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# CAMEL CLONING: ACHIEVEMENTS AND CONSEQUENCES

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## ABSTRACT

The natural process in producing an offspring is through *in vivo* fertilisation of an oocyte by sperm. Animal cloning via somatic cell nuclear transfer (SCNT) provided the possibility of producing live offspring independent of gametes' interaction. The procedure involves the reconstruction of an enucleated oocyte with a somatic cell followed by nuclear reprogramming of the differentiated diploid nucleus to an undifferentiated totipotent embryonic state. This allows the reconstructed embryo to grow and produce offspring nearly similar to the original animal that dedicated the somatic cell. SCNT is a very expensive technique and due to the numerous unknown signals involved in nuclear reprogramming and epigenetics, it has extremely low efficiency and low survival rates of offspring. Extensive use of this technique is associated with the reduction in genetic diversity. Nevertheless, it provides an opportunity to preserve an endangered breed of camel or to resurrect the deceased elite camel. This review concentrates on the efficiency of SCNT in dromedary camels and the possibility of using this technique in routine practice.

**Key words:** Animal cloning, camel, reproductive technologies

Based on the current status of animal reproductive technologies, the production of camel calves could be achieved by two main approaches: sexual and asexual. Sexual approach which relies on sperm and oocyte interaction, is the natural way of producing an offspring. Accordingly, several generations of reproductive technologies were used to assist the natural process of offspring production. Semen technology is the first generation of animal reproductive technologies that remains in the infancy stage in camels due to problems in the viscosity of semen. It could counteract semen processing and preservation and could prevent the production of camel calf using frozen semen (Niasari-Naslaji, 2023). The second generation of animal reproductive technologies that rely on the fertilisation of oocytes by sperm is the technique of embryo production, either *in vivo* or *in vitro* (Khatir and Anouassi, 2006; Anouassi and Tibary, 2013; Niasari-Naslaji and Nikjou, 2023). The *in vivo* production of embryos is very well-defined and routinely used in camel (Anouassi and Tibary, 2013; Ararooti *et al,* 2018; Niasari-Naslaji and Nikjou, 2023). However, *in vitro* embryo production has not had great progress since its introduction (Khatir and Anouassi, 2006), possibly due to the nature of follicle extrusion from the ovarian stroma, which might result in severe bleeding and adhesion following repeated ultrasound-guided transvaginal ovum pick-up and the problem of viscous semen that is not easy to be used for *in vitro* fertilisation.

Animal cloning is an asexual approach to producing offspring (Segers *et al,* 2019). Identical twins could occur naturally or through embryo splitting (Rahbaran *et al,* 2021). The birth of the first clone in mammals from an adult somatic cell provided a novel asexual approach to producing offspring (Campbell *et al,* 1996). Dolly was the only viable infant of 277 attempts created with mammary epithelial cells that developed into 29 early *in vitro* embryos. They were transferred into 13 surrogate females resulting in the production of a viable lamb. This indicated that the nucleus of the adult somatic cell could have a developmental competence nearly similar to the nucleus of the germ cell lineage. It took 14 years since the birth of Dolly, for the first dromedary camel cloned calf, Injaz, to be born using a similar approach (Wani *et al,* 2010). In 2017, the first Bactrian camel cloned calf was also born by interspecies SCNT (Wani *et al,* 2017). Since then, several articles published to optimise SCNT in camel (Wani, 2021; Hossein *et al,* 2023; Mansour *et al,* 2023; Moulavi and Hosseini, 2023). It was also claimed that the production of expensive camel calves could be possible via SCNT at the commercial scale (Olsson *et al,* 2021). The purpose of this review is not to provide the details of the technique for camel cloning, as this

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objective has already been accomplished (Wani, 2021; Moulavi and Hosseini, 2023). This review tries to summarise all results regarding camel cloning aiming at camel calf production to highlight the final result that one could expect using SCNT. Accordingly, the result of published articles was harmonised and a similar pattern in the report of result was followed to provide the possibility for comparing the results.

**The impact of camel cloning on genetic diversity and camel industry**

The existing genetic diversity in camels is the result of traditional camel breeding practices among ethnic groups. This in turn resulted in the production of different breed of camel (89 Dromedary camel breeds and 14 Bactrian camel breeds) according to cultural preferences throughout the centuries (Köhler-Rollefson, 2022). Extensive use of embryo transfer technology and camel cloning, in particular, could narrow the camel gene pool (Köhler-Rollefson, 2022) and possibly make the population vulnerable to particular diseases (Spielman *et al,* 2004). Therefore, any attempts to use cloning in the camel industry have to be conducted with great caution not to disturb the genetic diversity and not to disseminate particular diseases and abnormalities in camel. The genetic diversity allows us to select the racing camel with better performance, even better than those that died before. The expression of any particular traits such as meat and milk production, beauty and racing capabilities depend on the nature and the nurture of individuals. It might be possible to produce a camel calf very similar to the original donor camel genetically. However, it does not necessarily mean that such a cloned camel calf could perform as well as the original donor camel. Therefore, it is possible to produce a camel calf by the very expensive technique of SCNT which is as bad as any other camel calf in a group! According, trusting to the speculation that camel cloning could be used to produce animals with the highest potential for milk production or champions in racing and beauty contests and reintroduction of males of high genetic merit could be questionable. USA Food and Drug Administration addressed the safety of consumption of meat derived from cloned specimens (Animal Cloning, 2021). However, because the cost of obtaining such animals is extremely expensive, using cloned camel for meat consumption is not logical at the present time.

**Camel cloning achievements and consequences**

Very nice review articles on the scientific aspects and consequences of SCNT are available (Niemann, 2016; Malin *et al,* 2022; Mrowiec *et al,* 2022; French and Trounson, 2023). Production of an offspring through reproductive cloning is performed by *in vitro* or *in vivo* oocyte maturation, enucleation of the oocyte, reconstruction of the oocyte with somatic cell nuclear transfer, a fusion of somatic cell's nucleus (karyoplast) and oocyte (ooplast) followed by activation, *in vitro* embryo culture and transfer of the reconstructed embryo to a recipient animal. The first dromedary nuclear-transferred embryos derived from either adult fibroblasts or cumulus cells were successfully produced in 2008 (Khatir and Anouassi, 2008). Unfortunately, two detected pregnancies were lost by Day 60 following transfer and the success of producing the first cloned camel calf was postponed. Later on in 2010, the first camel calf, Injaz, was produced by reconstructing embryos following SCNT using cumulus cells of dromedary camel (Wani *et al,* 2010).

There are three main criteria for assessment of cloned animals including blastocyst development rate, birth rates and survival rate of newborns. In Table 1, blastocyst rates and birth rates in different studies were summarised. Out of more than 3000 reconstructed oocytes, 695 reached the blastocyte stage (23.1%). Of 915 recipients that received 1.67 blastocysts, on average, 105 were diagnosed pregnant by Day 60 of pregnancy (11.5%). The final achievement from more than 3000 reconstructed oocytes was 62 cloned camel calves from 2010 till 2023 (6.8%; Table 1). There is no information regarding the survivability and further performance of produced cloned camel calves. It was found that Injaz, the first cloned camel calf born on the 8th of April 2009, died several years ago. Early and late embryonic losses varied from 75 to 100%, were the main causes of failure in camel cloning.

Animal cloning remains inefficient compared with other assisted reproductive technologies, such as conventional embryo transfer, *in vitro* fertilisation, or artificial insemination. In general, the overall success rate from the creation of a viable and healthy camel calf remains at a similar and low level in literature similar to the achievements in other domestic animal species (Tsunoda and Kato, 2002; Oback, 2008; Czernik *et al,* 2019; Gouveia *et al,* 2020; French and Trounson, 2023). Despite the high cost and extreme difficulties, SCNT is often seen as a hope to restore extinct species or help preserve the endangered ones. Accordingly, the first cloned camel calf was produced from a decade-old vitrified tissue collected from a deceased champion show camel (Hossein *et al,* 2021).

**Table 1.** The result of camel cloning by SCNT technique from 2008 till 2023.

| Authors | Year | Karyoplast | Oocyte maturation | NT oocyte | Fusion (%)* | Cleaved (%)* | Blastocyst (%)* | Recipients | Mean no embryos transferred | Pregnant ≥Day 60 (%) | Offspring (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Khatir and Anouassi | 2008 | Adult skin fibroblast | *In vitro* | 369 | 247 (67) | 217 (59) | 52 (14) | 5 | --- | 1 (20) | 0 |
| | | Cumulus cells | *In vitro* | 363 | 225 (62) | 162 (45) | 55 (15) | 7 | --- | 1 (14) | 0 |
| Wani *et al* | 2010 | Cumulus cells | *In vivo* | 75 | 60 (79.8) | --- | 26 (34.7) | 26 | 2 | 4 (15) | 1(3.8) |
| | | Adult skin fibroblast-1 | *In vivo* | 98 | 87 (88.6) | --- | 29 (29.6) | 29 | 1.7 | 2 (6.9) | 0 |
| | | Adult skin fibroblast-2 | *In vivo* | 70 | 64 (92.1) | --- | 25 (35.7) | 45 | 1.8 | 4 (8.9) | 0 |
| | | Fetal fibroblast-1 | *In vivo* | 101 | 89 (88) | --- | 24 (23.8) | 24 | 1.6 | 2 (8.3) | 0 |
| | | Fetal fibroblast-2 | *In vivo* | 75 | 70 (92.8) | --- | 20 (26.7) | 15 | 1.5 | 0 | 0 |
| Wani and Hong | 2018a | Adult skin fibroblast | *In vivo* | 78 | 66 (85.2) | --- | 35 (44.9) | 19 | 1.8 | 2 (10.5) | 0 |
| | | Cumulus cells | *In vivo* | 72 | 68 (94.2) | --- | 23 (31.9) | 16 | 1.4 | 4 (25) | 3 (18.7) |
| | | Adult skin fibroblast-1 | *In vivo* | 86 | 75 (87.5) | --- | 21 (24.4) | 17 | 1.2 | 4 (23) | 3 (17.6) |
| | | Adult skin fibroblast-2 | *In vivo* | 73 | 60 (82.6) | --- | 30 (41.1) | 19 | 1.6 | 2 (10.5) | 0 |
| | | Adult skin fibroblast-3 | *In vivo* | 92 | 86 (94) | --- | 25 (27.1) | 22 | 1.1 | 2 (9.1) | 1 (4.5) |
| | | Adult skin fibroblast-4 | *In vivo* | 78 | 2 (79.3) | --- | 28 (35.9) | 19 | 1.5 | 1 (5.3) | 1 (5.3) |
| Wani and Hong | 2018b | Adult skin fibroblast-1 | *In vivo* | 68 | 55 (80.5) | --- | 18 (26.5) | 11 | 1.6 | 1 (9) | 1 (9) |
| | | Adult skin fibroblast-2 | *In vivo* | 79 | 67 (84.8) | --- | 25 (31.6) | 19 | 1.3 | 2 (10) | 2 (10) |
| | | Adult skin fibroblast-3 | *In vitro* | 43 | 39 (91.1) | --- | 10 (23.2) | 8 | 1.2 | 1 (12.5) | 1 (12.5) |
| | | Adult skin fibroblast-4 | *In vitro* | 32 | 24 (75.8) | --- | 24 (75.8) | 3 | 1.7 | 0 | 0 |
| Moulavi *et al* | 2020 | Adult skin fibroblast | HMC/ *In vitro* | --- | --- | --- | --- | 47 | 2.6 | 5 (10.6) | 4 (8.5) |
| | | Adult skin fibroblast | HMC/ *In vitro* | --- | --- | --- | --- | 15 | 2.4 | 1 (6.6) | 1 (6.6) |
| | | Adult skin fibroblast | Conventional/ *In vitro* | --- | --- | --- | --- | 40 | 2.7 | 3 (7.5) | 1 (2.5) |
| | | Adult skin fibroblast | Conventional/ *In vitro* | --- | --- | --- | --- | 5 | 2.4 | 1 (20) | 0 |
| Hossein *et al* | 2021 | Vitrified adult skin | *In vitro* | --- | --- | --- | --- | 31 | 1.1 | 5 (16.1) | 2 (6.4) |
| | | Vitrified adult skin | *In vivo* | --- | --- | --- | --- | 54 | 1.1 | 13 (24.1) | 9 (16.1) |
| Olsson *et al* | 2021 | Adult skin fibroblast | --- | --- | --- | --- | --- | 286 | --- | 31 (10.8) | 19 (6.6) |
| Son *et al* | 2022a | Adult skin fibroblast | *In vitro* | 517 | 362 (70.0) | 217 (41.9) | 73 (14.1) | 45 | 1.6 | 1 (2.2) | 1 (2.2) |
| | | Adult skin fibroblast | *In vivo* | 309 | 223 (72.2) | 183 (59.2) | 101 (32.7) | 62 | 1.5 | 10 (16.1) | 10 (16.1) |
| Son *et al* | 2022b | Adult skin fibroblast | *In vitro* | 326 | 239 (73.3) | 168 (51.5) | 51 (15.6) | 26 | 1.81 | 2 (7.7) | 2 (7.7) |
| Summary | | | | 3004 | 2208 | --- | 695 (23.1) | 915 | 1.67 | 105 (11.5) | 62 (6.8) |

*All percentages were calculated based on NT oocyte.

Reconstructed embryos produced following SCNT have numerous biological problems with several undesired consequences following transfer to recipients and questionable survivability after birth. The main cause of these problems and consequences could be due to the failure or incomplete nuclear reprogramming (Bourc'his *et al,* 2001; Yang *et al,* 2007) and epigenetic modifications (Reik *et al,* 2001; Couldrey and Wells, 2013; Alsalim *et al,* 2018; Gao *et al,* 2018) resulting in the great differences in gene expressions (Li *et al,* 2005; Vassena *et al,* 2007). As a result, besides failure in maternal recognition of pregnancy (Arnold *et al,* 2006), high early and late embryonic death and abortion rates (Hill *et al,* 2000; Chavatte-Palmer *et al,* 2012), problems associated with the implantation, placenta development and function and also abnormal offspring syndrome (Niemann, 2016). Later obesity, immunodeficiency, respiratory defects and early death (Campbell *et al,* 2007; Loi *et al,* 2016) and the low birth rate (Gouveia *et al,* 2020; Yang *et al,* 2007) could be expected. The survival of offspring is also questionable. Using a neonatal intensive care unit (NICU) could be an essential part of maintaining SCNT calves due to several predicated and unpredictable problems. Information on cattle has estimated that nearly one in three cloned calves dies within the first 6 months of life (Chavatte-Palmer *et al,* 2004). Large/abnormal offspring syndrome, respiratory failure, abnormal kidney development, and cardiovascular and liver pathologies are often reported (Chavatte-Palmer *et al,* 2004; Watanabe and Nagai, 2009). SCNT technique could produce an offspring suffering from several health hazard problems that prevent the newborn from continuing a normal life. This could be a subject for those scientists who are dealing with animal ethics to consider some regulations to stop the commercial application of camel cloning until it is considered as a safe technique to produce offspring. Unraveling the molecular mechanism underlying SCNT-mediated nuclear reprogramming is needed to enhance the development of cloned embryos (Wang *et al,* 2020).

## Conclusions

Several research groups using nearly similar and/or different approaches tried to clone camel. However, due to intrinsic problems associated with SCNT mainly because of epigenetic aspects of animal cloning involved in reprogramming the nucleus of an adult somatic cell, the final result of this technique is still extremely low and costly. With the small number of offspring produced by camel cloning during the last 13 years (not more than 70 cloned camels according to the published articles) without having any report on the health and survivability of cloned camel calves, any claims regarding the use of camel cloning to propagate elite racing, beauty and milking camel may not be valid and many years of innovative research with great investments are required for camel cloning to be recommended as a routine procedure in camel industry.

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