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The Potential Impacts of Assisted Reproductive Techniques in Camel Development and Future Prospects: A Review

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ABSTRACT

Dromedaries can be integrated efficiently into an extensive and intensive management systems for producing meat and milk, which necessitates application of assisted reproductive techniques for productive improvement. Different assisted reproductive techniques (ARTs) were applied in camels including artificial insemination (AI), *in vitro* embryo production, oocytes and embryo cryopreservation, embryo transfer and somatic cell nuclear transfer (SCNT) as well. The articles concerning ARTs application in camels were used for writing the manuscript and they were collected from google scholar, ScienceDirect, elsevier databases. The success of assisted reproductive techniques in camel species is limited compared to ruminants. The success of AI in camel is low due to differences in superovulation protocols and thick viscous consistency of seminal plasma, which immobilizes the sperm. The superovulation protocols were given variable results according to species and nutritive regimes. The oocyte maturation lasts 44-48 hours, which compromises the developmental competence of the resulting embryos. The resulting embryos were transferred to recipient females or cryopreserved. Furthermore, the germinal vesicle or MII matured oocytes could be used for camel somatic cell nuclear transfer and stem production. It could be concluded that ARTs protocols could be used for improvement of productive and reproductive performances.

Key words: Artificial insemination, ARTs, Cloning and cryopreservation, Embryos, Oocytes.

The world population is projected to reach 9.7 billions by the year 2050 and 10.4 billions by the year 2100 due to decreasing mortality and higher life expectancy. Therefore, the livestock revolution is necessitated due to the increase in demand for animal-sourced foods. Providing milk and meat food for humans is an urgent need through intensification and expansion of camel production, which started some decades ago (Gilbert *et al.,* 2021). Camels are considered the most important animal species adapted to survive in harsh environmental conditions in addition to their abilities to produce meat and milk for humans (Kakar *et al.,* 2008; Djenane and Aider, 2024). The number of camels are approximately estimated to be more than 35 million heads at world level (FAO 2013; Tharwat *et al.,* 2023). Although there is a continuous increase in the total number of camels in the world, the application of ARTs in camel's species is limited to scientific research and is still in its infancy (Skidmore, 2019).

Several studies in the last five decades were carried out to develop the ARTs in mammalian species (Mohammed *et al.,* 2005, 2008, 2010, 2019 a, 2019 b, 2022). The application of modern assisted reproductive techniques in camel species is an urgent necessity to improve camel productivity due to the low reproductive efficiency represented by the long gestation period and seasonal breeding. Camel gestation period ranges from 12.0 to 13.0 months (Almutairi *et al.,* 2010). Camel gestation lengths vary according to parity, month of conception and parturition and dead/live status of calves (Nagy and Juhasz, 2019).

Despite the great developments in modern assisted reproductive techniques in ruminants and other animals (Al Zeidi *et al.,* 2022a, 2022b; AlJubran *et al.,* 2023; Mohammed *et al.,* 2024 a,b,c,d,e,f), they still need to be developed in camels (Fig 1). Camel reproduction is poor compared to ruminant species due to several reasons such as delayed puberty, induced ovulation, seasonal breeding, poor signs of estrus, exclusive left uterine horn pregnancies, longer gestation period and prolonged anestrus following parturition (Derar *et al.,* 2014; Padalino *et al.,* 2016; Nagy and Juhasz 2019). The shape of camel uterus is bicornuate and the left horn is bigger than the right one.

Therefore, researchers adapted ARTs originally developed for ruminants to camels, including superovulation protocols, artificial insemination, culture media, *in vitro* embryo production, embryo transfer procedures and embryonic/somatic cloning (Al-Bulushi *et al.,* 2016). Some other techniques need to be explored in camels as semen and embryo sexing in addition to ovarian transplantation. Therefore, this article was designed to provide a comprehensive overview concerning application of ARTs in camels and their future prospects.

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The article was approved by the ethical committee of King Faisal University [KFU-2024-SEP-EA241000]. The criteria for considering studies for this review article including induction of ovulation, artificial insemination, production of embryos *in vitro*, transfer of embryos and embryonic/somatic cloning of male and female camels were collected form ScienceDirect, Elsevier and Google Scholar databases. Therefore, our targets are to highlight key aspects of ARTs application in camels and its effect on camel reproductive performance.

Oestrous cycle in camels

Full reproductive capacity of she-camels is reached at 3-6 years and they might be bred until 30 years of age (Belina *et al.,* 2021). She-camels are seasonally polyestrous species. The she-camel exhibited oestrous cycles during the short photoperiod but entered to anestrus during the long photoperiods (Purohit *et al.,* 2023). Therefore, she-camels melatonin treatment accelerated the breeding season by 2.5 months (El Allali *et al.,* 2018). In addition to the photoperiod, the environmental factors including humidity, temperature, food and rainfall had impact on onset of camel breeding season of different regions of the world (Ainani *et al.,* 2018). The estrous cycle in camels is incomplete if compared to ungulates. The average length of the estrous cycle has been reported to be 28 days in Sudan, 24.2 days in Egypt and 11-30 days in KSA. This variation in estrous cycle length is due to cyclic ovarian activity and the presence or absence of copulatory stimuli. The estrous cycle consists of proestrous, estrous and diapause phases. The breeding season of she-camels coincides with the rutting of males and both appear to respond to the same environmental conditions. Significant differences in ovarian activity were observed due to months and season. In addition, age had a significant effect on the presence of follicles over ovaries where the she-females of 6-10 year had the maximum number of ovarian follicles (Ashour *et al.,* 2017).

Changes occured during the camel estrous cycle in the level of ovarian and uterine activity and hormonal secretions (Bekkouche *et al.,* 2022; Purohit *et al.,* 2023). Ovarian activity include three stages: follicle growth stage, maturation stage and atresia stage. The follicle growth period lasts about ten and a half days (10.5±0.5 days), during which the follicles grow at a rate of 1 mm per day. The follicles continue to grow at a rate of 1.8 mm per day until reaching the maximum size, which ranges from 10 to 25 mm. The follicle remains at the maximum size for 2-3 days before entering the atresia stage, which lasts about 11.9±0.8 days. The size of the follicle has a positive relationship with the concentration of 17-α estradiol in the plasma. The mature follicle continues to grow to sizes ranging from 25 mm to 75 mm in the absence of ovulation. About a third of anovulatory follicles become hemorrhagic and even partially luteinized, leading to conflicting opinions about female care for superovulation. Anovulatory follicles typically regress within 8-45 days and do not always inhibit the development of a new follicular wave (Anouassi and Tibary, 2013).

Hormonal values of estradiol, LH and progesterone were significantly increased during the breeding season compared to those values during the non-breeding season (Amal *et al.,* 2019). Furthermore, the hormonal changes occurred during the estrous cycle according to follicle development and corpus luteum formation. The concentration of β-estradiol was 26.0±9.0 pg/ml during the follicular phase and the concentrations reached 30.8±5.1 pg/ml when the follicle were at its maximum growth size. The basal concentration of luteinizing hormone during the follicular phase was 2.7±1.2 ng/ml and 4 hours after insemination the concentration reached 6.9±1.0 ng/ml. The concentration of progesterone was low during the follicular

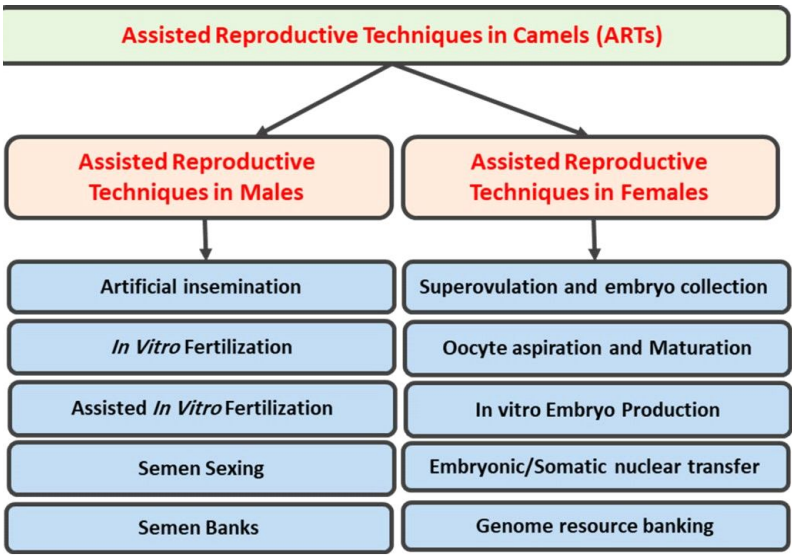

Fig 1: Assisted reproductive techniques in camels.

phase, reaching 0.36±0.28 ng/ml and then increased to 1.73±0.74 ng/ml on the third day after ovulation and then 2.4 ± 0.86 ng/ml on the seventh day after ovulation.

Induced ovulation in camels

The dromedary she-camel is an induced ovulatory species, in which ovulation occurred by manual cervical stimulation, mating with a normal or a vasectomized male. The factors that had been proposed to induce ovulation were visual, olfactory, mechanic and auditory stimuli (El Allali *et al.,* 2017). Some other studies have proposed biochemical components responsible for ovulation induction in semen called ovulation-inducing factor (Ratto *et al.,* 2005, 2006). This ovulation-inducing factor had been recognized from seminal vesicle as a neurotrophin, the β subunit of nerve growth factor (β-NGF) (El Allali *et al.,* 2017). β-NGF appeared to induce ovulation through an endocrine action where it might be absorbed through the endometrium to regulate the LH preovulatory surge. Therefore, administration of seminal plasma to intrauterine or intramuscular was found to induce LH surge followed by ovulation and corpus luteum formation. El Allali *et al.* (2017) suggest that β-NGF is a potent stimulator of GnRH and LH from hypothalamus of camels. Additionally, different hormonal protocols were applied for induction of ovulation in camels including prostaglandin F analogue, GnRH, LH and hCG hormones (El Allali *et al.,* 2017; Khalifa *et al.,* 2020; Ainani *et al.,* 2022). A single injection of 20 mg or 3000 IU of human chorionic gonadotropin (hCG) or GnRH analogue led to ovulation. Ovulation might not occur because these procedures do not stimulate the secretion of sufficient amounts of luteinizing hormone (LH) to cause ovulation. Finally according to Deen *et al.* (2003) and Vaughan and Tibary (2006), since ovulation occurs 28-48h after normal mating, the viscous seminal plasma can ensure the slow release of sperm in the fallopian tube to prolong the time to reach the oocytes.

Camel seminal plasma

Camel semen evaluation is a major challenge due to its thick viscous consistency, which immobilizes the sperm (Fig 2). The viscosity of camel semen was attributed to prostate and bulbourethral glands' secretions, which formed the seminal plasma (SP) and aided in fertilization process (Tibary and Anouassi *et al.,* 1997; Kershaw-Young and Maxwell, 2012; Kershaw-Young *et al.,* 2013). The results suggested that proteins are responsible for camel semen viscosity as mucin 5B. It is a member of the mucin protein family secreted by glandular epithelial cells. Moreover, camel SP contained important proteins for sperm viability and function (Kershaw-Young and Maxwell, 2012). Kershaw-Young and Maxwell (2011) examined the effect of alpaca SP on sperm functions in terms of sperm motility, acrosome and DNA integrity by incubating post-ejaculatory and epididymal sperm in SP at a concentration of 0, 10, 25, 50 and 100%. The study concluded that the presence of at least about 10% of seminal plasma was necessary to maintain motility, acrosome integrity and sperm viability. It has been found that camel seminal plasma improved sperm motility and longevity after thawing (Seyedasgari *et al.,* 2023). Desantis *et al.* (2021) found that camel seminal plasma altered surface glycoprofile of cryopreserved epididymal spermatozoa, which indicated that SP could be included in sperm processing protocols.

Artificial insemination

Artificial insemination in farm animals is an important tool to maximize the use of superior males and ensure rapid genetic improvements (Malafosse 1990). The challenges of AI in camels include difficulties in semen collection and semen handling due to the viscous nature of seminal plasma and semen liquefaction to evaluate and dilute the ejaculates (Skidmore *et al.,* 2018). Therefore, this section will discuss semen collection, the extenders used for storing fresh and freezed semen, site of insemination and timing of insemination and the problems that may hinder the increase of pregnancy rates using camel frozen semen (Malo *et al.,* 2016; Al Jubran *et al.,* 2023).

Different methods were used for camel semen collection including electroejaculation, artificial vagina and camel semen collection kit (CSCK) (Mansour 2023; Niasari-Naslaji 2023). Electro-ejaculation procedure requires heavy sedation of males using intramuscular detomidine hydrochloride injection (80 μg/kg BW). The camel artificial vagina procedure has been described as in other animals (Ziapour *et al.,* 2014). CSCK is a reliable semen collection technique with advantages over artificial vagina and electroejaculation techniques. The volumes of collected semen varied between 2-10 ml. The computer assisted sperm analysis (CASA) procedure was used for camel semen evaluation (Malo *et al.,* 2017a,b, 2018). The manual procedure was found to be more accurate for evaluating semen when compared to CASA method (Köse *et al.,* 2014).

The camel extenders used for semen cryopreservation include commercial egg yolk-free and egg yolk-supplemented tris-based (Abdel-Aziz Swelum *et al.,* 2019). AI procedure with fresh, liquid stored and frozen-thawed semen were either deposited in uterus body or tip of uterine horn (Al-Bulushi *et al.,* 2019). Semen deposition in the uterine body resulted in lower pregnancy rates compared to those deposited in the tip of uterine horn. Insemination of she-camels at the time of ovulation (0h) or 24 h later resulted in higher pregnancy rates compared to those inseminated at 30 h after ovulation. Camel pregnancy rates using fresh semen within 1.0-1.5 h of semen collection and diluted with several types of extenders ranged between 34.0-53.0 (Skidmore *et al.,* 2000; Morton *et al.,* 2013; Al-Bulushi *et al.,* 2019). In conclusion, the success of AI in camels is dependent on semen type and sperm dose, place of semen deposition and timing of insemination over ovulation. The continued progress of camel artificial insemination should be considered a priority due to its many advantages in camel improvement. There are challenges of camel semen cryopreservation. Thus, the AI procedures like semen analysis, semen dilution, freezing and liquefaction procedure, timing of insemination must be standardized with the resulting pregnancy rates.

Embryo production and embryo transfer

Embryo production either *in vivo* or *in vitro* and embryo transfer are assisted reproductive techniques that have witnessed great success in mammalian species. *In vivo* embryo production and transfer require oestrous synchronization, superovulation and embryo transfer techniques (Bó *et al.,* 2019). The main problems associated with camel superovulation include high failure rate of hormonal stimulation, early entry of oocytes into the luteal phase, variations in ovarian response among females and lack of response to repeated superovulation treatments (Manjunatha *et al.,* 2020). Selection and preparation of she-camel donors and recipients are important for successful embryo transfer through ultrasonic examination (Vettical *et al.,* 2019). Twenty percent in general of she-female donors and recipients have reproductive problems. It is preferable to choose females of 5-13 years of age with recently weaned calves. Development and pregnancy rates of *in vivo* produced embryos were higher than those *in vitro* produced embryos (Vettical *et al.,* 2019).

In vitro embryo production (IVP) procedure includes oocyte maturation, oocyte fertilization and culture of fertilized oocyte (Aljubran *et al.,* 2023; Mohammed *et al.,* 2024a, b,c). Oocyte maturation is the most important step for embryo production (Al Zeidi *et al.,* 2022a,b; Aljubran *et al.,* 2023). Oocyte maturation lasts 24h for ruminant oocytes compared to 44-48h for camel oocytes (Mohammed *et al.,* 2024a,b,c). This prolonged time of camel oocyte maturation *in vitro* compromised the development competence of the resulting oocytes (Mohammed *et al.,* 2022). Procedures of camel *in vitro* embryo production is less well-defined compared to those of other ruminant species where blastocyst formation rate was 21.77% (Saadeldin *et al.,* 2019; Wani, 2021).

It is imperative to transfer the embryos before hatching from the surrounding zona pellucida to prepared recipient females. The transferred embryos could be *in vivo* embryos, *in vitro* embryos, cloned embryos, or transgenic embryos. Pregnancy rates and parturition varied after embryo transfer, with the highest rates for *in vivo* embryos produced, followed by *in vitro* embryos produced and then very low rates for cloned embryos (Wani, 2021; Son *et al.,* 2022a,b). On the other hand, there has been no reports on the proven production of camel transgenic embryos yet.

Somatic nuclear transfer in camels

Somatic cell nuclear transfer is an area of interest in the field of stem cell research and regenerative medicine for the past 20 years (Gouveia *et al.,* 2020). Superior animals could be reproduced by somatic cell nuclear transfer (Wani *et al.,* 2017). Camel somatic nuclear transfer was first confirmed in 2010 (Wani *et al.,* 2010). Upon somatic nuclear transfer, the developed blastocysts of reconstructed oocytes could be used for producing embryonic stem cells. However, low efficiency (1-5%) in creating cloned offspring by somatic cell nuclear transfer in addition to abnormalities of cloned animals, were due to failure of epigenetic reprogramming. The recipient cytoplasts, the donor nuclei and culture conditions control the developmental

competence of cloned embryos and feti (Mohammed *et al.,* 2008, 2010, 2019, 2022, 2024a-f). The cytoplast of camel oocytes could reprogram and restore the telomere length in embryos produced by somatic cell nuclear transfer (Wani *et al.,* 2022). Further studies are still required in selecting recipient cytoplasts, donor nuclei and modifying culture conditions for improvement of cellular reprogramming and production of healthy cloned offspring.

CONCLUSION

Camels' reproductive performances can be significantly improved through assisted reproductive techniques including induced ovulation, artificial insemination, *in vitro* embryo production, oocytes and embryo freezing, embryonic/somatic cloning and ovarian transplantation. Future prospective studies are still required for further advancements and improvement.

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Conflict of interest

There is no conflict of interest for authors to declare.

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