Acta Entomology and Zoology

E-ISSN: 2708-0021
P-ISSN: 2708-0013
www.actajournal.com
AEZ 2025; 6(1): 20-24
Received: 23-11-2024
Accepted: 26-12-2024

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Application of Somatic Cell Nuclear Transfer (SCNT) in companion Animals: A review

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DOI: https://doi.org/10.33545/27080013.2025.v6.i1a.185

Abstract
The Somatic Cell Nuclear Transfer (SCNT) is an emerging technology that requires the nucleus from a donor somatic cell which is transferred to the enucleated oocyte *in vitro*. Since the first sheep had been cloned, the SCNT was a significant breakthrough of the last century, many countries and companies provide strong support to the development of this new biotechnology. SCNT has been continuously developed and used for diverse purpose in animals. However compare with other mammalian species, the SCNT used in dogs has developed slowly, due to some difficulties. As companion animials have played an important role in human society, this technique is moving from laboratory to commercial application to meet people's emotional needs. In this review, we summarise the application of SCNT for companion animal especially the current situation of commercial companion animal. Besides, we also concern about ethical focus on the cloning companion animal.

Keywords: Somatic cell nuclear transfer, companion animal, commercial, clone

Introduction
The companion animals are those physical, emotional, behavioral and social needs that can be readily met as companions in the home, or in close daily relationships with humans. Companion animals play a stabilizing and dynamic role in the family emotional system. They are also proved to proven important self-object needs such as self-cohesion, self-esteem, calmness, soothing and acceptance (Brown S-E. 2007) [1]. The dog, cat and horse are mostly companion animal species, but more exotic animals recently became popular. The global pet market has a promising future. In 2021, the global pet market was already over 200 billion dollars. (www.petkeen).
An artificial method called somatic cell nuclear transfer (SCNT) is a novel technique that is well-known as cloning. SCNT is the transplantation of a nucleus from a somatic cell into the denucleated oocyte. Since the first animal sheep "Dolly" was cloned in 1996(Campell *et al.*1996), owing to the rapid development of cloning technology, in the last 30 years, more than 20 mammalian species have been successfully cloned and reported. For example, the sheep (Wilmut *et al.* 1997) [5], cattle (Wells *et al.* 1999), goats (Baguisi *et al.* 1999) [7], pigs (Polejaeva *et al.* 2000) [8], horses (Galli *et al.* 2003) [9], Cats and dogs (Shin *et al.* 2002, Lee *et al.* 2005) [10, 11], and macaque monkey (Zhen Liu. 2018) [12]. The SCNT provides a great tool to copy the different species of certain individual who can accelerate the use of superior genotypes. With the help of SCNT, the high-quality traits of livestock, can be maintained to increase and the quantity of valuable products such as meat and milk. Helping decreases the emission of greenhouse gases (Niemann H. 2011) [13] and produces transgenic animals for medical applications (Bordigon. 2017) [14]. It provides a method to help preserve endangered species, and conserve biodiversity (Ambreen Iqbal. 2021) [15]. In addition, cloning companion animals is of immense emotional need and commercial interest.

Materials and Methods
Materials: the female dogs used as oocyte donors and embryo transfer recipients, surgical instruments, intravenous catheter, cell culture medium.
Method: after confirming the time of dogs estrus, blood was collected and progesterone levels in the blood were measured. Three days after ovulation, mature oocytes were surgically collected. Donor fibroblasts were obtained from an ear-skin biopsy of a female dog and cultured for two to five passages.

The chromosomes of the unfertilized canine oocytes were removed by micro manipulation, and a single donor cell was transferred into each enucleated oocyte. Transferred the active oocytes into the oviducts or uterine horns of recipient dogs at times appropriate to the embryos' developmental stages. The pregnancies we confirmed by ultrasound than until parturition.

Commercial applications of cloned pet

People's emotional need for companion animals has promoted the development of SCNT, particularly it's the commercialization. Many pet owners develop deep emotional bonds with their pets and may want to preserve that connection by cloning their pet. Cloning can provide a way to maintain a physical presence of a beloved pet that has passed away, especially in terminally ill or deceased pets (Olsson PO. 2022) [16]. Some pet owners may want to preserve the unique genetic traits of their pet, especially if the pet has rare or desirable characteristics. In some cases, cloning technology is used for the conservation of endangered species, and pet owners might be interested in contributing to such efforts (Jang G, 2010) [17]. High-profile celebrities and individuals with significant financial resources may choose to clone their pets as a form of investment or out of personal interest in the technology (Kim GA, 2012) [18]. Some owners even have an idea of having a cloned pet can be appealing due to the novelty and uniqueness of the concept. The demand for pet longevity is also expected to boost the market, with pets increasingly viewed as family members and a growing desire to extend their lives. From all these reason, SCNT provides the owners with a chance to recreate their beloved pets. Meanwhile, the development of commercial pet cloning has seen significant advancements in recent years. Rising technological advancements in biotechnology create lucrative opportunities for the pet cloning market. Recently with the Artificial Intelligence (AI) developing quickly, AI has a positive impact on the pet cloning market by enhancing the efficiency and precision of the cloning process. AI can analyze genetic data to identify optimal conditions for cloning, increasing success rates and reducing costs. Rising technological advancements in biotechnology create lucrative opportunities for the pet cloning market. Innovations in genetic engineering and reproductive technology have improved cloning success rates, reducing risks and uncertainties. At the same time, increasing consumer awareness is anticipated to drive the market, with scientific advancements becoming more accessible and widely understood (www.pet.biovenic.com). Some companies offer bio bank services of cells from living or recently deceased pets, to be used in case of death. The consumer demand for technique is intense. Nevertheless, high costs of pet cloning remain a restraint, as the complex and resource-intensive process leads to high costs that are often prohibitive for many potential customers. The global pet cloning market size was estimated at USD 4.03 Billion in 2023 and is projected to reach USD 16.56 Billion by 2032, with a compound annual growth rate (CAGR) of 17% during 2024-2032. This growth is attributed to the rising interest in wildlife conservation and the high emotional attachment to pets (www.dataintelo.com).

The greatest SCNT success has been achieved with the domestic cat. Following the birth of the first cloned kitten" CC" in 2002 (Shin. 2002). "CC" was produced using nuclear transfer of DNA from cells derived from a female domestic shorthair cat, "CC", which lived 18 years and died of kidney failure. Two years after first cloned cat, in 2004, Genetic savings and clone produced the first commercially cloned pet costed $50000, a Maine coon cat named "Little Nicky" who was cloned from a 17-year-old deceased pet cat (Piedrahita, J. A.2004) [21]. Other research and commercial groups have subsequently produced live clones (Ball 2005; Yin *et al*. 2005) [22, 23]. However, unlike domestic animal species, embryo development rates remain low, with only 0-10.5% of reconstructed embryos reaching the blastocyst stage (Gomez *et al*. 2003) [24]. Additionally, the post-implantation survival rate of clones is similarly poor across all species, with pregnancy rates per embryo ranging from 0.6% to 2.3% and live birth rates per embryo from 0.3% to 1.2% (Yin *et al*. 2006b) [25]. Similar to cats, the first cloned dog "Snuppy", was born in 2005. Through the cloning of Afghan hounds by nuclear transfer from adult skin cells into oocytes that had matured *in vivo*. Lee collected oocytes matured *in vivo* at metaphase II about 72 hours after ovulation by flushing the oviducts. On average, 12 oocytes were collected per female donor, resulting in a total of 1,095 reconstructed canine embryos being transferred into 123 recipients. One fetus miscarried and two others were carried to term (Lee *et al*. 2005) [11].

In 2006 a South Korean firm, Sooam Biotech Research Foundation began offering pet cloning to anyone willing to pay the $100,000 fee (www.veterinarypracticenews.com). The first commercial cloning of a pet dog appeared after 3 years of cloned dog. The owner in the US spends 50000 dollars on five identical copies of her beloved pit bull terrier. There is no doubt of the importance of the dogs as vital companion animals. They are the most loyal friends to mankind. The motivation of the clone dog is the dog-human relationship. Cloned dogs are important for fulfilling the emotional needs of dog owners. The company provides a pet tissue bank to preserve the cells cloning. These include the top company Viagenpets in the USA, Sinogene in China and RNL BIO, and Sooam Biotech in Korea. In 2015, ViaGen, a Texas company that had been cloning horses and livestock, expanded into replicating cats and dogs (www.times). In October of that year, two litters of kittens were successfully delivered. Viagenpets says it is now cloning "more and more pets every year", and has cloned "hundreds" since it first opened for business in 2015. The firm charges 50,000 dollars to clone a dog, 30,000 dollars for a cat, and 85000 dollars for a horse (www.BBC.com). That cost is out of the range of most of us, but a number of famous people have revealed in recent years that they have had their dogs cloned, or were planning to do so. ViaGen also offers a genetic material banking service. The preservation costs $1,600, plus $150 a year after 12 months. Pet owners can choose to clone at a later date or leave the cells in storage. ViaGen's client service manager said that's what the majority of our clients do: Simply store the cells for potential use down the road. There could be future cell-based therapies where these cells could be useful at some point. Those cells can be stored for decades (www.veterinarypracticenews.com).

Sinogene, as an early commercialization enterprise of pet cloning technology in China, realized commercialization in 2016, and completed the first commercial cloning cat "Garlic" in 2019. The initial price is approximately 200,000 yuan. Due to the increase in technical stability and the

emergence of major cloning companies, the current price is basically 120,000 to 150,000 yuan. Officials say the company's pet cloning business could reach 20 million (www.sohu.com). China's pet industry is developing rapidly, and the pet market in 2022 has enormous potential. China's pet cloning market is estimated to reach 4,000 dollars. The Korean company RNL Bio, accomplished the world's first commercial dog cloning in 2008. Another company Sooam Biotech clones many animals, including cattle and pigs for medical research and breed preservation, but is best known for its commercial dog service (en.sooam.com). With a client list including princes, celebrities and billionaires, the foundation offers owners protection against loss and grief with a cloning service that promises perfect replacement for a beloved pet. Since 2006, the facility has cloned nearly 800 dogs, commissioned by owners or state agencies seeking to replicate their best sniffer and rescue dogs. Despite the 100,000 dollars price tag, requests for the service have poured in from around the world, approximately half from North America. With a client list including princes, celebrities and billionaires (www.phys).

For horse cloning, it is more focused on the sport horse than the pet horse. The Haflinger foal Prometea, the first living cloned horse, was obtained in 2003 in an Italian laboratory (Galli. 2013) [33]. Reproductive cloning of the Pieraz and Quidam de Revel horses began in 2005. In 2007, the International Federation for Equestrian Sports ruled that cloned horses should be banned from the official competitions it organizes, believing that opening up participation to clones would be unjust and unfair to the competition. It revised its opinion in July 2012. Horse clones are now allowed in all FEI competitions (www.slate.fr). This reversal is seen as an important sign of recognition of the usefulness of clones in sport horse breeding (Galli. 2013) [33].

The Chinese institute originally created micropigs as models for human disease by applying a gene-editing technique. The make-up pigs were sold as pets, with each priced 1600 dollars. It is said that customers will be able to buy pigs with different coat colors and patterns, which the company says can be set by gene editing (Cyranoski D, 2015) [34]. The demand for cloning other exotic pets, such as reptiles, snakes and even chinchillas is too limited, and such animals were too small to justify the cost.

Application for working dog

Working dogs for different purposes such as search and rescue dogs, drug sniffer dogs, police dogs, and even cancer sniffer dogs are necessary for human society. Cloning technology has been used for breeding working dogs. It has been proven that cloned working dogs have similar anatomical, physiological, and neurological patterns and similar behavioral patterns, as those of their nuclear donors. In addition, it appears that cloned dogs can have similar lifespans to those of naturally bred dogs (Kim MJ. 2018) [35]. Korean custom service cloned 7 sniffer dogs from a retire elite drug sniffing dog and evaluated their sniffing ability (Choi J, 2014) [36]. They showed their talented drug detection and it saved the budget for selecting elite work dogs. Based on these promising and milestone results, the Korean government established a national cell bank for producing working dogs via SCNT.

Cloned dogs can be used to produce working dogs for cancer sniffing. After the first suggestion that a dog's scent detection ability could be used in the diagnosis of cancers in 1979, this proposition has been confirmed by many cancers including bladder, lung, prostate, ovarian, and colorectal cancers (Kim MJ, 2018) [35]. Similar research was conducted to evaluate the cancer sniffer ability of cloned dogs, and the cloned dogs also showed excellent ability to detect cancer. They had similarly excellent ability to detect cancer, with 93.9% sensitivity and 99.5% specificity for the detection of breast cancer (Kim *et al*. 2015) [37]. Studies have shown that SCNT can better overcome the problems of low efficiency and high investment in breeding qualified working dogs, and reduce the enormous cost of maintaining unqualified dogs.

Application for medical disease models

Dogs are a good model for human disease since dogs have very similar physiological and disease features compared to humans, including histological appearance, tumor genetics, molecular targets, biological behavior and responses to conventional therapies (Paoloni and Khanna. 2008) [38]. With the development and stabilization of canine cloning technology, cloned dogs are increasingly used in the development of human disease models. SNCT-based dog genome-editing can maintain genotype background, and phenotype and can be used to generate inbred dog models or to recover pathogenic mutations in purebred dogs to study diseases (Kim DE. 2022) [39]. Certain dog breeds appear to be spontaneous models of Parkinson's disease, which make them ideal models for human aging (Patronek GJ, 1997) [40]. Due to dog's brain being subjected to stress similar to humans, it is a good model for neurological disorders (Roth G, 2005) [41]. The SNCT-based transgenic and knockout technique has been widely used in dog models of human neurological diseases and muscular dystrophy (Oh HJ, 2017) [42].

Application for preserving endangered canid and felid species

At present, more and more species tend to be endangered or extinct, and it is urgent to protect biodiversity. The emergence of SCNT makes it possible to save endangered animals and revive extinct species. Using SCNT combined with a cell bank can promote the maintenance of genetic make-up species. Cell banks or biobanks can preserve biological samples of endangered species for the long term. Many somatic tissues of wildlife have been preserved, including some specials of threatened wild felids and canids. For instance, Iberian Lynx (Lynx pardinus) is in Europe (León-Quinto *et al*. 2014) [43], Leopard Panthera uncia) in Central Asia (Verma *et al*. 2012) [44], Asian golden cat is in Southeast Asia (Wittayarat *et al*. 2013) [45], and Jaguar (Panthera onca) is in America (Mestre-Citrinovitz *et al*. 2016) [46]. This technique has also been successfully used to preserve rare canid species and endangered canids such as wolves. The threatened species gray wolf (Kim MJ, 2007) and the Gyeongju donggyeong dog have been successfully cloned by SCNT (Choi YB, 2016) [48]. Interspecies somatic cell nuclear transfer (iSCNT) is also used for the cloning of endangered species for conservation. Many endangered animals have been cloned by iSCNT including the gray wolf and coyotes (Hwang *et al*. 2013) [49]. These results prove

that SCNT is an effective method to protect endangered canid and felid species.

Concerns about companion animal cloning

The application of SCNT biotechnology to companion animals has caused a series of issues, such as ethical problems, animal welfare problems, high failure rates and high cost. Due to the special relationship between humans and companion animals, the ethical issues raised mainly include death, friendship, individual uniqueness and wholeness, even lost embryos (Heðinsdóttir K, 2018) [50]. The success of cloning dogs is still low. Welfare issues are mainly related to surrogate mothers and cloned individuals, including health issues during pregnancy, parturition and dead embryos.

The application of cloning technology in pets urges people to consider whether it violates the nature of the friendship between humans and pets. Cloned pets may be the exact physical copy but without the shared history and experiment with the owner. The owner may suffer emotional dissatisfaction.

Conclusion

SCNT is a groundbreaking technique in the field of biotechnology that has opened up new possibilities in the realm of companion animals. The application of SCNT to companion animals is a significant milestone, as it offers a multifaceted approach to creating animals that are not only emotionally significant to their owners but also hold economic and genetic value. Emotionally, the ability to clone a beloved pet can provide comfort and continuity for those who have lost a cherished companion. Economically, cloning can be used to preserve the lineage of high-value animals, such as those with desirable traits in the breeding industry. Genetically, it allows for the propagation of specific genetic traits that may be beneficial for various purposes. As technology progresses, the use of SCNT in pet cloning is expected to become more sophisticated, addressing the emotional needs of pet owners by offering them the chance to replicate their pets' unique personalities and physical characteristics. This technology can also be instrumental in expanding the population of qualified elite working dogs, such as those used in search and rescue, law enforcement, and military operations. By cloning dogs with proven abilities and temperaments, we can ensure a consistent and high-quality force of working animals.

Furthermore, SCNT has the potential to generate more accurate human disease models. By cloning animals with specific genetic predispositions to certain diseases, researchers can study these conditions in a controlled environment, leading to advancements in medical treatments and a better understanding of disease mechanisms.

In terms of conservation efforts, SCNT can play a crucial role in preserving endangered species. By cloning individuals from endangered populations, we can increase genetic diversity and bolster the numbers of these species, which is vital for their survival in the wild. Despite the promise of SCNT, there are limitations and ethical considerations that must be addressed. Cloning can result in health issues for the cloned animals, and the success rate of cloning procedures is still relatively low. Additionally, there are ethical debates surrounding the commercialization of cloning and the welfare of the cloned animals. To overcome these limitations, ongoing research is essential to refine the techniques and improve the health outcomes for cloned animals.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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