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 dromedaries)

Binoy S. Vettical*, S.B. Hong, Muhammad A. Umer, Nisar A. Wani

Reproductive Biotechnology Centre, Post Box 299003, Dubai, United Arab Emirates

A B S T R A C T

In the present study, there was comparison of pregnancy rates with transfer of *in vivo*-produced embryos using multiple ovulation and embryo transfer (MOET) with *in vitro*-produced embryos by somatic cell nuclear transfer (SCNT) in dromedary camels. *In vivo*-produced embryos were collected from donors after super-stimulation of follicular development on day 7 after ovulation, while *in vitro*-derived embryos were produced using SCNT from *in vivo*-matured oocytes collected from camels after follicular development super-stimulation. As a result of estrous synchronization, all recipient camels for both groups were 1 day earlier in stage of estrous cycle than developmental status of embryos at the time of transfer. The animals into which embryos were transferred were monitored at 7-day intervals after embryo transfer for signs of pregnancy based on response to presence of a male and there was ultrasonic confirmation on days 35 and 60 subsequent to day of estrus in recipient animals. A greater proportion of recipients ($P < 0.05$) were considered pregnant based on response to male presence when there was transfer of MOET- (76.8 ± 3.2) compared with SCNT- (26.4 ± 2.4) derived embryos on day 14. There was no difference in pregnancy losses in subsequent weeks until day 60 between groups. There were also no differences in calving rates of females in which MOET- (91.7%) and SCNT- (93.3%) derived embryos were transferred. These results indicate pregnancies at day 60 with SCNT-derived embryos are sustained for the remainder of gestation periods similar to when there was transfer of MOET-derived embryos in dromedary camels.

1. Introduction

Dromedary camels (Camelus dromedaries) are reproductively inefficient due to the long gestation period of 380 ± 10 days (Khanvilkar et al., 2009), thus producing a calf once every 2 years. Embryo transfer technology is being used to produce elite animals in this species, but its application has not been fully explored. There are many reports on multiple ovulation and embryo transfer (MOET) in camels with the pregnancy rates ranging from 19% to 44% when there is transfer of embryos (Anouassi and Tibary, 2013). There, however, is very limited research regarding pregnancies when there is *in vitro* production of embryos using either *in vitro* fertilization (IVF; Khatir and Anouassi, 2006) or somatic cell nuclear transfer (SCNT) (Wani et al., 2010, 2017, 2018). *In vitro* production of embryos using SCNT has improved in most of the animal species including camels, but the pregnancy rates with use of

SCNT-derived embryos are less than desired when compared with transfer of MOET-derived embryos (Tveden-Nyborg et al., 2005; Pontes et al., 2009). There has been a large demand for production of camels to be elite racing champions, elite bulls for food (*e.g.*, milk) production and winners in exhibition competitions with use of SCNT-derived embryos during the last decade, however, the pregnancy rates have been less than desirable with transfer of SCNT-derived embryos resulting in a lack of application of this technology to its fullest extent.

To the best of our knowledge, there are no earlier reports on comparison of pregnancy rates with *in vivo*- compared with *in vitro*-produced embryos in any of the camelid species. The objective of this study, therefore, was to compare and study the pregnancy rates with transfer of *in vivo*-derived embryos produced using MOET and *in vitro*-derived embryos produced using SCNT in dromedary camels, with an aim to ascertain the reasons for differences in the pregnancy rates with use of embryos that were derived using these two procedures and to identify specific areas where there needs to be future research efforts for enhancement of the pregnancies when there is transfer of SCNT-derived embryos.

2. Materials and methods

2.1. Animals

Female dromedary camels aged 5–14 years all having at least one previously normal parturition, maintained at the Reproductive Biotechnology Centre, were used for the studies. All the client animals were also housed in the center along with other experimental animals with the same management conditions at least 2 months before the experimental period. All the camels were in evaluated and determined to be in good physical condition and were fed hay and water *ad libitum*. The studies were conducted during the physiological breeding season for camels in the Arabian Peninsula region (November to March). All procedures that were conducted in this study were reviewed and approved by Animal Ethics Committee of Central Veterinary Research Laboratory, in accordance with the regulations of the Ministry of Climate Change and Environment, the government of United Arab Emirates (Permit Number 550353).

2.2. In vivo embryo production by MOET

There was super-stimulation of ovarian follicular development of donor animals using the procedures previously described (Vettical et al., 2016). In brief, there was induction of ovulation with an injection of Gonadotropin-Releasing Hormone (GnRH) (Receptal; Intervet International GmbH, Feldstrasse 1a, Unterschleissheim, Germany). Animals were treated with an injection of 2500 IU Pregnant Mare Serum Gonadotropin (PMSG; Folligon; Intervet International GmbH, Feldstrasse 1a, Unterschleissheim, Germany) as a single administration on day 4 after ovulation, and with 400 mg NIH Follicle Stimulating Hormone (FSH; Folltropin; Bioniche Animal health, Belleville, Ontario, Canada) administered twice daily in decreasing doses of 80 mg x 2, 60 mg x 2, 40 mg x 2, 20 mg x 2 for 4 consecutive days. Donors were then assessed daily from Day 8 (day of initiation of treatment period) and mated with a fertile bull when majority of follicles were of a mature size of 1.3 to 1.7 cm in diameter. Embryos were collected non-surgically on day 7 after ovulation by flushing both uterine horns using a 2-way high recovery special flushing catheter (Bioniche Animal health, Belleville, Ontario, Canada). Individual uterine horns were flushed by filling and recovering the flushing media (Vigro complete flush; Bioniche Animal health, Belleville, Ontario, Canada) repeatedly for 10 to 12 times depending upon the volume of the uterine horn. The flushed medium was assessed for embryos and each embryo was evaluated and graded to determine the developmental stage and morphology. All hatched blastocysts with normal appearance were graded as transferable, while those with degenerative or abnormal morphology were graded as non-transferable (Skidmore, 2005; Vettical et al., 2016).

2.3. In vitro embryo production by SCNT

In vivo matured oocytes were collected from donor camels using an ultrasonically-guided ovum pick-up device (OPU) by conducting procedures that were described previously (Wani and Skidmore, 2010). In brief, there was super-stimulation of ovarian follicular development of donor camels using the same protocol as previously described in this manuscript for *in vivo* embryo production. Animals were injected with 20 µg of GnRH (Receptal, Intervet International GmbH, Feldstrasse 1a, Unterschleissheim, Germany) 26 h before the scheduled OPU. The aspirated fluid was searched for COCs using a stereo-zoom microscope. The mature oocytes were used for *in vitro* embryo production with SCNT using procedures that were previously described (Wani et al., 2017, 2018). In brief, serum deprived donor cells were transferred into the perivitelline space of enucleated oocytes with a 20-µm glass micro-injection pipette. The couplets were washed in fusion medium (0.3 M mannitol, 0.1 mM MgSO4, 0.05 mM CaCl2, 0.05% PVA) and fused by applying two DC pulses of 100 V for 15 µs each using an Eppendorf electroporator. Electrofused couplets were then transferred to Hepes-TCM-199 for evaluating fusion success after 30 min. Reconstructed couplets were activated at 1 h post-fusion with 5 mM ionomycin followed by treatment with 6 dimethyl aminopurine (6-DMAP) for 4 h (Wani, 2009). The activated oocytes were then cultured *in vitro* for 7 days and all the blastocysts that were recovered were transferred to estrous synchronized recipients.

2.4. Synchronization of stage of reproductive cycle in recipients and embryo transfer

As a result of estrous synchronization, all recipient camels for both groups were 1 day earlier in stage of estrous cycle than the developmental status of embryos at the time of transfer. Recipients were selected based on the size of ovarian follicles from a large

pool of camels and ovulation was induced with a single injection of GnRH (Vettical et al., 2016). On day 6 after ovulation, the animals were positioned in sternal recumbence and assessed for the presence of Corpus Luteum (CL) by trans-rectal ultrasonic evaluation. The camels that were classified as having a large and functional CL were used as recipients for embryo transfer. A single embryo was transferred into the uterine horn of each recipient in both the groups using the recto-vaginal technique. The recipients with embryos were monitored and there was assessment every 7 days for signs of pregnancy using males to evaluate whether there were symptoms of behavioral estrus using the procedures that were previously described (Wani et al., 2018). Recipients that expressed symptoms of behavioral estrus were evaluated using trans-rectal ultrasonography for confirmation that the animals that expressed these symptoms were not pregnant. Recipients with tail cocking during the period when the females were exposed to bulls were evaluated for pregnancy using ultrasonic-guided scanning (USG) on Days 35 and 60.

2.5. Experimental design

In Experiment 1, the data from the last 4 years (2014-18) were used to compare the pregnancy percentages on and before Day 60 in recipients in which there was transfer of embryos obtained using MOET and SCNT. A total of 220 embryos were obtained using MOET and transferred into 220 recipients while 725 embryos were produced using SCNT and were transferred to 725 recipients at seven replicated time periods. Pregnancy was assessed until Day 90 before the animals were returned to their owners.

Experiment 2 was conducted in the 2017-18 time period, during which a total of 12 and 15 pregnancies were confirmed on Day 60 after there had been transfer of MOET- and SCNT-derived embryos. These animals were assigned to treatments at random before embryo transfers occurred and were housed together in a pen separate from other animals throughout the gestation period.

2.6. Statistical analysis

The results are presented as a mean percentage $\pm$ SEM in Table 1 and as Means $\pm$ S.E.M in Table 2. The data were analyzed using an ANOVA with the Fisher protected least significant difference test as well as t-test being conducted ($P \leq 0.05$). All the percent data were arcsine transformed before analysis.

3. Results

As data in Table 1 indicate, there was a greater proportion of recipient females ($P < 0.05$) that were classified as being pregnant based on detection of symptoms of estrus when the treated females were exposed to males in the group in which there was transfer of embryos produced using MOET (76.8 ± 3.2%) compared with the group utilizing embryos produced using SCNT (26.4 ± 2.4%) on day 14, (Days 6 and 7 being the day of embryo transfer for SCNT and MOET groups, respectively). All the animals, which were assessed to not be pregnant based on the female responses to the male (23.2 ± 3.2% and 73.6 ± 2.4% of recipients in which MOET- and SCNT-derived embryos were transferred, respectively) were confirmed to be pregnant or not pregnant using ultrasonic examination. There was no difference ($P > 0.05$) in pregnancy losses between treatment groups in subsequent weeks until Day 60.

In Experiment 2, there were no difference in the percentage of pregnancies that were retained throughout the gestational period when there was transfer of MOET- (91.7%) and SCNT- (93.3%) derived embryos. The gestational length was slightly greater in recipients with embryos produced using MOET (389.5 ± 1.7 days) when compared with recipients in which SCNT-derived embryos had been transferred (373.0 ± 3.6 days). All the calves were developed at the Research Center using the same management conditions for all animals for 6 months until the calves were returned to the clients.

4. Discussion

To the best of our knowledge, this is the first report in camels comparing pregnancy rates from *in vivo* produced embryos using MOET and *in vitro* produced embryos utilizing SCNT that were transferred to estrous synchronized recipient females. There was a markedly lesser pregnancy rate in recipient camels in which there was transfer of SCNT derived embryos compared with *in vivo* produced embryos. The results of the present study are consistent with those in other studies, where there was a lesser percentage pregnancy when there was transfer of *in vitro*-produced compared with when there had been transfer of *in vivo*-produced embryos in

Table 1
Dromedary camel (Camelus dromedaries) pregnancy establishment and loss after transfer of SCNT- and MOET-derived embryos in the first 60 days after transfer to estrous synchronized recipients; Values are shown a % mean $\pm$ S.E.M; n indicates the total number of embryo transfers in each group.

Source of embryo	Pregnancies sustained to			Pregnancies lost between Day 14-60
	Day 14	Day 35	Day 60	
MOET ($n = 220$)	76.8 ± 3.2[a]	55.2 ± 3.1[a]	55.2 ± 3.1[a]	21.6 ± 2.7
SCNT ($n = 725$)	26.4 ± 2.4[b]	8.0 ± 1.4[b]	8.0 ± 1.4[b]	18.4 ± 2.2

Different superscripts with lower−case letters (a–b) indicate difference ($P < 0.05$) between two different sources of embryos; Multiple ovulation and embryo transfer (MOET), somatic cell nuclear transfer (SCNT).

Table 2

Dromedary camel (Camelus dromedaries) pregnancies at day 60 and those maintained for the entire gestation period and mean gestational length after there was transfer of MOET- and SCNT-derived embryos; Values shown as mean $\pm$ S.E.M indicates the gestation length in days.

Source of Embryo	Pregnancies		Gestation length (days $\pm$ SEM)
	D60	Reached full term (%)	
MOET ($n = 12$)	12	11 (91.7)	389.5 $\pm$ 1.7[a]
SCNT ($n = 15$)	15	14 (93.3)	373.0 $\pm$ 3.6[b]

Different superscripts with lower−case letters (a–b) indicate difference ($P < 0.05$) Multiple ovulation and embryo transfer (MOET), somatic cell nuclear transfer (SCNT).

sheep (Tveden-Nyborg et al., 2005), cattle (Pontes et al., 2009), and mice (Wenqiang et al., 2016). In a previous study with cattle, there was no difference in day 30 pregnancy rates when there was transfer of SCNT-derived and control embryos, however, the majority of pregnancy loss after transfer of SCNT-derived embryos occurred during the initial ⅓ of the gestation period from days 30 to 90 of pregnancy (Hill et al., 2000). In the present study in dromedary camels, the pattern of pregnancy loss was different than that in this previous study. There was a greater proportion of recipients non-pregnant at Day 14 when there was transfer of SCNT-derived embryos ($P < 0.05$) compared with recipients in which there was transfer of *in vivo*-produced embryos. After pregnancy was established in camels in the present study, however, there was no difference in subsequent pregnancy losses between the groups, which is similar to results reported for *Bos indicus* cattle where the embryonic losses between Days 30 and 60 were similar when there was transfer of *in vitro*- and *in vivo*-derived embryos (Pontes et al., 2009). There could be many reasons for this early embryonic loss when there was transfer of SCNT-derived embryos in the present study including the fewer cell numbers in embryos that have been reported in cattle when there was use of this technique for embryo production (Koo et al., 2002) and abnormal epigenetic reprogramming of the somatic cell nucleus (Young et al., 2001; Shi et al., 2003) leading to failure of implantation. In dromedary camels, by day 14 of pregnancy, most of the trophoblast becomes closely apposed to the luminal epithelium of the endometrium to form an epitheliochorial placenta with microvillar integration at some locations in the uterus and by day 25 there is a well-developed microvillar junction formed between the fetal and maternal tissues (Skidmore et al., 1996). One of the major reasons for pregnancy losses in cattle between days 30 and 90 are the relatively few numbers of barely discernible cotyledonary areas and rudimentary development of the placenta with lesser vascularization as compared to what exists during a normal pregnancy. There are many other reports, in other species as well where the period of implantation is critical for maintenance of pregnancy and that the relatively lesser pregnancy percentages rate when there is transfer of SCNT-derived embryos results from a lesser cell differentiation and embryo development when there was transfer of SCNT-derived embryos (Van et al., 1997; Koo et al., 2002; Tveden-Nyborg et al., 2005). In a study with sheep, all *in vivo*-derived embryos had normal gastrulation development whereas with *in vitro*-cultured (IVC) and SCNT-derived embryos the process of gastrulation was impaired by day 13 of pregnancy. Also, on days 17 and 19 of pregnancy, when there was transfer of *in vivo*-derived embryos there were more somites and a greater development of the allantois than with IVC- and SCNT-derived embryos (Tveden-Nyborg et al., 2005). The lesser development of SCNT-derived embryos could be due to lack of certain species-specific proteins, growth factors or enzymes in the *in vitro* embryo culture media. There are reports on the effects of oviduct-specific proteins on maturation (Mastroianni et al., 1970) and fertilization (Martus et al., 1998) of gametes. Enzymes (Grippo et al., 1994) and growth factors (Gandolfi, 1995) in the oviduct fluid have also been reported to have a beneficial effect on maturation and fertilization of gametes. Das et al. (2013) reported the beneficial effects in cattle of oviduct-specific proteins (cOSPs) as a media supplement for cleavage and blastocyst formation. There are no reports in camels where oviduct fluids have been studied for improving the *in vitro* culture media. Interestingly embryo aggregation has also been reported to increase the cell number (Lee et al., 2007) of SCNT-derived embryos and there are greater pregnancy rates in cattle after transfer of SCNT-derived embryos when there was embryo aggregation during the culture period. In the present study, there was no use of aggregated embryos so the effects of these media culture conditions cannot be evaluated.

In the present study, there was loss of only one pregnancy from each group, from day 90 of pregnancy during the remainder of the gestation period. There, therefore, were no differences in pregnancies being maintained for the entire period of gestation after pregnancy establishment on day 14 between the two groups, indicating all SCNT-derived embryos had the capacity to implant and for normal gestation development to occur. Interestingly, there was a slightly different gestation length between the recipients into which there was transfer of SCNT- and *in vivo*-produced embryos. In females where there was transfer of *in vivo*-produced embryos there was a gestation period of 389.5 $\pm$ 1.7 days, while in recipients where there had been transfer of SCNT-derived embryos there was a shorter gestation period of 373.0 $\pm$ 3.6 days, although the gestation period in both groups was in normal range for the species. The reasons for the shorter gestation period in recipients in which there had been transfer of SCNT-derived embryos could be related to a lesser extent or disrupted placentation as has been reported in many species (Heyman et al., 2002; Bertolini and Anderson, 2002). The epigenetic reprogramming and disproportionate gene expression in cloned embryos (Daniels et al., 2000; Young et al., 2001; Shi et al., 2003) could be the reason for an abnormal placental development and thus earlier parturition, when compared to females in which there had been transfer of *in vivo*-produced embryos.

The SCNT-derived embryos are generally incompetent in suppression of a few somatic genes inherited from donor cells (Teperek and Miyamoto, 2013). The numbers and functions of the mis-regulated genes effect the outcome of each cloned embryo (Rodriguez-Osorio et al., 2012). Histone modification, DNA methylation (Xinxin et al., 2018), and aberrant hypermethylation of histone H3 lysine 9 (H3K9) have been reported to affect the development of SCNT-derived cloned embryos (Yu-Mei et al., 2018). Abnormal

epigenetic reprogramming of somatic cell nuclei could impair pre- and post-implantation development of SCNT embryos. There are studies in which there have been attempts to overcome abnormal reprogramming in many species such as in sheep (Yu-Mei et al., 2018), mice (Shogo et al., 2018), goats (Chengquan et al., 2018), pigs (Long et al., 2018), cattle (Byungkuk et al., 2018), buffalo (Papori et al., 2018), and buffalo-cattle interspecies SCNT (Husamaldeen et al., 2018). Wenqiang et al. (2016) reported that co-injection of Kdm4b can restore the transcriptional profile of SCNT-derived embryos and enhance the blastocyst development and production of cloned mice.

5. Conclusion

In conclusion, there was no difference in the pregnancy loss and the outcome, after the establishment of pregnancy on Day 14, in females in which there was transfer of SCNT-derived and *in vivo*-derived embryos by MOET in dromedary camels. There was a greater pregnancy loss as a result of embryonic death within 14 days of the gestation period when there was transfer of SCNT-derived compared with *in vivo*-derived embryos. Further studies are required to investigate various factors that are involved in the process of proper reprogramming of the somatic cell nucleus in the reconstructed embryos to improve development *in vitro* and implantation after transfer to estrous-synchronized recipients. Study of follicular and oviduct fluid in camels may help in understanding the specific requirement for *in vitro* culture of SCNT-derived embryos. Studies are also needed to assess whether aggregation of SCNT-derived embryos could help in improving the cell numbers and thus implantation after transfer to recipients.

Declaration of Competing Interest

We declare that there is no conflict of interest.

Acknowledgments

References

Anouassi, A., Tibary, A., 2013. Development of a large commercial camel embryo transfer program: 20 years of scientific research. Anim. Reprod. Sci. 136, 211–221.

Bertolini, M., Anderson, G.B., 2002. The placenta as a contributor to production of large calves. Theriogenology 57, 181–187.

Byungkuk, M., Jung, S.P., Yong-Kook, K., 2018. Determination of oocyte-manipulation, zygote-manipulation, and genome-reprogramming effects on the transcriptomes of bovine blastocysts. Front. Genet. 9, 143.

Chengquan, H., Ruizhi, D., Tingchao, M., Yan, L., Biao, W., Peng, M., Lu, Z.Q.Z., Fusheng, Q., Jun, L., Yong, Z., et al., 2018. Overexpression of Tet3 in donor cells enhances goat somatic cell nuclear transfer efficiency. FEBS J. 285, 2708–2723.

Daniels, R., Hall, V., Trounson, A.O., 2000. Analysis of gene transcription in bovine nuclear transfer embryos reconstructed with granulosa cell nuclei. Biol. Reprod. 63, 1034–1040.

Das, S.K., Sharma, A.K., Mohapatra, S.K., Bhatia, V., Chatterjee, A., Mohanty, A.K., et al., 2013. Purification of cattle oviduct specific proteins and their effect on *in vitro* embryo development. Livestock Sci. 152, 88–93.

Gandolfi, F., 1995. Functions of proteins secreted by oviductal cells. Microsc. Res. Tech. 32, 1–12.

Grippo, A.A., Anderson, S.H., Chapman, D.A., Henault, M.A., Killian, G.J., 1994. Cholesterol phospholipid and phospholipase activity of ampullary and isthmic fluid from the bovine oviduct. J. Reprod. Fertil. 102, 87–93.

Heyman, Y., Chavatte-Palmer, P., LeBourhis, D., Camous, S., Vignon, X., Renard, J.P., et al., 2002. Frequency and occurrence of late-gestation losses from cattle cloned embryos. Biol. Reprod. 66, 6–13.

Hill, J.R., Burghardt, R.C., Jones, K., Long, C.R., Looney, C.R., Shin, T., et al., 2000. Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. Biol. Reprod. 63, 1787–1794.

Husamaldeen, A., Farnoosh, J., Nima, T.V., Mohammad, H.N., Amir, N., 2018. Effect of DNA and histone methyl transferase inhibitors on outcomes of buffalo-bovine interspecies somatic cell nuclear transfer. Cell. Reprogram. 20, 256–267.

Khanvilkar, A.V., Samant, S.R., Ambore, B.N., 2009. Reproduction in camel. Vet. World 2, 72–73.

Khatir, H., Anouassi, A., 2006. The first dromedary (Camelus dromedarius) offspring obtained from *in vitro* matured, *in vitro* fertilized and *in vitro* cultured abattoir derived oocytes. Theriogenology 65, 1727–1736.

Koo, D.B., Kang, Y.K., Choi, Y.H., Park, J.S., Kim, H.N., Oh, K.B., Son, D.S., Park, H., Lee, K.K., Han, Y.M., et al., 2002. Aberrant allocations of inner cell mass and trophectoderm cells in bovine nuclear transfer blastocysts. Biol. Reprod. 67, 487–492.

Lee, S.G., Park, C.H., Choi, D.H., Kim, H.S., Ka, H.H., Lee, C.K., et al., 2007. *In vitro* development and cell allocation of porcine blastocysts derived by aggregation of *in vitro* fertilized embryos. Mol. Reprod. Dev. 74, 1436–1445.

Long, J., Qing, G., Guang-Lei, Z., Xiao-Xu, X., Mei-Fu, X., Qi-Rong, L., Zhao-Bo, L., Jun-Xia, W., Xi-Jun, Y., Jin-Dan, K., et al., 2018. The histone deacetylase inhibitor, CI994, improves nuclear reprogramming and *in vitro* developmental potential of cloned pig embryos. Cell. Reprogram. 20, 205–213.

Martus, N.S., Verhage, H.G., Mavrogianis, P.A., Thibodeaux, J.K., 1998. Enhancement of bovine oocyte fertilization *in vitro* with a bovine oviductal specific glycoprotein. J. Reprod. Fertil. 113, 323–329.

Mastroianni Jr, L., Urzua, M., Stambaugh, R., 1970. Protein patterns in monkey oviductal fluid before and after ovulation. Fertil. Steril. 21, 817–820.

Papori, S., Yadav, A.S., Selokar, N.L., Dharmendra, K., Dhaka, S.S., Yadav, P.S., et al., 2018. Epigenetic status of buffalo fibroblasts treated with sodium butyrate a chromatin remodeling agent. Tissue Cell 50, 51–58.

Pontes, J.H.F., Nonato-Junior, I., Sanches, B.V., Ereno-Junior, J.C., Uvo, S., Barreiros, T.R.R., Oliveira, J.A., Hasler, J.F., Seneda, M.M., et al., 2009. Comparision of embryo yield and pregnancy rate between *in vitro* and *in vitro* methods in the same Nelore (*Bos indicus*) donor cows. Theriogenology 71, 690–697.

Rodriguez-Osorio, N., Urrego, R., Cibelli, J., Eilertsen, K., Memili, E., 2012. Reprogramming mammalian somatic cells. Theriogenology 78, 1869–1886.

Shi, W., Zakhartchenko, V., Wolf, E., 2003. Epigenetic reprogramming in mammalian nuclear transfer. Differentiation 71, 91–113.

Shogo, M., Huihan, W., Lan, J., Falong, L., Kumiko, A.I., Xiaoji, W., Kimiko, I., Lin, Y., William, P., Jeannie, T.L., Atsuo, O., Li, S., Yi, Z., et al., 2018. Loss of H3K27me3 imprinting in somatic cell nuclear transfer embryos disrupts post-implantation development. Cell Stem Cell 23, 343–354.

Skidmore, J.A., 2005. Reproduction in dromedary camels: an update. Anim. Reprod. 2, 161–171.

Skidmore, J.A., Wooding, F.B.P., Allen, W.R., 1996. Implantation and early placentation in the one-humped camel (Camelus dromidarius). Placenta 17, 253–262.

Teperek, M., Miyamoto, K., 2013. Nuclear reprogramming of sperm and somatic nuclei in eggs and oocyte. Reprod. Med. Biol. 12, 133–149.

Tveden-Nyborg, P., Peura, T.T., Hartwich, K.M., Walker, S.K., Maddox-Hyttel, P., 2005. Morphological characterization of pre- and peri-implantation in vitro cultured somatic cell nuclear transfer and *in vitro* derived ovine embryos. Reproduction 130, 681–694.

Van, S.A., Ysebaert, M.T., de Kruif, A., 1997. Relationship between timing of development, morula morphology, and cell allocation to inner cell mass and tro-phectoderm in *in vitro*-produced bovine embryos. Mol. Reprod. Dev. 47, 47–56.

Vettical, B.S., Hong, S.B., Wani, N.A., 2016. Multiple ovulation and embryo transfer (MOET) in camels: an overview. Asian Pac. J. Reprod. 5, 102–104.

Wani, N.A., 2009. *In vitro* embryo production in camel (Camelus dromidarius) from *in vitro* matured oocytes fertilized with epididymal spermatozoa stored at 4°C. Anim. Reprod. Sci. 111, 69–79.

Wani, N.A., Hong, S.B., Vettical, B.S., 2018. Cytoplast source influences development of somatic cell nuclear transfer (SCNT) embryos *in vitro* but not their devel-opment to term after transfer to synchronized recipients in dromedary camels (Camelus dromedarius). Theriogenology 118, 137–143.

Wani, N.A., Skidmore, J.A., 2010. Ultrasonographic-guided retrieval of cumulus oocyte complexes after super-stimulation in dromedary camel (Camelus dromedaries). Theriogenology 74, 436–442.

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.A.H., Wernery, R., Skidmore, J.A., 2010. Production of the first cloned camel by somatic cell nuclear transfer. Biol. Reprod. 82, 373–379.

Wenqiang, L., Xiaoyu, L., Chenfei, W., Yawei, G., Rui, G., Xiaochen, K., Yanhong, Z., Jingyi, L., You, W., Wenchao, X., Su, W., Jiqing, Y., Wei, L., Tao, C., Hong, W., Yong, A., Shaorong, G., et al., 2016. Identification of key factors conquering developmental arrest of somatic cell cloned embryos by combining embryo biopsy and single-cell sequencing. Cell Discov. 2, 16010.

Xinxin, L., Xudong, A., Li, B., Dongfang, L., Xuefei, L., Zhuying, W., Shorgan, B., Guangpeng, L., et al., 2018. VPA selectively regulates pluripotency gene expression on donor cell and improve SCNT embryo development. In Vitro Cell. Dev. Biol. Anim. 54, 496–504.

Young, L.E., Fernandes, K., McEvoy, T.G., Butterwith, S.C., Gutierrez, C.G., Carolan, C., et al., 2001. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. Nat. Genet. 27, 153–154.

Yu-Mei, Z., En-En, G., Qian-Qian, W., Hao, T., Jian, H., 2018. Effects of histone methyltransferase inhibitor chaetocin on histone H3K9 methylation of cultured ovine somatic cells and development of preimplantation cloned embryos. Reprod. Toxicol. 79, 124–131.