG treatment (McKinnon *et al.,* 1994) [14] or a double dose of GnRH (Skidmore *et al.,* 2009) [20] are commonly used to synchronise the estrus cycles of the donor and recipient. Superovulation protocols using FSH alone or in combination with eCG have produced reliable follicular responses and increased embryo yield.

4.2.2 Embryo Collection and Evaluation

Embryos are collected either surgically or non-surgically, preferably at the morula stage. Non-surgical uterine flushing using Foley catheters is widely practised and less invasive (McKinnon *et al.,* 1994) [14]. Collected embryos are evaluated morphologically and graded based on size, symmetry, and cellular integrity.

4.2.3 Embryo Cryopreservation

Embryos can be preserved using slow-freezing or vitrification techniques. Vitrification has demonstrated higher post-thaw survival and lower cellular damage compared to slow-freezing. However, further refinement is required to improve pregnancy outcomes following the transfer of cryopreserved embryos (Skidmore *et al.,* 2009) [20].

4.3 Transfer of Embryo

There are two methods for the transfer of the embryo: one is Surgical embryo transfer, and the other is a non-surgical method. Surgical embryo transfer in the dromedary and Bactrian camels is done via the left flank laparotomy. The embryo is transferred into the uterine cavity through a puncture made in the exteriorized horn by a Pasteur pipette. The non-surgical technique for embryo transfer consists of placing the embryo directly into the uterine lumen through the cervix using a regular bovine insemination gun.

4.4 *In vitro* Fertilisation and ICSI

In vitro fertilisation (IVF) involves the collection of cumulus-oocyte complexes, *in vitro* maturation, fertilisation, and embryo culture. Oocytes can be obtained from

slaughterhouse ovaries or via ultrasound-guided transvaginal ovum pickup. Collect the COCs from the ovaries by a different method (Fathi *et al.*, 2018) [10]. For USG-guided transvaginal ovum pickup donor has to be stimulated by the eCG or FSH, or a combination. After collecting the oocytes grading of the oocytes was done based on the cumulus layer. *In vitro* maturation is influenced by culture media, protein supplementation, growth factors, and follicle size. Maturation media for dromedary camel oocytes can be supplemented with any FCS (fetal calf serum), EDS (estrus dromedary serum) and BSA without any significant differences in the maturation rates (Wani and Wernery, 2010) [24]. A supplementation of 20 ng/ml of EGF in the maturation medium seems to be optimal and improves the nuclear maturation of dromedary camel oocytes (Wani and Wernery, 2010) [24]. Supplementation of caffeine and 0.5 mg/mL of L-carnitine to maturation media has been reported to enhance the maturation rates and developmental potential of such oocytes in dromedary camels (Fathi *et al.*, 2017) [9]. The time of sperm-egg interaction, the medium utilised, temperature and concentration of the sperm used play an essential role in IVF (Wani, 2021) [25]. Intracytoplasmic sperm injection (ICSI) is an alternative approach, particularly useful when semen quality is poor (Wani *et al.*, 2010) [24].

5. Conclusions and Future Perspectives

Assisted reproductive technologies have significant potential to enhance camel reproductive efficiency and genetic improvement. Advances in semen collection, preservation, AI, embryo transfer, and IVF have improved success rates, although challenges remain due to unique camel reproductive traits. Continued research and refinement of protocols are necessary to make these technologies more reliable, cost-effective, and commercially viable. Wider adoption of ARTs will play a crucial role in sustainable camel production and conservation of valuable camel genetic resources.

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