

Development of somatic cell nuclear transfer biotechnology for cloning of animals: a mini-review

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Article information	ABSTRAK
Article history:	Transfer inti sel somatik (SCNT) pada hewan kloning penting untuk bioteknologi reproduksi, dapat digunakan untuk konservasi alam liar, terancam punah atau hampir punah, dan juga untuk meningkatkan sumber daya genetik ternak lokal yang unggul. Begitu banyak publikasi yang menunjukkan keberhasilan kloning hewan dengan SCNT untuk berbagai keperluan, serta perkembangan metode kloning hewan. Tulisan ini akan mencoba mengulas hasil penelitian kloning hewan yang berhasil dan terbatas pada kebutuhan non-terancam (ternak dan hewan liar) dan satwa liar yang terancam. Jenis penelitian ini adalah tinjauan pustaka. Penelitian ini dilakukan dalam beberapa tahap yaitu identifikasi, skrining abstrak, seleksi teks lengkap, dan penulisan mini review. Hasil bioteknologi SCNT untuk kloning hewan dapat digunakan untuk pengawetan sumber daya genetik plasma nutfah hewan liar, peningkatan produksi ternak unggul dan cara-cara konservasi hewan terancam.
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Keywords:	Somatic cell nuclear transfer (SCNT) in cloning animals is essential for reproductive biotechnology. It can be used for the conservation of wild, endangered, or critically endangered, and also to improve superior local livestock genetic resources. Many publications have been shown the success of animal cloning with SCNT for various purposes, as well as the development of animal cloning methods. This paper will attempt to review the results of successful and limited animal cloning research on the needs of non-threatened (livestock and wild animals) and threatened wildlife. This research was a kind of literature review. The research was conduct in several phases. These are identification, abstract screening, full-text selection, and mini-review writing. The results of SCNT biotechnology for animal cloning can be used for the preservation of wild animal germplasm genetic resources, increasing the production of superior livestock, and ways of conserving threatened animals.
Animal biotechnology	
Cloning	
SCNT	
Somatic cell	
Threatened animal	

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INTRODUCTION

In general, several reproductive biotechnology techniques such as artificial insemination (AI), embryo transfer (ET), in vitro fertilization (IVF), and animal cloning (Ciptadi, 2007) can help preserve animals, one of which includes the cloning of the somatic cell nucleus transfer. Somatic Cell Nuclear Transfer (SCNT) cloning is important biotechnology evidence for the conservation of endangered wild animals and preventing the loss of genetic resources of a species (Fatira et al., 2019; Hajian et al., 2011; Holt et al., 2004) as well as for conservation efforts (preservation) of superior local livestock genetic resources (Ciptadi, 2007).

SCNT provides reprogramming somatic cells into totipotent embryos and generates viable animals, such as cloned amphibians and mammals (Chen et al., 2020). To clone one individual, must from the egg cells. First, must be removed nuclear of egg cells that would become a targeted cell. Then, the nuclear of the clone's egg cell passed towards targeted egg cell before (it doesn't have nuclear anything), it called by cloned egg cells. Cloned egg cells must be transferred to the uterus to develop the embryos. Some somatic cell has the chance to create clone embryos that totipotent specifically (Tajuddin et al., 2015).

SCNT techniques to reprogram somatic cells that have more benefits. Such as so many treatments during the process of nuclear transfer, the donor can be from any other types of somatic cells (Chen et al., 2020), the potential of targeted (cloned) egg cell can load all of the genetic resources. Besides, SCNT techniques also provide good livestock products with high genetic resources, so they can increase the productivity of agricultural products (Tajuddin et al., 2015). So, SCNT more potent than any other reproductive biotechnological technique.

Cloning is an organism that is genetically identical to one another (aggregates), either a group of cells, tissue, or an individual produced asexually (in vitro). Cloning can take the form of cloning of cells, tissues, or whole individuals (Tajuddin et al., 2015). Cloning can also be in the form of embryo cloning, which is obtained from the results of nucleus transfer donors that have the potential for applications in various fields, including medical, livestock production, and conservation of threatened wildlife. The success of animal cloning began with the cloning of Dolly's sheep, a clone resulting from the transfer of somatic cell nuclei to each other. Since then, several animals, such as livestock, rats, pigs, and goats, have been cloned (Ciptadi, 2007; Rojas et al., 2005).

The large numbers of publications that show the success of animal cloning for various purposes, as well as the development of animal cloning methods, make it necessary to compare any existing research to provide an evaluation of the success of animal cloning methods. The success of animal cloning depends on the treatment given before, during, and after the cloning process. Based on the study of existing literature, this paper will attempt to review the results of successful and limited animal cloning research on the needs of non-threatened (livestock and wild animals) and threatened wildlife.

METHODS

This research was a kind of literature review. The research method refers to Putri et al. (2018), consists of several stages which are carried out comprehensively. These stages are carried out to get the results of an in-depth review based on the selected articles. The flow diagram of this research is described in more detail in Figure 1.

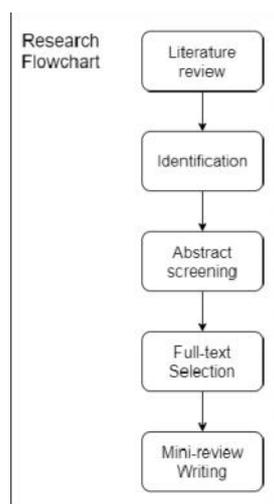


Figure 1. Research flowchart

The research flow begins with a literature study of the Scopus indexed journals, Google Scholar, and we get 50 articles. These articles are based on further searches based on the keywords in each article. The search was limited to the keywords "cloning; somatic cell nuclear transfer; animal cloning". Subsequent searches identify titles that match the scope of the mini-review in this article. Identify the title in terms of the keywords for each article chosen. Of the 50 articles, the titles identified for further stages were carried out based on the suitability of the title with the topic of cloning reproduction biotechnology with the SCNT method.

After the appropriate article titles are selected, screening for each selected title is based on the abstract to obtain 20 suitable articles. The abstract of each article becomes a detailed description of the article's discussion. The selected abstract was adapted to the purpose of writing this mini-review, which discusses the success of animal cloning using the SCNT method from time to time.

The next step is to read the full text of each article and a mini-review process. These 20 articles have gone through comprehensive stages in their selection. Limitation of the scope of discussion in this mini-review had done by selecting the article that has the recent coverage of the success of animal cloning by the SCNT method. This mini-review article only discusses briefly the various previous studies (20 articles) related to the success of animal cloning using the SCNT method, but it would be better to understand that each article that is made into a mini-review can be searched for the article in question and then carried out full-text reading for a thorough understanding of the article.

RESULTS AND DISCUSSION

1. Successfully Animal Cloned

a. Importance of SCNT Cloning

Somatic cell nuclear transfer (SCNT) has a major role in animal reproduction biotechnology, namely as a reproductive biotechnology method that can increase livestock yields (Selokar *et al.*, 2019), bio-conservation strategies for endangered animals, critically endangered animals, and safeguarding animal genetic resources (Fatira *et al.*, 2019; Hajian *et al.*, 2011). SCNT is carried out in several mammals. With the help of reproductive technology, genetic copies are obtained from individual single somatic cell donors. SCNT can be done to maintain the genetics of an existing species and epigenetically (Ogura, 2020).

Reproductive cloning, the production of identical offspring through SCNT, can save endangered wildlife species. Cloning of endangered mammals, including species with external embryonic development, is possible (Holt *et al.*, 2004). Cloning of differentiated SCNT to previously enucleated oocytes is a promising technique for producing cloned embryos with high genetic value (Rojas *et al.*, 2005).

b. *Evidence of the Success of SCNT Cloning Technique*

A suitable SCNT cloning technique can produce embryos from endangered species. Huemul is a native Andean deer that has been declared as an endangered species, has great patrimonial value, and is Chile's national emblem. The use of assisted reproductive and procreation biotechnology can assist conservation programs oriented towards protecting endangered species of deer (Rojas *et al.*, 2005).

Cloning with SCNT can be intraspecific and interspecific (iSCNT; interspecific Somatic Cell Nuclear Transfer) for the conservation of wild mammals' biodiversity of germplasm; nuclei cell reprogrammed to produce cells induced by pluripotent cells. Many wild mammals are cloned, but due to the difficulty of obtaining cytoplasmic (or cytoplasm) donor cells, it becomes a major problem in the cloning process. Donor cell nuclei (karyoplast) are obtained from the skin of live or post-mortem individuals, then stored in somatic cell banks. Cloning of different species, such as wild carnivores and ungulates, can be successful through the iSCNT technique even though it is not easy (Borges & Pereira, 2019).

2. Development of Cloned Animals

Since Dolly cloned, the first cloned mature animal in 1996 from 6 years old mammary gland cell Finn Dorset (white sheep) in lowest nutrition medium, so the cell stopped divided, research of cloned animals has increased. We provide the successfully cloned animals with authors, years, and results. It can be seen in Table 1.

Table 1. History of the development of successfully animal cloned.

No.	Authors and Year	Cloned	Results and source of cloned cell
1	Arat <i>et al.</i> (2011)	Anatolian Grey cow	1 male individual from fibroblast cells, 3 female individuals from granulosa cells, and 1 female individual from cartilage cells.
2	Ciptadi (2007)	<i>B. taurus</i> cow	1 male <i>B. Taurus</i> from mammary gland somatic cell and fetal fibroblast epithelial cell.
3	Dantas <i>et al.</i> (2020)	Cow (for livestock)	15 individuals (10 females, 5 males).
4	Wells <i>et al.</i> (1999)	Friesian cow	10 fetus birth with Caesar surgeon, cloned from 16 re-clone blastocysts (granulosa mural cells).
5	Deng <i>et al.</i> (2020)	Goat	1 embryo from SCNT parental embryo.
6	Boquest <i>et al.</i> (2002)	Pig	1 individual from fibroblast cells of frozen fetus in liquid nitrogen for 2 years.
7	Hajian <i>et al.</i> (2011)	Mouflon Esfahan	1 individual of <i>Ovis orientalis isphahanica</i> mouflon from cryofibroblast (from genome bank).
8	Selokar <i>et al.</i> (2019)	Murrah superior bull	1 individual from the donor of tail skin biopsy and seminal plasma.
9	Lu <i>et al.</i> (2018)	Bull	1 individual transgenic <i>Bubalus bubalis</i> .
10	Kim <i>et al.</i> (2017)	Dog	3 individuals from ASCs (adipose-derived mesenchymal stem cells).
11	Song <i>et al.</i> (2019)	Cat	1 individual birth from SCNT, 3 individuals' birth from CICT techniques.
12	Ogura (2020)	Mouse	1 individual tried and birth successfully, from nucleus <i>B6D2F1</i> -female cells, 12 individuals from B6x129 F1 Sertoli cells.
13	Riaz <i>et al.</i> (2011)	Mouse	1 male individual chimera from injection of <i>TetraCT ES</i> (F-Tetra 1) cells into diploid blastocysts.
14	Song <i>et al.</i> (2020)	Mouse	mouse embryo cloned SCNT dan CICT technique and manipulated (<i>reprogramming</i>) of the embryo.
15	Fatira <i>et al.</i> (2019)	Fish	the embryo of <i>Acipenser gueldenstaedtii</i> from multiple donor somatic cells and <i>Acipenser ruthenus</i> sterlet as a non-enucleated egg cell of the recipient.
16	Hou <i>et al.</i> (2016)	Fish	611 individuals have cloned <i>Paralichthys olivaceus</i> from induction of meioinogenesis in homozygote diploid egg from mitoginogenesis.

a. Cow

Researchers have successfully cloned farm animals such as Anatolian Gray's endemic cow cloning, according to Arat *et al.* (2011) using different types of donor cells (fibroblasts, cartilage, and granulosa cells) cryopreserved in a gene bank and oocytes aspirated from the ovaries of Holstein Cows as the source of the recipient cytoplasm. The cloning results consisted of one male from fibroblasts, three females from granulosa cells, and one female from the cloned cartilage cells, which were born healthy with normal birth weight. No calf is lethal after birth. The results showed that the cloned calves had the same microsatellite alleles in 11 loci with their nucleus cell donors. However, the mtDNA from five cloned calves Anatolian Gray had a different haplotype from the donor cells, and mtDNA heteroplasmy could not be detected in any of the clones. The birth of healthy clones indicated that haplotype differences between the cells and the donor oocytes did not affect development before or after implantation of SCNT-derived embryos. This study proves that the Anatolian Gray Cattle, which is a critically endangered species, can be preserved (Arat *et al.*, 2011).

According to Ciptadi (2007), for the successful birth of a *B. taurus* bull male, which was the result of iSCNT cloning on *B. taurus* cow oocyte gaur, which was transferred to the recipient broodstock of domestic cattle. The production method of embryo cloning using somatic cell donors in various livestock species was reconstituted using mammary gland somatic cell donors and fetal fibroblast epithelial cells. One of the advantages of this technology is that it can be used to implement genome resource banks, which are very useful for conserving the genetic diversity of endangered animal species or accelerate the improvement of the genetic quality of superior livestock (Ciptadi, 2007). Cloning of 15 cattle (10 females, 5 males) was also successfully carried out (Dantas *et al.*, 2020).

According to Wells *et al.* (1999), they have cloned Friesian dairy cows with high livestock genetic resources. The SCNT technique uses mural granulosa cells, which are totipotent. This cloning succeeded in producing ten calves performed by cesarean section, and all of them survived. After the transfer of 16 chronographed blastocysts, embryo survival was monitored at day 60 by 38%. DNA analysis said the calves were genetically identical to the donor cattle. Missed chances of survival during pregnancy may be due to placental dysfunction at some stage. Further research is needed to modify specific genetics for biomedical or livestock purposes (Wells *et al.*, 1999).

b. Goat

Deng *et al.* (2020) cloned goat embryos with control of DNA and histone methylation. The cloned goat embryo is still in the development stage to become an individual born whole and normal. It is necessary to further develop this research to produce cloned filial (small goats) that are intact and can survive.

c. Pig

Boquest *et al.* (2002) report successfully cloning piglets from fetal fibroblast cells that have been cultured in liquid nitrogen for two years by inducing the donor cell nucleus to the oocyte cytoplasm for approximately three days before chemical activation. The cultured fibroblast cells were mixed in a calcium-free medium to become enucleated oocytes excreted from superovulation. This research focuses on the presence of the $\alpha(1,3)$ -galactosyltransferase gene, which can be used for transplantation in humans in the future.

d. Sheep

Hajian *et al.* (2011) explained that in vitro ripened and enucleated oocytes in domestic sheep can be used to clone the Esfahan mouflon *Ovis orientalis isphahanica*, which is vulnerable to extinction. Cryofibroblast deflected from the mouflon (derived from the genome bank), and domestic sheep were prepared and cultured in vitro and used as a karyotype. The somatic cells used from *Ovis orientalis isphahanica* and domestic sheep have been frozen for two years. The freeze-zone SCNT technique used domestic sheep oocytes ripened and enucleated in vitro induced by donor nuclei *Ovis orientalis isphahanica* and domestic sheep nuclei cells. The cloned blastocyst of *Ovis orientalis isphahanica* was then transferred into the uterus of a domestic sheep by dissection (Hajian *et al.*, 2011). The cloned sheep need to be considered for their health. Since the incident of Dolly's sheep, it is important to pay attention to monitoring the health of sheep; such as

Sinclair *et al.* (2016) conducted research related to musculoskeletal monitoring, metabolic tests, and blood pressure of 13 cloned sheep aged 7-9 years, including four from Dolly's lineage.

e. *Bull*

The cloning of Murrah's superior bull, which is a very high value in livestock production, was successfully carried out through the SCNT technique. Donor cells were derived from a tail skin biopsy and seminal plasma. Development, hematology, plasma biochemistry, and reproductive organs are all normal and intact (Selokar *et al.*, 2019). The transgenic *Bubalus bubalis* cloning was also successfully carried out using the SCNT technique, according to Lu *et al.* (2018).

f. *Dog and Cat*

The birth of the world's first cloned dog, *Snuppy*, was a huge success in mammal cloning. This study succeeded in giving birth to 3 puppies cloned *Snuppy* by SCNT from ASCs (*adipose-derived mesenchymal stem cells*) (Kim *et al.*, 2017). Also, cat cloning was successfully by Song *et al.* (2019) on an SCNT basis. Cytoplasm injection (CICIT; *cytoplasm injection cloning technology*) is performed to develop cloned embryos in vitro.

g. *Mice (Mouse)*

According to Ogura (2020), it was related to mouse cloning by SCNT using female nucleus cell donor *B6D2F1-female*. Somatic cell donors can come from different cell types and different genotypes. In contrast, Riaz *et al.* (2011) managed to clone a mouse from a fertilized egg. The cell nuclei were reprogrammed then cultured in vitro and in vivo after transferring chromosomes from embryonic stem cells into tetraploid mouse embryos. The SCNT cloned mice had normal chromosomes and intact genome composition. The possibility to improve the quality of cloned mice with the SCNT technique has been developed and is very useful for biomedical research purposes as an experimental animal (Ogura, 2020). Song *et al.* (2020) cloned mouse embryos by reprogramming the cloned embryos' cytoplasmic volume. Cytoplasm injection (CICIT; *cytoplasm injection cloning technology*) was developed to increase reprogramming of the SCNT.

h. *Fish*

Cloning primitive Russian sturgeon fish, *Acipenser gueldenstaedtii*, could save this endangered species. Fatira *et al.* (2019) tried to clone the primitive fish *Acipenser gueldenstaedtii* by SCNT with multiple donor somatic cells and the *Acipenser ruthenus* sterlet as recipient non-nucleated egg cells. Injection of multiple donor somatic cells into single manipulated cells (reprogramming) can promote embryonic development. In the future, similar engineering experiments can save endangered species (Fatira *et al.*, 2019). The cloning of *Paralichthys olivaceus*, a kind of fish, has also been done by Hou *et al.* (2016); cloning comes from the induction of meiotinogenesis in eggs from homozygous diploid mitotinogenesis. When the clones reached their peak maturity of the gonads, meiotinogenesis was re-induced to produce a second-generation cloning group of *Paralichthys olivaceus*. After three months, 611 individuals survived.

Besides, the various negative implications that may arise in the development of cloning using the SCNT technique include: 1) cloning can be used to create dominant groups or certain races through the selection of individuals with desired traits. Morally, this is still contradictory; 2) on a large scale, it is possible to reduce genetic diversity; 3) cloned results are susceptible to disease; 4) cloning is possible to shorten the life expectancy of the individual clone; 5) religiously, some people still believe how the cloned zygote is from the "soul" side (Tajuddin *et al.*, 2015). Various studies related to ethics may need to be carried out to discuss the positives and negatives of this SCNT cloning process. On the other hand, the development of SCNT can be a breakthrough for animal husbandry and rescue endangered wild animals.

CONCLUSIONS

Based on the discussion above, it can be concluded that the results of SCNT biotechnology for animal cloning can be used for the preservation of wild animal germplasm genetic resources, increasing the production of superior livestock, and ways of conserving threatened animals. Various suitable and purposeful SCNT methods have had a high success rate in animal cloning

efforts. These methods can be applied to both farmed and wild animal germplasm and the conservation of endangered animals.

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