OPEN ACCESS
Journal of Animal Health and Production

Review Article

Dromedary Camel (*Camelus dromedarius*) Cloning Using Somatic Cell Nuclear Transfer (SCNT) Current Knowledge and Future Perspectives

Fahad A. Alshanbari[1], Abdelrahman M. A. Elseory[2,3]*

[1]Department of Medical Biosciences, College of Veterinary Medicine, Qassim University, Buraydah 51452, Saudi Arabia; [2]Department of Anatomy, College of Veterinary Medicine, King Faisal University, Saudi Arabia, P.O. Box 400 Al-Ahsa 31982, Saudi Arabia; [3]Department of Anatomy, Faculty of Veterinary Medicine, University of Khartoum. PO Box: 32, Postal Code: 13314 Shambat, Khartoum North, Sudan.

Abstract | The dromedary camel is an important species for milk and meat production as well as transportation, racing, and beauty competitions in many countries around the world. Camel milk marketing has rapidly increased globally in the past decade. Improvement of reproduction technologies such as artificial fertilization is still limited in the dromedary due to physiological difficulties such as sperm collection and ovulation occurring during mating. Superior dromedary camels with excellent genetic backgrounds are essential targets for selecting and cloning due to their superior fitness. Also, dromedary camels with excellent morphological features; as well as production and aesthetic camels are crucial resources for future use as cell sources for camel cloning. Somatic cell nuclear transfer (SCNT) has been achieved in many attempts to produce clone camels. This review highlights current knowledge of SCNT in the species, limitations of the technique, and possible usage of genetic engineering (GE) technology in the dromedary camel.

Keywords | Animals, Somatic cell nuclear transfer, Dromedary, Cloning

Received | February 04, 2024; **Accepted** | June 30, 2024; **Published** | October 01, 2024
***Correspondence** | Fahad A. Alshanbari, Department of Medical Biosciences, College of Veterinary Medicine, Qassim University, Buraydah 51452, Saudi Arabia; **Email:** shnbry@qu.edu.sa
Citation | Alshanbari FA, Elseory AMA (2024). Dromedary camel (*Camelus dromedarius*) cloning using somatic cell nuclear transfer (SCNT) current knowledge and future perspectives. J. Anim. Health Prod. 12(4): 535-544.
DOI | http://dx.doi.org/10.17582/journal.jahp/2024/12.4.535.544
ISSN (Online) | 2308-2801

INTRODUCTION

Dromedary camels (*Camelus dromedarius*) are traditionally important as a source of food and as a means of transportation in many desert regions according to their unique adaptability to harsh environmental conditions (Ali et al., 2009; Wu et al., 2014). In modern times, camel racing sport and camel beauty festivals in the Middle East have led to increased interest in improving camel genetic makeup (Khalafalla et al., 2021). Unlike other domestic mammalian species, dromedaries exhibit low reproductive performance, mainly due to short breeding seasons (Marai et al., 2009). Some other factors also decrease the dromedary reproductive efficiency, such as long inter-calving interval (17-20 months), long pre-pubertal period (4-5 years of age), long gestation period (12-13 months), and long lactating period (12.5 months) (Marai et al., 2009; Ali et al., 2018; Nagy et al., 2022). Controlled breeding techniques, including conventional embryo transfer, artificial insemination, *in-vitro* fertilization, and cloning, have been applied to mammalian species, including dromedaries. Unlike other mammals, dromedary camel-assisted reproduction experienced several problems including variable estrous behaviour and follicular development (Skidmore, 2011; Skidmore, 2018).

Furthermore, semen sampling and semen storage for artificial insemination is more difficult in the dromedary than in other domestic species due to the nature of semen plasma (Skidmore, 2018).

Reproduction organs (testis and ovaries) produce gametes with haploid genetic materials (1n) during which meiosis takes place and recombination events occur (McGeady et al., 2017). The regular way to reproduce is to have two haploid gametes (1n from each of the sperm and the egg) fuse to produce the zygote (2n) (McGeady et al., 2017). On the other hand, normal cells have a diploid number of chromosomes (2n). They divide by mitosis, where no proof of recombination events occurs (Sanchez et al., 2021). Scientists use somatic cell nuclei (2n) of cells cultured from animal tissues in which these cells can be preserved in liquid nitrogen for years or even decades as a source of genetic materials replacing the haploid ovum nucleus (1n) to produce the zygote. This method is called somatic cell nuclear transfer (SCNT).

The first cloned animal was achieved in sheep using SCNT in 1996 (Campbell et al., 1996). After that, cloning in mammalian species has been improved over the years, including cows (Cibelli et al., 1998), mice (Wakayama et al., 1998), goats (Baguisi et al., 1999), pigs (Polejaeva et al., 2000), rabbits (Chesné et al., 2002) and cats (Kitiyanant et al., 2003). Additionally and importantly for our purposes, the dromedary camel, too, has been cloned (Wani et al., 2010). In this review, we aim to review current knowledge of camel cloning, issues related to camel cloning procedure and successes, and possible usage of genetically engineered technologies in the dromedary camel cloning.

Reproduction cycle of the dromedary camel

Dromedary camel life span reaches 29 years and is quite long compared to other mammalian species (Hoffman and Valencak, 2020). Figure 1 shows dromedaries from different ages during the dromedary life cycle. Farrag and others showed that dromedary camel maturation takes 4-5 years (Farrag et al., 2019). Dromedary camels are seasonal breeders that start their season in late fall until late spring with 5 to 7 month intervals depending on the environmental temperature and can be influenced by other factors such as rain and the brightness of the sun (Skidmore, 2011). Females ovulate only after mating as they are induced ovulators. The fertility rate was estimated at 60% and 50% of Saudi Arabian and Algerian dromedary females, respectively (Ali et al., 2018; Gherissi et al., 2020).

The first reproduction calf takes 12-13 months, in which the female camel's age will be 5-6 years after the gestation period. The second reproduction cycle usually goes to the following year, when the female will be 7-8 years of age. In some cases, however, the female can reproduce in the same rutting season because the birth of the first calf is at the beginning of the season (Ali et al., 2018). If the female fails to conceive successfully or fails to get pregnant in the rutting season, breeders must wait until the next season, which is five months at least.

Other factors affecting pregnancy might also lead to delays in the reproduction cycle of the dromedary (Ali et al., 2018). This delay adversely affects breeders due to the long period of waiting. Alternative reproduction technologies are available, such as *in-vitro* embryo transfer and cloning. Even though these techniques are costly and limited, some breeders are still interested in using them mainly because they want to reproduce dromedaries from certain precious individuals.

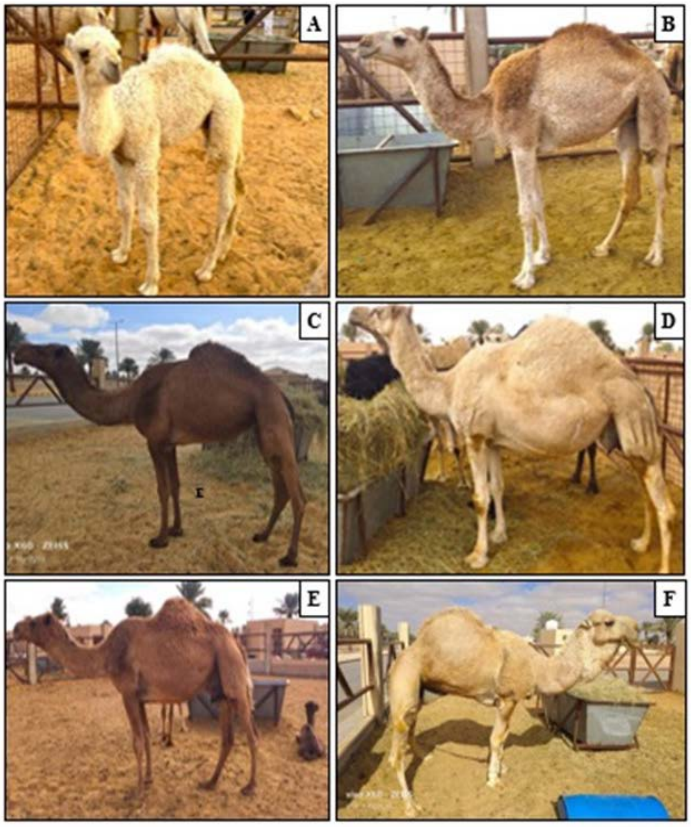

Figure 1: Different ages of dromedary camels representing the simple life cycle of the dromedary. A: 3 months old calf; B: 2.5 years old juvenile (immature male); C: 4.5 years old female (unmated female); D: 12 years old pregnant female (8 months pregnancy); E: 8 years old female with newborn calf and F: 18 years old fertile bull in reproduction season.

Steps to clone a dromedary camel

Cloning a camel is a complex process that requires the availability of somatic cells derived from cell culture created from a donor camel, a dromedary mature oocyte collected from dromedary females, and/or a dromedary female in the gestation period as a recipient (Figure 2). This complexity underscores the need for expertise and knowledge in the process.

Procedure of SCNT

Selection of cell donor and cell type

This is one of the most critical steps because the newborn cloned will be identical to the donor camel. In this step, it is vital to select a highly valuable dromedary (or other camelid species) that has a documented record of achievement, has a highly productive phenotype, or has beauty characteristics (Saadeldin et al., 2019; Son et al., 2021b). A tissue sample is generally obtained from the skin or other organs to generate a fibroblast cell line. Usually, skin is preferred because it is easier to take a biopsy, less harmful to the animals, lowers the risk of infection, and heals quickly (Son et al., 2021b).

Once the tissue was obtained, cell culture was established using cell culture protocol, that was reviewed elsewhere (Segeritz and Vallier, 2017). Briefly, the tissue biopsy was washed several times. Then, it was transferred to a flask with a cell culture medium, 10% fetal bovine serum, and antibiotics. Subsequently, the flask was incubated in a 38°C incubator supplied with humidified 5% CO_2. Cells will start replicating out of the tissue, producing a fibroblast cell line, and once the confluency of the cells reaches 25%, tissue biopsy can be removed and stored for future use. Usually, cells grow for two passages before being used in the following steps. Cells were removed from the flask to a tube and stored in liquid nitrogen as a source of nuclear donors.

Oocyte collection

Oocytes were collected from either slaughterhouses or lived dromedary females in all reports of camel cloning, as described below. Firstly, the *in-vitro* method, an efficient process, involved collecting ovaries from the slaughterhouse. These collected ovaries were kept in a normal buffer solution until they arrived at the laboratory. Then, follicles were picked with a diameter of at least 2 mm. After that, the follicles were transferred to cultured media incubated at 38°C and supplemented with polyvinylpyrrolidone (PVP) to reach the maturation phase (MII oocyte). Secondly, oocytes collected from fertile dromedary females (*in-vivo*) were observed by injecting the females with stimulation hormone to produce multiple mature oocytes. The widely used hormones were follicular stimulating hormone (FSH) and gonadotropin hormone (GnRH). Initially, the dromedary females were injected with FSH to enhance ovaries for producing multiple mature oocytes. Beyond that, after 9-10 days of the first injections, the females were injected with GnRH to release the mature oocytes. After one day of the GnRH injection, mature oocytes were picked using single-lumen needles or similar tools. Subsequently, mature MII oocytes were incubated at 38° C and supplemented with PVP for one day to reach their maturity size.

SCNT

Once all materials are ready, the process of SCNT can begin (Figure 2). Before starting the procedure, the mature oocyte was treated by removing cumulus cells, zona pellucida, and the first polar body surrounding the ovum. Removal of the ova constructions was done by gently pipetting the oocyte with hyaluronidase or similar enzymes, which was required to ease the oocyte enucleation and SCNT.

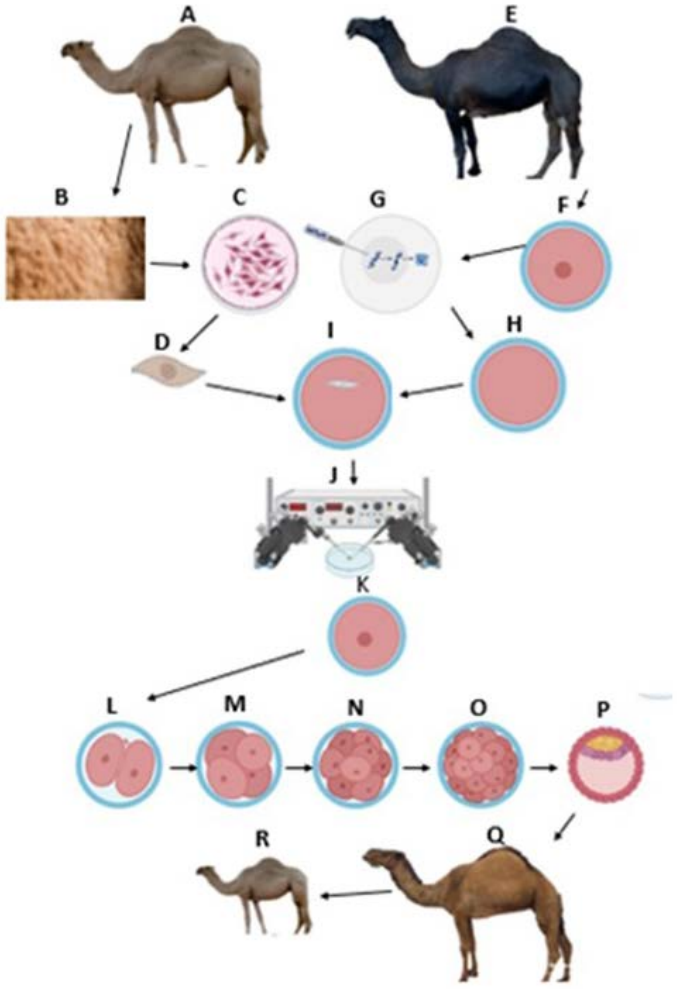

Figure 2: A: A donor dromedary to be cloned; B: Skin tissue; C: Fibroblast created from skin tissue; D: A fibroblast cell picked from the cell culture; E: Dromedary female; F: Oocyte picked from the female; G: Microneedle used for enucleation; H: Insertion of the fibroblast into the oocyte; I: Insertion of the picked fibroblast into the oocyte; J: Electroshock to activate the cloned oocyte and fibroblast; K: Cloned zygote; L: 2 cells cleavage; M: 4 cells cleavage; N: 8 cells cleavage; O: Morula; P: Blastula; Q: Transfer blastula to the recipient; R: Cloned embryo that is identical to A.

Nucleus removal

Oocyte enucleation (miotic spindle complex) was achieved using a micropipette under the microscope after staining the nucleus with Hoechst stain (Figure 2). One issue accompanied by enucleation was the removal of a portion of

the oocyte cytoplasm. Moulavi and Hosseini accomplished a handmade protocol to enucleate the genetic materials from the ova using a disposable micropipette (Moulavi and Hosseini, 2019). This protocol assisted in removing the ova genetic materials and reduced the cytoplasm materials. Related issues to minimize the amount of the oocyte cytoplasm are discussed below.

SCNT of donor nucleus to enucleated oocyte

Fibroblast cells generated from a donor dromedary were used to transfer their genetic materials to the enucleated oocyte. For this step, a fibroblast was picked and attached to the oocyte wall in a plate with normal solution media. Subsequently, in the media, the oocyte and fibroblast were fused using electrophoresis or chemical stimulant materials. In this step, the genetic material from the donor cell was activated to start developing in the enucleated oocyte and producing a cloned zygote (Figure 2).

Cleavage and blastocyst formation

Somatic cells undergo reprogramming after the artificial activation; an ova contains genetic factors that initiate cell proliferation. Subsequently, cleavage (an embryonic stage after the ova nucleus fuses with a sperm nucleus in a regular reproduction path) started by dividing the zygote cell (Figure 2). Embryos were examined with a microscope to ensure cleavage was initiated. Once the developed cloned embryo reached the morula stage, cells started forming a blastula in which the first cell specialization was initiated in the developing embryo (Figure 2). Cleavage and blastocyst formation took 6-7 days after SCNT. The embryos were then ready to be transferred to the recipient (Figure 2).

Embryo transfer and pregnancy examination

A group of dromedary females was chosen to receive the cloned embryos (Figure 2). First, they were injected with prostaglandin F2 alpha to prepare the uterus for adapting the blastocytes. Then, blastocytes were transvaginally transferred to the left horn of the synchronized recipient's uterus. Chemical pregnancy tests were preferred to examine pregnancy 15 days before embryo transfer using blood or urine samples from the recipients and available commercial pregnancy kits. After 30 and 90 days of Embryo transfer, an ultrasound diagnosis could be performed for pregnancy examination. After 90 days, abortion could be seen if a pregnant recipient loses the fetus. Subsequently, the cloned calf will be born after a gestation period of 360-390 days post-embryo transfer.

Dromedary camel cloning

The first successful cloning of the dromedary camel was reported in 2010 (Wani et al., 2010), in which the first live cloned dromedary was born in 2009. Thus, attempts at dromedary camel cloning started before 2008 when few trials failed to clone a dromedary (Khatir and Anouassi, 2008; Wani et al., 2008). In the past decade, however, cloning techniques and methodology have been improved significantly to clone a mammalian species in general and the dromedary in specific (Wani and Hong, 2018; Czernik et al., 2019; Moulavi and Hosseini, 2019, 2023). Subsequently, successful dromedary camel cloning was achieved in several attempts along with improving the results (Table 1) (Wani et al., 2018; Wani and Hong, 2018; Vettical et al., 2019; Moulavi et al., 2020; Hossein et al., 2021, 2023; Olsson et al., 2021; Son et al., 2021a).

Table 1: A comprehensive summary review of published reports about the dromedary SCNT.

Cell source	Oocyte source	SCNT	Cleavage	Blastocyst	number of produced cloned camel	Publication
Fibroblasts from skin, granulosa cells	*In vitro*	732	385	106	0	(Khatir and Anouassi, 2008)
Granulosa cells	*in vitro* matured from slaughterhouse	380	89	12	0	(Wani *et al.*, 2008)
Cumulus cells, skin fibroblasts, fetal fibroblasts	*in vivo*	363	265	139	2	(Wani *et al.*, 2010)
Skin fibroblasts	*in vivo*	92	77	28	1	Conference
Skin fibroblasts, cumulus cells	*in vivo*	329		104	8	(Wani and Hong, 2018)
Skin Fibroblast	*in vivo* and *in vitro*	222	148		4	(Wani *et al.*, 2018)
Skin fibroblasts, cumulus cells	*in vitro*	350	77	49	2	(Moulavi and Hosseini, 2019)
Skin fibroblasts	*in vivo* and *in vitro*	331			7	(Moulavi *et al.*, 2020)
Skin fibroblast	*in vivo* and *in vitro*	1033	560	442	19	(Olsson *et al.*, 2021)
Skin fibroblast	*in vivo* and *in vitro*	329	234	96	7	(Hossein *et al.*, 2021)
Skin fibroblast	in vivo	195	154	55	4	(Son *et al.*, 2021a)

One of the subjects discussed the source of oocytes used in the study. Dromedary oocytes for many of these studies were collected from either ovaries of camels from slaughterhouses or live dromedaries using stimulated hormones to release mature oocytes. These studies showed a significant difference between oocytes collected from live dromedaries and oocytes collected from the slaughterhouse (Wani et al., 2018; Wani and Hong, 2018; Ali et al., 2019; Vettical et al., 2019). Several factors impact *in-vitro* oocyte maturation (after oocyte collection in humans), such as weak responses to medium components to reach secondary oocyte (Yang et al., 2021). This weak response to hormones and growth components might be a significant reason for reducing successful embryonic development. One of the primary responses is the activation gene expression of certain genes as a response to a hormone or an activation factor, as discussed below (Salhab et al., 2013). However, collecting oocytes from the slaughterhouse has benefits, such as collecting oocytes requiring only a few steps compared to in vivo collection. Furthermore, more oocytes can be collected from ovaries that will be disposed of as waste (Landeo et al., 2022).

One issue to be considered is that many reports used duplicate transfers, meaning they transferred two or more blastocysts to one recipient. However, dromedary camels are monotocous animals, so twin births are naturally rare (Hossein et al., 2023). In contrast, they ensure that at least one blastocyst is implanted and developed successfully. Recent reports have shown that many successful clonings have been achieved, mainly due to improvements in the cloning methodology (Table 1), thus the percentage of successful cloning still has room for improvement.

Camelid cloning using dromedary camel as a host

Interspecies cloning within the family of camelids has been demonstrated by the successful cloning of a Bactrian camel in a dromedary camel (Wani et al., 2017). This achievement showed the possibility of saving the extinction of endangered camelids such as the wild camel (*Camelus ferus*). As it is known that all camelid species have 74 chromosomes and also have similar karyotypes (Avila et al., 2014; Wu et al., 2014), it was possible to use a cell source from one species and clone it in another species. Even interbreeding between camelid species was applied, and a hybrid camel was reproduced (Cebra et al., 2014). In addition, the dromedary and llama have been hybridized and reproduced a hybrid (Jones et al., 2008). These similarities can benefit the species as wild camelids face extinction due to climate change and a decrease in habitat due to human population growth and poaching.

Issues related to camel cloning

Reproducing mammalian clones has been done for the past two decades. However, the number of oocytes used to clone a mammalian species is considerably high, whereas the number of reproduced clones is low (Gouveia et al., 2020; Malin et al., 2022). Even though there is a significant improvement in the methodology, the number of successful clonings, including dromedary camels, is still lacking (Table 1). For example, Hossein and colleagues used 640 oocytes, where only seven individuals were born alive (Hossein et al., 2021). Furthermore, all other reports have shown lower efficiencies in camel cloning. Scientists have been investigating the reason behind the low efficiency of SCNT. Their investigations include the donor cell, source of the oocyte, abortion rate, and developmental abnormalities. Thus, one of the major problems that causes embryonic development failure is epigenetics (Gouveia et al., 2020).

Genetic regulation and mechanisms are critical in embryonic development for generating different cell types and organs. Epigenetic programming means that each cell expresses specific genes depending on the cell type, whereas the remaining genes in that cell remain silent or not expressed (Basu and Tiwari, 2021). These genes are repressed due to histone modification, DNA methylation, X chromosome inactivation, and genetic imprinting. Moreover, embryonic development requires a series of cell proliferation and then cell differentiation. These changes are necessary because the morula is generated in the first days of cloned embryos. Then, the first cell differentiation happens by forming the blastocyte (inner cell mass (ICM) and trophoblast). Subsequently, the major part of the ICM forms the first three embryonic lyres (ectoderm, mesoderm, and endoderm) from which all other organs form. The minor part of ICM and trophoblast form the placenta. These changes in cell type during embryonic development require epigenetic reprogramming in which the cell goes from a general embryonic cell to a specific cell.

One key factors is DNA methylation. In SCNT, DNA methylation and re-methylation do not interact with genetic factors in the oocyte resulting in failing embryonic development (Basu and Tiwari, 2021). This failure is due to the lower response of the SCNT zygote compared to the normal fertilized zygote.

Another epigenetic factor is histone remodeling and chromatin structure, which regulate gene expression (Basu and Tiwari, 2021). Chromatin factors regulate gene expression, which gives accessibility to express certain genes. One of the key enzymes is DNase I hypersensitive site, which interacts with specific DNA sequences to activate certain genes that behave regularly in normal embryos but not in SCNT embryos due to the specialized genome of somatic cells which already have been accomplished.

Histon regulation is controlled by histone deacetylase (Hdac) and histone acetyltransferase (Hat) proteins (Basu and Tiwari, 2021). This gene promotes gene inactivation and activation, respectively. Histon modification has a decreased impact on SCNT due to lower Hdac and Hat enzymes, which leads to failure of cell proliferation and cell specialization in developed cloned embryos.

Another factor that affects the SCNT clone's development is imprinting, in which paternal or maternal genes are expressed (Basu and Tiwari, 2021). These expressions balance the fetal gene expression, which is essential for embryonic development and is critical mainly after fertilization, where specific genes are expressed and cause developmental defects due to an imbalance in gene expression. Developmental defects such as placenta malformation, organogenesis and embryo abortion; result in the death of the embryo. However, in normal fertilization status, and during gametogenesis, recombination events occur in which paternal and maternal sister chromatids exchange genetic materials. This exchange will erode the normal imprinting in the natural cells reproducing gametes and will produce a new imprinting status for normally developed embryos.

In female mammals, X chromosome inactivation occurs randomly during embryonic development (Basu and Tiwari, 2021). Briefly, females have two X chromosomes, and only one is active. The paternal X chromosome is active in some tissues, whereas the maternal X chromosome is active in other tissues. Their Xist gene is expressed during normal embryonic development. However, in SCNT-developed embryos, the Xist gene is already activated, leading to an imbalance in genetic variation, affecting the embryo and placenta development.

All these genetic factors cause genetic defects and abnormalities. Epigenetic reprogramming adversely impacted the SCNT embryo development leading to a reduction of successful cloning of dromedary camels and other mammalian species. However, the reprogramming of somatic cells has been reported to enhance genetic remodeling and increase the efficiency of SCNT (Gouveia et al., 2020). It has also been seen that histone deacetylase inhibitor (HDACi) decreased DNA methylation and improved SCNT fetal development. HDACi showed an increase in the cleavage rate and blastocyst formation. Improving the quality of the cleavage and blastocyst formation played a significant role in enhancing the efficiency of SCNT in the dromedary camel. These techniques have not been used in the dromedary, thus, genetic studies have shown few genes associated with different phenotypic traits in the species.

Possible usage of advanced technologies to genetically engineered dromedary camels

Genetic engineering (GE) has been used in domestic animals to increase production and pharmaceutical products in livestock (Klinger and Schnieke, 2021). This makes it possible to use different genetic variations of species or xenogeneic between species to engineer the genome of the somatic cell genetically. The technique was first developed in 1980 by microinjection of purified DNA into a mouse genome (Gordon et al., 1980). After the successful transformation of genetic material into mice, mammalian genetic engineering entered a new era. One of the more recent techniques is clustering regularly interspaced short palindromic repeats (CRISPR) that are easy to design (Uddin et al., 2020). CRISPR can target a specific gene of interest and edit the sequence of that gene. The method was applied to somatic cell culture, where CRISPR was introduced to the cell line (Navarro-Serna et al., 2020).

GE was applied in different domestic species to enhance the production of athletic and aesthetic features of cloned animals. GE was used in goats in which mammary gland-regulated genes were edited to increase milk yield, skeletal muscle growth, and carcass weight (Skrzyszowska and Samiec, 2021). Another example of GE in livestock was generating sheep with cystic fibrosis (CF) disorder as a model to study CF in humans (Fan et al., 2018). Also, a gene called Myostatin, responsible for muscle growth and performance, was edited in horses using CRISPR/Cas9 techniques to produce a cloned horse (Moro et al., 2020).

Possible usage of genetic markers in dromedary camel

Dromedary camels are used for milk, meat, riding, racing, and aesthetic reasons. Camel milk consumers have increased globally (Nagy et al., 2022). Production techniques such as marker-assisted selection (MAS) and marker-assisted production (MAP) were limited in this species. SCNT was achieved in the dromedary camel, and the cloning techniques have significantly improved over the last decade (Moulavi and Hosseini, 2019, 2023). Genetic studies have shown several genes associated with different phenotypes. Milk production genes have been studied in the dromedary, and only one report showed leptin, κ-casein, and αs1-casein polymorphisms associated with milk yield and reproductive traits in the dromedary (Al-Sharif et al., 2022a). Further investigation is still necessary to identify milk production traits in the dromedary. Also, growth hormone, diacylglycerol O-acyltransferase 1 and melanocortin four receptor genetic variants were associated with increased body weight in the dromedary camel (Al-Sharif et al., 2022b).

Coat color phenotype was one of the most important traits in the dromedary for its involvement in aesthetic reasons. Few reports stated the association between coat color phenotypes and genetic markers. For example, four coat color genes were identified in this species. Melanocortin 1 receptor and agouti signaling protein genetic variations were associated with white and black coat color in the dromedary, respectively (Almathen et al., 2018; Alshanbari et al., 2019). Proto-oncogene c-KIT gene frameshift deletion was associated with spotting phenotype in the dromedary (Holl et al., 2017). Also, Tyrosinase polymorphisms were associated with light brown dromedaries (Alshanbari, 2023). Finally, one study showed that FGF5 frameshift mutation is related to the fiber length -of dromedary hair (Maraqa et al., 2021).

Even though MAS and MAP have improved in the past decade, genetic marker development is still behind that of other domestic species. This delay was due to traditional breeding systems applied by most breeders. However, systematic breeding practices have been improved in the past few years and genetic studies of the dromedary camels have been significantly increased. These improvements should assist selective breeding programs for this species.

CONCLUSIONS AND RECOMMENDATIONS

SCNT is less efficient due to many factors, including epigenetic reprogramming. However, extensive SCNT research has increased the success of cloning in this species. High-performance and more valuable individuals are recommended to be cloned. In conclusion, this review highlighted the current knowledge of dromedary camel cloning, the limitations of the techniques used, and the possible use of advanced GE methods for this animal.

SCNT is an expensive technique that requires many steps and equipment. As a result, a precise process is strongly recommended.

Donor cells

This is the most important step in the process of cloning a camel. High-production, performance, and aesthetic dromedaries should be selected for the procedure.

Oocyte donors

Oocytes should be collected from normal females with a healthy history. Oocytes contain mitochondrial DNA (mtDNA) that is responsible for relevant activities. mtDNA contains 37 genes, of which 13 are responsible for oxidation phosphorylation and regulate adenosine triphosphate (ATP) activity. ATP is the energy source for cells. The rest of the genes are responsible for the production of ribosomal and translator RNA, which is essential for protein building in the cell. Therefore, all cells will be created from the donor oocyte containing maternal mtDNA derived from the oocyte.

Media supplementation

Media should be supplemented with antioxidant reagents such as vitamin C. Also, scriptaid should be added as it is necessary for histone modification. The latter reduces histone acetylase activity.

Implantation

Only one high-quality blastostyle should be implanted in one recipient, with no duplication. As discussed earlier, more than one implantation may lead to the failure of both embryos to develop.

ACKNOWLEDGMENTS

The authors would like to thank the Deanship of Scientific Research, Qassim University, for supporting the publication of this research. I would also like to thank Dr. Z. Ibrahim and Prf. A. Zaki for their help and assistance in writing this review and Douglas Baum for English language editing.

NOVELTY STATEMENT

The innovative aspect of this work summarizes the state of the art regarding stem cell research in camels, along with the limitations and possible uses of the method in genetic engineering.

AUTHOR'S CONTRIBUTION

FA prepared the outlines of the review and wrote it. AE prepared the figures and tables as well as assisted in editing the final draft.

Funding

This research received no funding.

List of abbreviations

SCNT: somatic cell nuclear transfer; GE: genetic engineering; PVP: polyvinylpyrrolidone; FSH: follicular stimulating hormone; GnRH: gonadotropin hormone; ICM: inner cell mass; Hdac: histone deacetylase; Hat: histone acetyltransferase; HDACi: histone deacetylase inhibitor; CRISPR: clustered regularly interspaced short palindromic repeats; MAS: marker assisted selection; MAP: marker assisted production; CF: cystic fibrosis; mtDNA: mitochondrial DNA; ATP: adenosine triphosphate.

Conflicts of interest

The authors have declared no conflict of interest.

REFERENCES

Ali, A., Baby, B., Vijayan, R., (2019). From desert to medicine: A review of camel genomics and therapeutic products. Front. Genet., 10: 17. https://doi.org/10.3389/fgene.2019.00017

Ali, A., Derar, D., Alsharari, A., Alsharari, A., Khalil, R., Almundarij, T.I., Alboti, Y., Al-Sobayil, F., (2018). Factors affecting reproductive performance in dromedary camel herds in Saudi Arabia. Trop. Anim. Health Prod., 50: 1155-1160. https://doi.org/10.1007/s11250-018-1545-3

Ali, I., Chaudhry, M.S., Farooq, U., (2009). Camel rearing in Cholistan desert of Pakistan. Pak. Vet. J., 29: 2.

Almathen, F., Elbir, H., Bahbahani, H., Mwacharo, J., Hanotte, O., (2018). Polymorphisms in MC1R and ASIP genes are associated with coat colour variation in the Arabian camel. J. Heredity, 1: 7. https://doi.org/10.1093/jhered/esy024

Alshanbari, F., (2023). Variants in the tyrosinase *(TYR)* gene are associated with coat color in the dromedary (*Camelus dromedarius*). Int. J. Vet. Sci., 12: 695-701.

Alshanbari, F., Castaneda, C., Juras, R., Hillhouse, A., Mendoza, M.N., Gutiérrez, G.A., Ponce de León, F.A., Raudsepp, T., (2019). Comparative FISH-mapping of MC1R, ASIP, and TYRP1 in new and old world camelids and association analysis with coat color phenotypes in the dromedary (*Camelus dromedarius*). Front. Genet., 10: 340. https://doi.org/10.3389/fgene.2019.00340

Al-Sharif, M., Radwan, H., Hendam, B., Ateya, A., (2022a). DNA polymorphisms of FGFBP1, leptin, κ-casein, and αs1-casein genes and their association with reproductive performance in dromedary she-camels. Theriogenology, 178: 18-29. https://doi.org/10.1016/j.theriogenology.2021.11.001

Al-Sharif, M.M., Radwan, H.A., Hendam, B.M., Ateya, A.I., (2022b). Exploring single nucleotide polymorphisms in GH, IGF-I, MC4R and DGAT1 genes as predictors for growth performance in dromedary camel using multiple linear regression analysis. Small Rumin. Res., 207: 106619. https://doi.org/10.1016/j.smallrumres.2022.106619

Avila, F., Das, P.J., Kutzler, M., Owens, E., Perelman, P., Rubes, J., Hornak, M., Johnson, W.E., Raudsepp, T., (2014). Development and application of camelid molecular cytogenetic tools. J. Heredity, 105: 858-869. https://doi.org/10.1093/jhered/ess067

Baguisi, A., Behboodi, E., Melican, D.T., Pollock, J.S., Destrempes, M.M., Cammuso, C., Williams, J.L., Nims, S.D., Porter, C.A., Midura, P., Palacios, M.J., Ayres, S.L., Denniston, R.S., Hayes, M.L., Ziomek, C.A., Meade, H.M., Godke, R.A., Gavin, W.G., Overström, E.W., Echelard, Y., (1999). Production of goats by somatic cell nuclear transfer. Nat. Biotechnol., 17: 456-461. https://doi.org/10.1038/8632

Basu, A., Tiwari, V.K., (2021). Epigenetic reprogramming of cell identity: Lessons from development for regenerative medicine. Clin. Epigenet., 13: 144. https://doi.org/10.1186/s13148-021-01131-4

Campbell, K.H., McWhir, J., Ritchie, W.A., Wilmut, I., (1996). Sheep cloned by nuclear transfer from a cultured cell line. Nature, 380: 64-66. https://doi.org/10.1038/380064a0

Cebra, C., Anderson, D.E., Tibary, A., Van Saun, R.J., Johnson, L.W., (2014). Llama and Alpaca Care-E-Book: Medicine, Surgery, Reproduction, Nutrition, and Herd Health. Elsevier Health Sciences.

Chesné, P., Adenot, P.G., Viglietta, C., Baratte, M., Boulanger, L., Renard, J.P., (2002). Cloned rabbits produced by nuclear transfer from adult somatic cells. Nat. Biotechnol., 20: 366-369. https://doi.org/10.1038/nbt0402-366

Cibelli, J.B., Stice, S.L., Golueke, P.J., Kane, J.J., Jerry, J., Blackwell, C., Ponce, de León F.A., Robl, J.M., (1998). Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science, 280: 1256-1258. https://doi.org/10.1126/science.280.5367.1256

Czernik, M., Anzalone, D.A., Palazzese, L., Oikawa, M., Loi, P., (2019). Somatic cell nuclear transfer: Failures, successes and the challenges ahead. Int. J. Dev. Biol., 63: 123-130. https://doi.org/10.1387/ijdb.180324mc

Fan, Z., Perisse, I.V., Cotton, C.U., Regouski, M., Meng, Q., Domb, C., Van Wettere, A.J., Wang, Z., Harris, A., White, K.L., Polejaeva, I.A., (2018). A sheep model of cystic fibrosis generated by CRISPR/Cas9 disruption of the CFTR gene. JCI Insight, 3: 19. https://doi.org/10.1172/jci.insight.123529

Farrag, F., Shalaby, N., Moustafa, T., Abdel-Salam, A., Ismail, S.A., (2019). Evalution of productive, reproductive, longevity and lifetime traits of camel in Egypt. J. Anim. Poult. Prod., 10: 203-214. https://doi.org/10.21608/jappmu.2019.53487

Gherissi, D.E., Monaco, D., Bouzebda, Z., Bouzebda, F.A., Gaouar, S.B.S., Ciani, E., (2020). Camel herds reproductive performance in Algeria: Objectives and thresholds in extreme arid conditions. J. Saudi Soc. Agric. Sci., 19: 482-491. https://doi.org/10.1016/j.jssas.2020.09.002

Gordon, J.W., Scangos, G.A., Plotkin, D.J., Barbosa, J.A., Ruddle, F.H., (1980). Genetic transformation of mouse embryos by microinjection of purified DNA. Proc. Natl. Acad. Sci., 77: 7380-7384. https://doi.org/10.1073/pnas.77.12.7380

Gouveia, C., Huyser, C., Egli, D., Pepper, M.S., (2020). Lessons learned from somatic cell nuclear transfer. Int. J. Mol. Sci., 21(7): 2314. https://doi.org/10.3390/ijms21072314

Hoffman, J.M., Valencak, T.G., (2020). A short life on the farm: Aging and longevity in agricultural, large-bodied mammals. Geroscience, 42: 909-922. https://doi.org/10.1007/s11357-020-00190-4

Holl, H., Isaza, R., Mohamoud, Y., Ahmed, A., Almathen, F., Youcef, C., Gaouar, S., Antczak, D.F., Brooks, S., (2017). A frameshift mutation in KIT is associated with white spotting in the Arabian camel. Genes, 8(3): 102. https://doi.org/10.3390/genes8030102

Hossein, M.S., Kang, M., Olsson, P.O., Kim, H., Bae, Y., Kim, H.S., Noh, J.Y., Hwang, K.I., Son, Y.B., Jeon, Y.I., (2023). Production of somatic cell nuclear transfer derived cloned calves in the United Arab Emirates. Emirates J. Food Agric., 35: 4.

Hossein, M.S., Yu, X., Son, Y.B., Jeong, Y.I., Jeong, Y.W., Choi, E.J., Tinson, A.H., Singh, K.K., Singh, R., Noura, A.S., (2021). The resurrection of mabrokan: Production of multiple cloned offspring from decade-old vitrified tissue collected from a deceased champion show camel. Animals, 11(9): 2691. https://doi.org/10.3390/ani11092691

Jones, C.J., Skidmore, J.A., Aplin, J.D., (2008). Placental glycosylation in a cama (camel-llama cross) and its relevance to successful hybridisation. Mol. Phylogenet. Evol., 49(3): 1030-1035. https://doi.org/10.1016/j.ympev.2008.09.024

Khalafalla, A.I., Hussein, M.F., Bornstein, S., (2021). Evolution, distribution, and economic importance of the camels. Infectious Diseases of Dromedary Camels: A Concise

Guide. Springer, pp. 1-19. https://doi.org/10.1007/978-3-030-79389-0_1

Khatir, H. and Anouassi, A., (2008). Preliminary assessment of somatic cell nuclear transfer in the dromedary (*Camelus dromedarius*). Theriogenology, 70: 1471-1477. https://doi.org/10.1016/j.theriogenology.2008.06.094

Kitiyanant, Y., Saikhun, J., Pavasuthipaisit, K., (2003). Somatic cell nuclear transfer in domestic cat oocytes treated with IGF-I for *in vitro* maturation. Theriogenology, 59: 1775-1786. https://doi.org/10.1016/S0093-691X(02)01235-9

Klinger, B., Schnieke, A., (2021). 25th anniversary of cloning by somatic-cell nuclear transfer twenty-five years after dolly: How far have we come? Reproduction, 162: F1-F10. https://doi.org/10.1530/REP-20-0652

Landeo, L., Zuñiga, M., Gastelu, T., Artica, M., Ruiz, J., Silva, M., Ratto, M.H., (2022). Oocyte quality, *in vitro* fertilization and embryo development of alpaca oocytes collected by ultrasound-guided follicular aspiration or from slaughterhouse ovaries. Animals, 12(9): 1102. https://doi.org/10.3390/ani12091102

Malin, K., Witkowska-Piłaszewicz, O., Papis, K., (2022). The many problems of somatic cell nuclear transfer in reproductive cloning of mammals. Theriogenology, 189: 246-254. https://doi.org/10.1016/j.theriogenology.2022.06.030

Marai, I.F., Zeidan, A., Abdel-Samee, A., Abizaid, A., Fadiel, A., (2009). Camels'reproductive and physiological performance traits as affected by environmental conditions. Trop. Subtrop. Agroecosyst., 10: 129-149.

Maraqa, T., Alhajeri, B., Alhaddad, H., (2021). FGF5 missense mutation is associated with dromedary hair length variation. Anim. Genet., 52: 848-856. https://doi.org/10.1111/age.13132

McGeady, T.A., Quinn, P.J., Fitzpatrick, E.S., Ryan, M.T., Kilroy, D., Lonergan, P., (2017). Veterinary embryology. John Wiley & Sons.

Moro, L.N., Viale, D.L., Bastón, J.I., Arnold, V., Suvá, M., Wiedenmann, E., Olguín, M., Miriuka, S., Vichera, G., (2020). Generation of myostatin edited horse embryos using CRISPR/Cas9 technology and somatic cell nuclear transfer. Sci. Rep., 10(1): 15587. https://doi.org/10.1038/s41598-020-72040-4

Moulavi, F., Hosseini, S.M., (2019). Development of a modified method of handmade cloning in dromedary camel. PLoS One, 14: e0213737. https://doi.org/10.1371/journal.pone.0213737

Moulavi, F., Hosseini, S.M., (2023). A modified handmade cloning method for dromedary camels. In somatic cell nuclear transfer technology. Springer, US. pp. 283-303. https://doi.org/10.1007/978-1-0716-3064-8_16

Moulavi, F., Asadi-Moghadam, B., Omidi, M., Yarmohammadi, M., Ozegovic, M., Rastegar, A., Hosseini, S., (2020). Pregnancy and calving rates of cloned dromedary camels produced by conventional and handmade cloning techniques and *in vitro* and *in vivo* matured oocytes. Mol. Biotechnol., 62: 433-442. https://doi.org/10.1007/s12033-020-00262-y

Nagy, P.P., Skidmore, J.A., Juhasz, J., (2022). Intensification of camel farming and milk production with special emphasis on animal health, welfare, and the biotechnology of reproduction. Anim. Front., 12(4): 35-45. https://doi.org/10.1093/af/vfac043

Navarro-Serna, S., Vilarino, M., Park, I., Gadea, J., Ross, P.J., (2020). Livestock gene editing by one-step embryo manipulation. J. Equine Vet. Sci., 89: 103025. https://doi.org/10.1016/j.jevs.2020.103025

Olsson, P.O., Tinson, A.H., Al-Shamsi, N., Kuhad, K.S., Singh, R., Son, Y.B., Jeong, Y., Jeong, Y.W., Cai, L., Sakaguchi, K., Kim, S., Choi, E.J., Yu, X., Hwang, W.S., (2021). Blastocyst formation, embryo transfer and breed comparison in the first reported large scale cloning of camels. Sci. Rep., 11(1): 14288. https://doi.org/10.1038/s41598-021-92465-9

Polejaeva, I.A., Chen, S.H., Vaught, T.D., Page, R.L., Mullins, J., Ball, S., Dai, Y., Boone, J., Walker, S., Ayares, D.L., Colman, A., Campbell, K.H., (2000). Cloned pigs produced by nuclear transfer from adult somatic cells. Nature, 407: 86-90. https://doi.org/10.1038/35024082

Saadeldin, I.M., Swelum, A.A.A., Noreldin, A.E., Tukur, H.A., Abdelazim, A.M., Abomughaid, M.M., Alowaimer, A.N., (2019). Isolation and culture of skin-derived differentiated and stem-like cells obtained from the Arabian camel (*Camelus dromedarius*). Animals, 9(6): 378. https://doi.org/10.3390/ani9060378

Salhab, M., Dhorne-Pollet, S., Auclair, S., Guyader-Joly, C., Brisard, D., Dalbies-Tran, R., Dupont, J., Ponsart, C., Mermillod, P., Uzbekova, S., (2013). *In vitro* maturation of oocytes alters gene expression and signaling pathways in bovine cumulus cells. Mol. Reprod. Dev., 80(2): 166-182. https://doi.org/10.1002/mrd.22148

Sanchez, A., Reginato, G., Cejka, P., (2021). Crossover or non-crossover outcomes: tailored processing of homologous recombination intermediates. Curr. Opin. Genet. Dev., 71: 39-47. https://doi.org/10.1016/j.gde.2021.06.012

Segeritz, C.P., Vallier, L., (2017). Cell culture: Growing cells as model systems *in vitro*. Basic science methods for clinical researchers, Academic Press. pp. 151-172. https://doi.org/10.1016/B978-0-12-803077-6.00009-6

Skidmore, J., (2018). Reproduction in dromedary camels: An update. Anim. Reprod., 2(3): 161-171.

Skidmore, J.A., (2011). Reproductive physiology in female old world camelids. Anim. Reprod. Sci., 124(3-4): 148-154. https://doi.org/10.1016/j.anireprosci.2010.08.023

Skidmorem J.A., Malom C.M., Crichtonm E.G., Morrellm J.M., Pukazhenthim B.S., (2018). An update on semen collection, preservation and artificial insemination in the dromedary camel (*Camelus dromedarius*). Anim. Reprod. Sci., 194: 11-18. https://doi.org/10.1016/j.anireprosci.2018.03.013

Skrzyszowska, M., Samiec, M., (2021). Generating cloned goats by somatic cell nuclear transfer. Molecular determinants and application to transgenics and biomedicine. Int. J. Mol. Sci., 22(14): 7490. https://doi.org/10.3390/ijms22147490

Son, Y.B., Jeong, Y.I., Jeong, Y.W., Yu, X., Olsson, P.O., Cai, L., Choi, E.J., Hossein, M.S., Singh, K.K., Rajesh, S., (2021a). Comparison of pregnancy outcomes following the transfer of early-developmental stage embryos and blastocysts produced by somatic cell nuclear transfer in *Camelus dromedarius*. Anim. Reprod. Sci., 233: 106842. https://doi.org/10.1016/j.anireprosci.2021.106842

Son, Y.B., Jeong, Y.I., Jeong, Y.W., Yu, X., Cai, L., Choi, E.J., Hossein, M.S., Tinson, A., Singh, K.K., Rajesh, S., Noura, A.S., Hwang, W.S., (2021b). Vitrification of camel skin tissue for use as a resource for somatic cell nuclear transfer in *Camelus dromedarius*. *In Vitro* Cell. Dev. Biol. Anim., 57(5): 487-492. https://doi.org/10.1007/s11626-021-00590-6

Uddin, F., Rudin, C.M., Sen, T., (2020). CRISPR gene therapy: Applications, limitations, and implications for the future. Front. Oncol., 10: 1387. https://doi.org/10.3389/fonc.2020.01387

Vettical, B.S., Hong, S., Umer, M.A., Wani, N.A., (2019). Comparison of pregnancy rates with transfer of *in vivo* produced embryos derived using multiple ovulation and embryo transfer (MOET) with in vitro produced embryos by somatic cell nuclear transfer (SCNT) in the dromedary camel (*Camelus dromedarius*). Anim. Reprod. Sci., 209: 106132. https://doi.org/10.1016/j.anireprosci.2019.106132

Wakayama, T., Perry, A.C., Zuccotti, M., Johnson, K.R., Yanagimachi, R., (1998). Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature, 394(6691): 369-374. https://doi.org/10.1038/28615

Wani, N., Skidmore, J., Wernery, U., (2008). 55 Preliminary studies on the development of reconstructed embryos after nuclear transfer in dromedary camel (*Camelus dromedarius*). Reprod. Fert. Dev., 21(1): 128-128. https://doi.org/10.1071/RDv21n1Ab55

Wani, N.A., Hong, S.B., (2018). Source, treatment and type of nuclear donor cells influences in vitro and in vivo development of embryos cloned by somatic cell nuclear transfer in camel (*Camelus dromedarius*). Theriogenology, 106: 186-191. https://doi.org/10.1016/j.theriogenology.2017.10.010

Wani, N.A., Hong, S., Vettical, B.S., (2018). Cytoplast source influences development of somatic cell nuclear transfer (SCNT) embryos in vitro but not their development to term after transfer to synchronized recipients in dromedary camels (*Camelus dromedarius*). Theriogenology, 118: 137-143. https://doi.org/10.1016/j.theriogenology.2018.05.021

Wani, N.A., Vettical, B.S., Hong, S.B., (2017). First cloned Bactrian camel (*Camelus bactrianus*) calf produced by interspecies somatic cell nuclear transfer: A step towards preserving the critically endangered wild Bactrian camels. PLoS One, 12(5): e0177800. https://doi.org/10.1371/journal.pone.0177800

Wani, N.A., Wernery, U., Hassan, F., Wernery, R., Skidmore, J., (2010). Production of the first cloned camel by somatic cell nuclear transfer. Biol. Reprod., 82(2): 373-379. https://doi.org/10.1095/biolreprod.109.081083

Wu, H., Guang, X., Al-Fageeh, M.B., Cao, J., Pan, S., Zhou, H., Zhang, L., Abutarboush, M.H., Xing, Y., Xie, Z., Alshanqeeti, A.S., Zhang, Y., Yao, Q., Al-Shomrani, B.M., Zhang, D., Li, J., Manee, M.M., Yang, Z., Yang, L., Liu, Y., Zhang, J., Altammami, M.A., Wang, S., Yu, L., Zhang, W., Liu, S., Ba, L., Liu, C., Yang, X., Meng, F., Wang, S., Li, L., Li, E., Li, X., Wu, K., Zhang, S., Wang, J., Yin, Y., Yang, H., Al-Swailem, A.M., Wang, J., (2014). Camelid genomes reveal evolution and adaptation to desert environments. Nat. Commun., 5(1): 5188. https://doi.org/10.1038/ncomms6188

Yang, H., Kolben, T., Meister, S., Paul, C., van Dorp, J., Eren, S., Kuhn, C., Rahmeh, M., Mahner, S., Jeschke, U., von Schönfeldt, V., (2021). Factors influencing the *in vitro* maturation (IVM) of human oocyte. Biomedicines, 9(12): 1904. https://doi.org/10.3390/biomedicines9121904