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Facultad de Ciencias Agropecuarias

Universidad Nacional de Trujillo

REVIEW

Somatic cell nuclear transfer in domestic and wild ruminants: Overcoming epigenetic barriers to improve cloning efficiency and biodiversity conservation

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Received: 9 February 2026. Accepted: 13 September 2026. Published: 24 September 2026.

Abstract

Somatic cell nuclear transfer (SCNT) is a powerful reproductive biotechnology with wide-ranging applications in livestock breeding, biodiversity conservation, regenerative medicine, and developmental biology. Despite remarkable progress in mammalian cloning, the efficiency of SCNT remains low, particularly in wild ruminants, as only a small proportion of reconstructed embryos develop to terms. There is growing evidence that incomplete epigenetic reprogramming, including aberrant DNA methylation, histone modifications, and dysregulated embryonic gene expression, is a key factor in developmental failure and postnatal abnormalities. This review provides a comprehensive overview of recent advances in SCNT in domestic and wild ruminants, covering donor cell selection, oocyte competence, nuclear reprogramming, embryonic development, placental abnormalities, pregnancy loss, and offspring health. It also summarizes current strategies aimed at improving cloning efficiency through epigenetic modulation and the optimization of SCNT protocols. Special attention is given to the challenges associated with applying SCNT to wild ruminant species, where limited access to high-quality gametes and donor cells, combined with species-specific reproductive limitations, further compromise the success of cloning. Finally, the technical and biological challenges that still limit the routine application of SCNT were analyzed, and the importance of future research on characterizing donor cells, cell cycle synchronization, and epigenetic reprogramming was highlighted.

Keywords: Cloning; Ruminants; Epigenetic modification; Nuclear transfer; Donor cells.

DOI: https://doi.org/10.17268/sci.agropecu.2026.054

Cite this article:

Laines-Arcce, I. M., Bellido-Quispe, D. K., Pinzón-Osorio, C. A., Contreras Huamani, M., Negreiros, N. A. B., de Souza, F. K. P., Chaves, M. S., Melo, L. M., & Freitas, V. J. F. (2026). Somatic cell nuclear transfer in domestic and wild ruminants: Overcoming epigenetic barriers to improve cloning efficiency and biodiversity conservation. *Scientia Agropecuaria, 17*(3), 795-813.

1. Introduction

In the realm of reproductive biotechnologies, various methods such as artificial insemination (AI), *in vitro* fertilization (IVF), embryo transfer (ET), and animal cloning have emerged (Ciptadi, 2007; Zhu et al., 2025; de Aguiar et al., 2026). Cloning, specifically, entails the asexual reproduction process resulting in genetically identical copies of an organism, whether animal or plant. While this biotechnology signifies a significant technological leap, it remains an experimental technique necessitating further refinement for widespread adoption in agricultural and social settings (Tajuddin et al., 2015). Cloning via somatic cell nuclear transfer (SCNT) is based on the principles of nuclear equivalence preservation of the genome during cell differentiation and cell plasticity a cell's ability to be reprogrammed and have its differentiation redirected (Smith & Murphy, 2004).

SCNT is of particular importance in the conservation of threatened wildlife and the preservation of species' genetic resources (Gu et al., 2026). Allowing, in addition to obtaining descendants, the understanding of the physiological and embryonic aspects of reproduction and the obtaining of descendants of genetically valuable animals (Fatira et al., 2019; Hajian et al., 2011; Holt et al., 2004). The concept of SCNT was initially proposed by Speman (1938) and later demonstrated by Briggs and King (1952) in amphibians. Gurdon's in 1962 revealed the possibility of reversing the differentiated state of a vertebrate cell through factors present in the ooplasm, introducing the concept of nuclear reprogramming, as well as Gurdon and Uehlinger (1966) in African clawed frog showing the potential for reprogramming of differentiated cells using adult animals.

In mammals, a SCNT was described in rabbits in 1975 (Bromhall, 1975), with subsequent studies in ruminant species such as sheep (Willadsen, 1986) and cattle (Prather et al., 1987), where clones were produced using blastomeres as donor sources of core. As the technique evolved, it became evident that fully differentiated cells could be used in nuclear transfer programs.

The birth of Dolly the sheep (Wilmut et al., 1997) demonstrated that the genome of a fully differentiated mammalian cell could be restored to complete totipotency, marking a significant advancement in cell reprogramming. Then, several mammalian species were successfully cloned from different cell types, albeit with low efficiency, through SCNT. Since then, numerous animals have been cloned using this technology across various species, including cattle (Cibelli et al., 1998; Kato et al., 1998), goats (Baguisi et al., 1999), gaur (Lanza et al., 2000), buffaloes (Shi et al., 2007), cervids (Berg et al., 2007; Magalhães et al., 2020; Melo et al., 2022; Venegas et al., 2006), and camelids (Wani et al., 2018; Wani et al., 2017; Wani et al., 2010) as well as in other species, and more recently, second-generation sheep clones have been produced from amniotic cells derived from first-generation gene-edited animals (Zhu et al., 2025). Despite its successes and use of interspecies somatic cell nuclear transfer (iSCNT) as an alternative, it once uses oocytes from different species (Lanza et al., 2000; Galli & Lazzari, 2008; Wani et al., 2017), as recently illustrated by the establishment of interspecies SCNT-derived, transgene-free induced pluripotent stem cells for the conservation of the wild boar germplasm resource (Gao et al., 2025). The main argument for the application of iSCNT is the rapid decrease in the number of species.

The technique involves several stages such as: obtaining nucleus donor cells, including alternative and previously underused sources such as seminal somatic cells (Ferreira et al., 2025), recipient oocytes, chromatin removal (enucleation), cell cycle synchronization between donor cells and cytoplasm, electrofusion between donor cells and oocytes, activation of the reconstituted oocyte, *in vitro* culture, inovulation and development of the embryos (Saini et al., 2015) on the processes in the production of clone animals by SCNT (Figure 1). The SCNT faces technical obstacles limiting its practical application. These challenges include low cloning efficiency and abnormalities observed in extraembryonic tissues such as the placenta of cloned embryos (Ogura et al., 2013). Furthermore, abnormalities may persist in cloned animals' post-birth, including obesity, immunodeficiency, respiratory defects, and premature death (Loi et al., 2016; Ogura et al., 2013), although these phenotypes are not passed on to offspring (Fulka Jr et al., 2004; Wakayama et al., 2013).

Efforts to enhance cloning efficiency have been hindered by limited understanding of reprogramming barriers. Recent analyses of transcriptome and epigenetic changes during reprogramming in SCNT have shed light on molecular defects, offering insights to overcome critical barriers to epigenetic reprogramming (Liu et al., 2016; Matoba et al., 2014; Matoba et al., 2018), including the modulation of histone deacetylase activity, such as HDAC3 inhibition, to improve epigenetic reprogramming and early development of cloned embryos (Song et al., 2026), and the derivation of extra-embryonic endoderm stem cell lines from SCNT-derived blastocysts, which provides a further *in vitro* model for studying reprogramming outcomes across embryonic and extraembryonic lineages (Li et al., 2026).This review provides an overview of the progress and challenges associated with SCNT in domestic and wild ruminant species. It also covers obstacles at different stages of the technique, from donor cell selection to placental abnormalities and health problems, offering insights into future research directions and potential improvements.

For this review, a wild ruminant includes any artiodactyl (member of the mammalian order Artiodactyla) possessing a rumen, reticulum, omasum, or isthmus homologous to the omasum, and abomasum. Ruminants also possess certain skeletal features, such as loss of upper incisors, the presence of incisiform lower canines, and fusion of the cubiod and navicular bones in the tarsus, that are useful in fossil identification (Gentry, 2000) but that are not of primary consideration here.

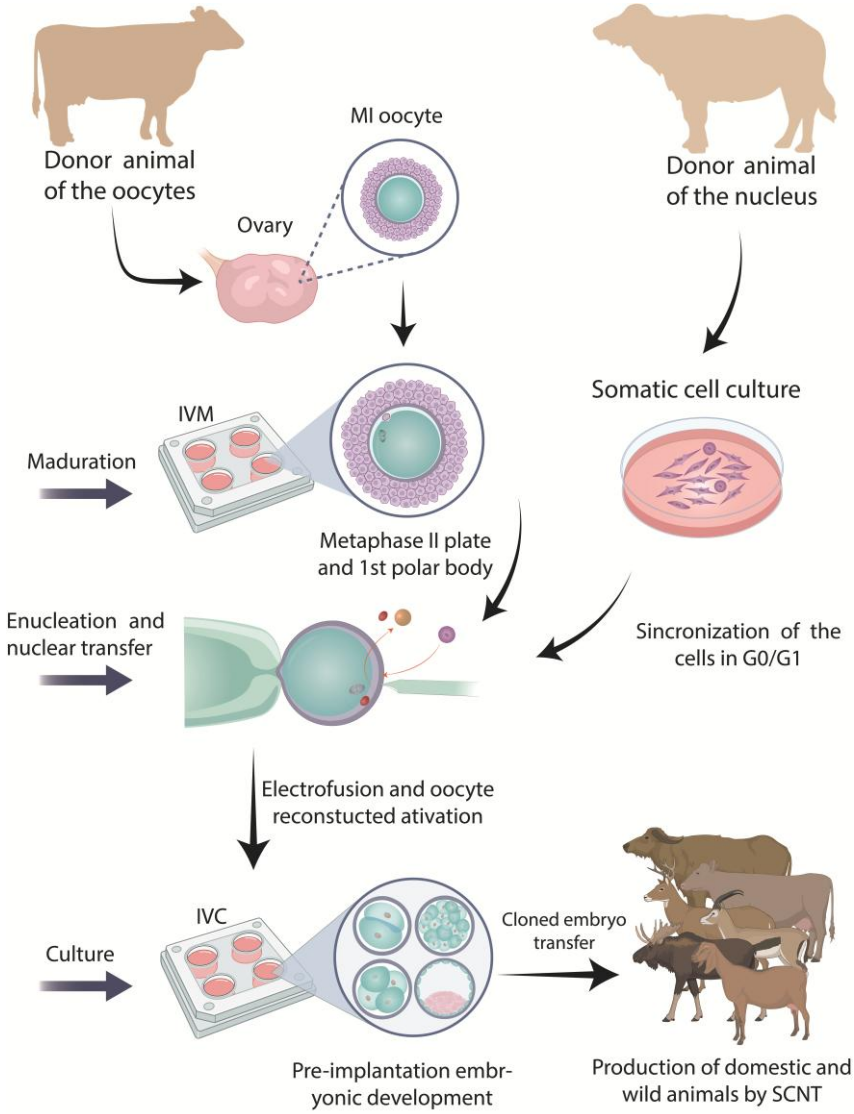

Figure 1. Schematic illustration of the production process of domestic and wild ruminants cloned by somatic cell nuclear transfer (SCNT).

2. Deficiencies and improvements in SCNT protocols

Despite the production of embryos through cloning in the 1980s, obtaining viable cloned animals remained a distant dream, and most researchers were ambivalent until the successful cloning of Dolly the sheep in 1996 (Wilmut et al., 1997). This historic and revolutionary achievement marked the first successful production of a cloned animal and garnered attention sparking a new wave of research in the field. Based on the knowledge gained from this pioneering technique, scientists have been able to apply cloning methods to achieve various genetic advancements. This has led to the cloning of many different animals, including chimeric animals (Cibelli et al., 1998). However, despite these advancements, the SCNT protocols still present several deficiencies and areas for improvement.

There are multiple factors related to cloning efficiency, with oocyte availability being very important for maturation and *in vitro* culture and therefore critical to the success of SCNT cell reprogramming (Galli & Lazzari, 2008). The events required for nuclear reprogramming to occur are varied and complex, and verification of their occurrence is highly rigorous as development progresses from oocyte activation to embryo development (Oback, 2008).

The reduced efficiency may be due to the failure of the oocyte to restore the totipotent state of the transferred nucleus through the nuclear reprogramming process (Meissner & Jaenisch, 2006). Thus, a minority of cloned embryos achieve complete reprogramming compatible with full-term development, while a large proportion of them show epigenetic dysregulation and abnormal gene

expression in the pre- and post-implantation phases (Sawai, 2009). Some, several attempts have been made to improve the outcomes of SCNT with limited effects, except for the use of some chromatin remodeling agents, such as TSA (Luo et al., 2013), or the use of embryonic stem cells as a donor nucleus (Xie et al., 2014) (Figure 2). Kawamura et al. (2009) performed a study on induced pluripotent stem (iPS) cells, indicating an essential role of the tumor suppressor gene p53 in nuclear reprogramming.

Another problem with SCNT is the incompatibility between mitochondrial DNA (mtDNA), which is responsible for encoding some of the subunits of the electron transfer chain responsible for ATP production. Furthermore, proper coordination between mtDNA and genomic DNA is essential for embryo development, since some factors are required for the replication, transcription, and translation of mtDNA encoded by nuclear DNA (Loi et al., 2011). Some mtDNA haplotypes strengthen embryonic development, proving the importance of compatibility between nuclear and mitochondrial DNA for embryonic development (Loi et al., 2011). Thus, some closely related species have already been successfully produced, such as cattle/guinea pig (Lanza et al., 2000) and sheep/mouflon (Loi et al., 2001).

After fertilization, the newly established genome starts to become transcriptionally active at different embryonic stages, depending on the species. Maternally expressed transcription factors, accumulated and stored in the cytoplasm of the oocyte, are responsible for activating the embryonic genome (Hu et al., 2010). Initial experiments with iSCNT between different species (bovine/pig and ovine/pig) showed that the transferred genome was not transcribed correctly in the host cytoplasm, leading to a halt in embryonic development (Fulka et al., 2008). However, more recent studies on the activation of the embryonic genome have proven to be very useful for iSCNT. As long as if the complete sequences of the main genes involved are identified, these genes can be microinjected into the donor nucleus to promote the maternal/zygotic transition in embryos originating from iSCNT (Loi et al., 2011).

Another factor is the availability of females to be used as recipients within the population of an endangered species, which is certainly limited. The genetics of the embryo recipient can be an even more important obstacle than the incompatibility between mitochondrial and genomic DNA or even the activation of the embryonic genome. Therefore, narrowing the boundaries between species before embryo transfer is a fundamental requirement for the successful multiplication of endangered genotypes (Loi et al., 2011).

To date, few studies have been successfully performed in extinct species. In this regard, the first animal derived from an extinct subspecies was obtained using fibroblasts from skin biopsies collected before death from the last female *Capra pyrenaica pyrenaica*. After one year of cryopreservation, these cells were used as karyoplasts when fused with the cytoplasts of a domestic goat to reconstruct embryos.

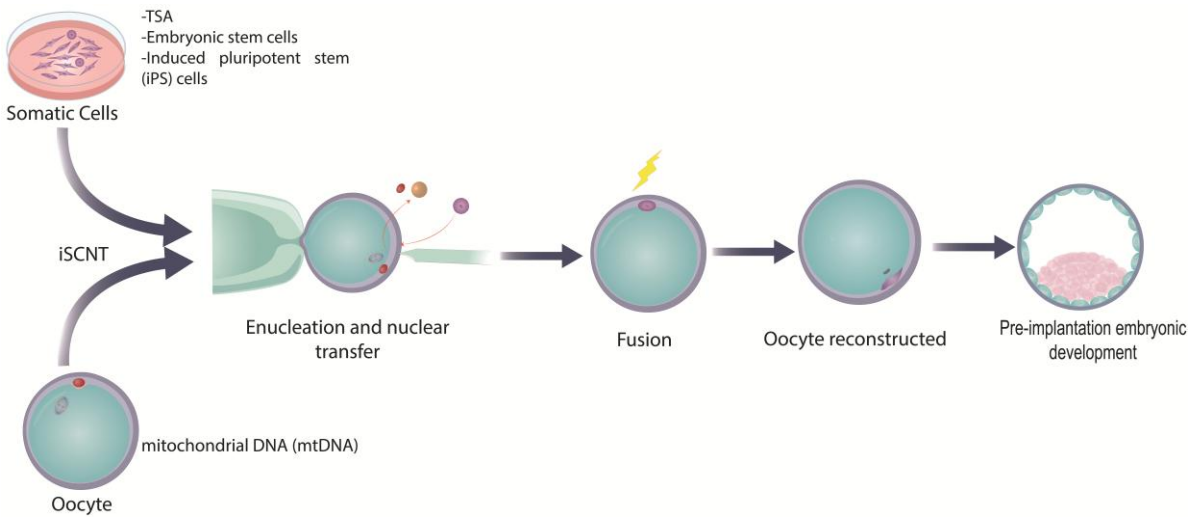

Figure 2. Limitations and Improvements in SCNT.

The results showed 5 pregnancies after transfer, of which only one reached term, which, shortly after birth, the animal died of pulmonary complications (Folch et al., 2009). In 2007, Li et al., found that the use of fibroblasts and *cumulus* cells from wild yak (*Bros grunniens*) of iSCNT.

3. Factors interfering in the success of SCNT/iSCNT

Despite the production of embryos through cloning in the 1980s, obtaining viable cloned animals remained a distant dream, and most researchers were ambivalent until the successful cloning of Dolly the sheep in 1996 (Wilmut et al., 1997). This historic and revolutionary achievement marked the first successful production of a cloned animal and garnered attention sparking a new wave of research in the field. Based on the knowledge gained from this pioneering technique, scientists have been able to apply cloning methods to achieve various genetic advancements. This has led to the cloning of many different animals, including chimeric animals (Cibelli et al., 1998). However, despite these advancements, the SCNT protocols still present several deficiencies and areas for improvement. Therefore, it is necessary to take into account certain points to improve the SCNT, as seen in Figure 3.

3.1. Donor cell

There is a vast array of scientific literature documenting diverse success rates in animal cloning, with the belief that the efficacy of SCNT may hinge on numerous biological factors related to the cell serving as the nucleus donor. These factors include age, cell origin, cell cycle phase, number of passages, telomere length, telomerase activity, or the capacity to differentiate into various tissue types (Rakha, 2015). Furthermore, challenges such as oocyte incompetence (poor cytoplasmic maturation), mitochondrial incompatibility, and oocyte trauma due to micromanipulation may also impact success rates. However, arguably the most crucial factor is the nucleus of the donor cell. This is not only because the cloned animal will essentially be a genetic replica of the donor's genome but also because the epigenetic state of the donor cell influences its reprogrammability by the cytoplasm of the enucleated metaphase II (MII) oocyte. Since the landmark achievement of the first successful cloning in an animal using differentiated adult somatic cells (Wilmut et al., 1997), various sources of somatic cells have been explored to enhance success rates in generating pre-implantation and post-implantation embryos, as well as pre- and post-natal development, ultimately leading to the birth of healthy offspring (Kato et al., 2000).

To date, abundant evidence suggests that somatic cells from diverse origins and various tissues can serve as nucleus donors (karyoplasts) for SCNT in both domestic and wild ruminants (Table 1). These tissues include mammary gland cells (Wilmut et al., 1997), fetal fibroblasts (Cibelli et al., 1998), adult animal fibroblasts (Lee et al., 2003; Saini et al., 2015; White et al., 1999), leukocytes (Galli et al., 1999), cumulus cells (Kato et al., 2000; Pan et al., 2014), oviductal cells, liver cells, muscle cells, kidney cells, heart cells (Kato et al., 2000), granulosa cells (Loi et al., 2001), semen-derived somatic cells (Nel-Themaat et al., 2008), testicular spermatic cord cells (Hoshino et al., 2009), epithelial cells (Saini et al., 2015), urine cells (Madheshiya et al., 2015), bone marrow mesenchymal stem cells (Kato et al., 2004; Kwong et al., 2014), adipose tissue-derived mesenchymal stem cells (Da Silva et al., 2016; Savy et al., 2021), amniotic fluid mesenchymal stem cells (Da Silva et al., 2016), skin-derived precursor cells (Xiao et al., 2016), muscle-derived satellite cells (Li et al., 2022), among others.

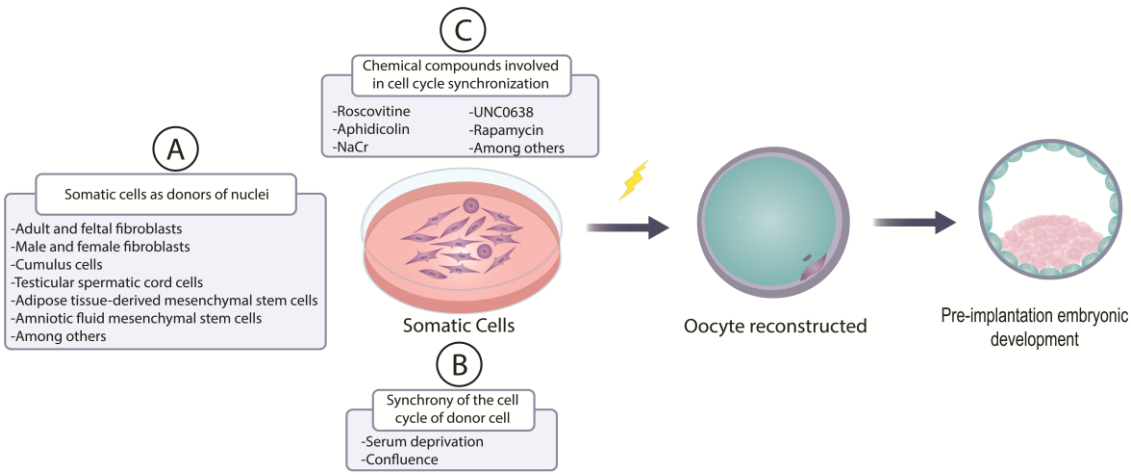

Figure 3. Key points to increase the production of clone embryos through somatic cell nuclear transfer (SCNT). A) Somatic cells as donors of nuclei, B) synchrony of the cell cycle, C) chemical compounds used to synchronize the cycle.

Table 1
Summary of some successful species on domestic and wild ruminants by SCNT

Domestic ruminants				
Species			Results	Authors
	Nucleus donor	Oocyte donor		
Sheep	Mammary gland cells	Sheep	Nascimento da ovelha Dolly	(Wilmut et al., 1997)
Goat	Fetal transgenic cell line	Goat	3 pups were born	(Baguisi et al., 1999)
Bovine	Fetal fibroblast	Bovine	3 transgenic calves	(Cibelli et al., 1998)
	Cumulus cells	Bovine	8 calves 4 died at birth	(Kato et al., 1998)
	Thawed testicular cells	Bovine	5 pregnancies and 4 cloned calves developed to term	(Hoshino et al., 2009)
	Fetal fibroblast	Bovine	10 healthy calves born	(Su et al., 2025)
Buffalo	Fetal fibroblast	Buffalo	3 animals born	(Shi et al., 2007)
Dromedary	Cumulus cells	Dromedary	1 calf born	(Wani et al., 2010)
	Fibroblasts and cumulus cells	Dromedary	A total of 9 calves born	(Wani & Hong, 2018)
	Fibroblast cells	Bovine	4 Full term calf delivered	(Wani et al., 2018)
	Adult fibroblasts	Dromedary	7 clones were born	(Moulavi et al., 2020)
	Fibroblast cells	Dromedary	11 live births	(Son et al., 2022)
Bactrian	Fibroblast cells	Dromedary	1 calf, died on day 7 postpartum	(Wani et al., 2017)
	Fibroblast cells	Dromedary	2 calf, died on day 7 postpartum	(Mansour et al., 2023)
Sheep	Amniotic cells	Sheep	3 second-generation cloned sheep produced by re-cloning; all 3 survived to date with normal vital signs	(Zhu et al., 2025)
Wild ruminants				
Species			Results	Authors
	Nucleus donor	Oocyte donor		
Sheep	Ovis ammon-fibroblast cells	Sheep	2 pregnancies that were lost on day 59	(White et al., 1999)
	Ovis ammon-fibroblast cells	Sheep	(22.1%) blastocyst	(Pan et al., 2014)
	Ovis canadensis mexicana-fibroblast cells	Sheep	14.3% blastocyst	(Hernández Martínez et al., 2020)
	Ovis canadensis mexicana-fibroblast cells	Sheep	16% blastocyst	(Vazquez-Avendaño et al., 2017)
	Mouflon-granulosa cells	Sheep	1 lamb, apparently normal	(Loi et al., 2001)
Goat	Esfahan mouflon-Fibroblast cells	Sheep	2 lambs, both died shortly after birth	(Hajian et al., 2011)
	Capra ibex-ibroblast cells	Goat	(11%) blastocyst	(Wang et al., 2007)
	Pyrenean ibex-adult fibroblast	Goat	1 born, died shortly after birth	(Folch et al., 2009)
Gaur	Gaur-adult fibroblast	Bovine	25% pregnancies, none term	(Lanza et al., 2000)
	Fibroblast cells	Goat, Bovine, Buffalo	blastocyst: gaur-bovine (12.10%), gaur-buffalo (5.10%) and gaur-goat (3.21%).	(Tasripoo et al., 2018)
Yak	Adult fibroblasts	Bovine	Aggregated blastocyst: Yak-bovine (40.38 %), blastocyst: Yak-bovine (25.34 %)	(Yauri et al., 2026)
Banteng	Adult fibroblast	Bovine	2 calves, 1 survived long term	(Janssen et al., 2003)
	Adult male and female fibroblasts	Bovine	(15%–28%) blastocysts 17% pregnancies, none term	(Sansinena et al., 2005)
Wild buffalo	Fibroblastic and epithelial cells	Buffalo	(50.6 vs 20.5%) blastocyst	(Saini et al., 2015)
	Fibroblast cells	Buffalo	(38.7%) blastocyst	(Priya et al., 2014)
Tragelaphus eurycerus isaaci	Fibroblast cells	Bovine	24% blastocyst	(Lee et al., 2003)
Takin	Fibroblast cells	Bovine	5% blastocyst	(Li et al., 2006)
Tibetan antelope	Fibroblast cells	Goat	(11%–25%) morula, (0%–11.1%) blastocyst	(Zhao et al., 2007)
Elande	semen-derived somatic cells	Bovine	21% Morula and blastocyst	(Nel-Themaat et al., 2008)
Arabian oryx	Fibroblast cells	Bovine	9.23% blastocyst	(Ammari et al., 2022)
Deer	Fibroblast cells	Deer	1 calf born	(Westhusin, 2003)
	Fibroblast cell	Bovine	4% blastocyst	(Venegas et al., 2006)
	Fibroblast cells	Bovine, goat	Morula-goat/goat:40%, Deer/goat:50% and blastocyst: 0%	(Magalhães et al., 2020)
	Fibroblast cells	Bovine	Blastocyst-cattle/cattle:11.3% Deer/cattle: 5.9%	(Melo et al., 2022)

All these cell types demonstrated development up to the morula and blastocyst stage, but only a few managed to generate viable offspring after transfer. Conversely, Saini et al. (2015) demonstrated that fibroblasts are easier to reprogram than those of epithelial origin, despite originating from the same tissue in iSCNT experiments, resulting in higher success rates. However, it has long been recognized that the developmental potential of cloned embryos derived from differentiated cells is lower, whereas less differentiated cells tend to be more effective as nucleus donors (Li et al., 2003). Additionally, different cell types possess unique epigenetic states characteristic of the tissue of origin, resulting in varying reprogramming potential upon fusion with the cytoplasm of a mature oocyte. However, the optimal cell type for cattle remains unclear (Savy et al., 2021). Some studies suggest that there is no significant difference in the development rate or quality of the embryo when using less differentiated donor cells, such as mesenchymal stem cell (MSCs) from adipose tissue compared to adult fibroblasts (Savy et al., 2021). Conversely, Da Silva et al. (2016) did not achieve successful term pregnancies with skin fibroblast cells, in contrast to less differentiated cells like MSCs from amniotic fluid and adipose tissue. Similarly, successful pregnancies were achieved using MSCs from bone marrow (Kato et al., 2004). Additionally, MSCs from caprine bone marrow exhibited greater *in vitro* development up to the blastocyst stage compared to ear fibroblasts (Kwong et al., 2014). These findings support the notion that less differentiated cells are generally more effective as nucleus donors due to their ease of reprogramming. It is important to note that different cell types possess unique epigenetic states characteristic of the tissue of origin, influencing their reprogramming potential upon fusion with the cytoplasm of a mature oocyte. However, in horses, inconsistent results have been reported. While more differentiated cells like fibroblasts have resulted in successful offspring until birth, less differentiated cells such as iPSCs and UC-MSCs have not yielded offspring, although they may exhibit higher rates of blastocyst formation (Olivera et al., 2016).

3.2. Cell cycle synchrony

It is now recognized that nuclear donor cells in the G0/G1 phase are crucial and necessary to maintain correct chromosomal ploidy and synchronization prior to transfer, thereby enhancing the success of animal production through SCNT. Cell cycle arrest in the G0/G1 phase is particularly beneficial for reprogramming. Nucleus donor cells are typically naturally in the G0/G1 stage of the cell cycle and can be synchronized through various methods, including serum deprivation (Gibbons et al., 2002; Selokar et al., 2012; White et al., 1999; Wilmut et al., 1997), inhibition via contact or confluence (Folch et al., 2009; Selokar et al., 2012), control of cell division time through "shake-off" of active cells (Goto et al., 2013; Kasinathan et al., 2001), and the use of cell cycle inhibiting chemicals such as roscovitine (Cho et al., 2005; Gibbons et al., 2002; Selokar et al., 2012; Sun et al., 2008), aphidicolin (Shi et al., 2007), crotonato de sodio (NaCr) (Zhao et al., 2024), UNC0638 (Wang et al., 2017), rapamycin (Xu et al., 2021), among others.

Initially, Dolly the sheep was cloned from cells likely in the G0/G1 phase of the cell cycle. Wilmut et al. (1997) suggested the use of cultured cells without serum to induce exit from the growth cycle (G0) by reducing serum concentration, asserting that this was necessary to support cloned embryonic development to term. Subsequently, Cibelli et al. (1998) utilized populations of dividing cells, proposing that the G0 stage was not necessary due to serum deprivation, and that calves could be produced from actively dividing fibroblasts. In another study by Kasinathan et al. (2001), the G0 (confluence) and G1 (shake-off) phases of the cell cycle were evaluated as nucleus donors, resulting in successful term pregnancies from the G1 phase. These findings indicate the importance of the donor cell cycle stage for SCNT, particularly during late fetal development, with cells in the G1 interphase supporting higher development rates than those in G0 (Goto et al., 2013; Kasinathan et al., 2001).

Although confluence or serum deprivation are the most used methods to interrupt the G0/G1 phase of the cell cycle, serum deprivation only increases a proportion of cells in the G0/G1 phase compared to the control. Consequently, it raises levels of reactive oxygen species (ROS), induces apoptosis and DNA damage in donor cells, and leads to abnormal chromosome duplication (Xu et al., 2021). This is likely one of the most influential reasons for the low rate of pre- and post-implantation embryonic development, high fetal mortality, and abnormal fetal development due to donor cell damage.

Likewise, in the cloning of wild ruminants using SCNTi, fibroblast cells were mainly used with synchronization methods such as confluence, serum deprivation, and active division as nuclear donors. In SCNTi, oocytes can reprogram the nucleus of a differentiated cell even between different species or genera (Melo et al., 2022). However, the efficiency of intraspecific cloning development tends to sur-

pass that of interspecific cloning (Li et al., 2006; Nel-Themaat et al., 2008; Tasripoo et al., 2018; Wang et al., 2007). Priya et al. (2014) reported no variation in blastocyst rates in both wild and domestic buffalo, similarly, when cow oocytes were used for *A. oxys* (Ammari et al., 2022). In interspecies cloning, the factors that could contribute to improved outcomes are not yet fully known, but a comprehensive understanding of the molecular mechanisms involved in reprogramming could provide insights for improvements. Despite the evolutionary distance between these species, morulas and blastocysts have already been obtained using this technique (Li et al., 2006; Melo et al., 2022; Nel-Themaat et al., 2008; Tasripoo et al., 2018; Wang et al., 2007), along with the birth of full-term animals (Folch et al., 2009; Hajian et al., 2011) with normal appearances (Loi et al., 2001). Undoubtedly, there is hope that SCNT can help preserve or alleviate endangered species, for which it is necessary to understand and apply the generated knowledge, such as the selection and synchronization of donor cells to improve blastocyst rates.

On the other hand, over the years, there has been a growing need to seek new alternatives for synchronizing the cell cycle, combined with reducing damage and increasing the arrest of donor cells in the G0/G1 phase before transfer to the oocyte cytoplasm (Xu et al., 2021; Wang et al., 2017; Yagcioglu et al., 2023).

3.2.1. Strategies to improve the efficiency of cell cycle synchrony

Roscovitine has been used as a specific inhibitor of cyclin-dependent kinase (CDK) to improve synchronization in the G0 (quiescent) or G1 phase of the cell cycle in somatic cells such as ruminant fibroblasts (Yagcioglu et al., 2023). Thus, it has been shown to lead to higher blastocyst rates compared to serum deprivation or confluence (Selokar et al., 2012). However, Gibbons et al. (2002) observed lower blastocyst rates with roscovitine compared to serum inhibition, but they obtained more live calves, suggesting that the use of roscovitine for synchronization before transfer is feasible to improve survival rates in late-developing embryos.

Similarly, a study by Shi et al. (2007) demonstrated that live offspring obtained using aphidicolin plus serum starvation in fibroblasts and granulosa cells resulted in the arrest of more cells in the G0/G1 phase with a lower proportion of cells exhibiting DNA fragmentation. This treatment also increased the number of embryos derived from these cells that developed into blastocysts, suggesting that aphidicolin plus serum deprivation could be a potential method for synchronizing donor cells in the G0/G1 phase.

The effect of sodium crotonate (NaCr) on the cell cycle has also been tested. Li et al. found that increasing the treatment time and concentration leads to progressive inhibition of cell cycles in different fibroblasts. Specifically, the group treated with 20 mM showed a higher proportion of cells in the G0/G1 phase and a lower proportion of cells in the G2/M phase compared to the group treated with 10 mM. Additionally, somatic cells treated with NaCr could improve cleavage rates by SCNT (Li et al., 2022). Recently, SCNT of donor cells cultured in 5 mM NaCr showed a 38.1% blastocyst development rate, significantly higher than the 25.2% observed in the control group (Zhao et al., 2024). (Li et al., 2022).

UNC0638 is another reported chemical compound that affects the cell cycle. This compound significantly increased the proportion of cells in the G0/G1 phase of the cell cycle compared to the untreated group, while the number of cells in the G2/M and S phases decreased (Wang et al., 2017). Furthermore, rapamycin is another compound known to increase cell cycle arrest in the G0/G1 phase and can even reduce levels of apoptosis, making the cells more available for nuclear transfer at the desired stage and of good quality to enhance embryonic development (Xu et al., 2021).

4. Epigenetic reprogramming

Epigenetics refers to the differential alteration of gene expression without any change in the DNA sequence, which is conferred by the physical and biochemical properties of chromatin. This is achieved through two main pillars, which vary according to cell type: DNA methylation status and histone modifications, influenced by various factors triggering these changes (Long et al., 2014). As mentioned, each cell type bears a specific programming or epigenetic mark of differentiation. After transferring to the enucleated MII oocyte, the donor cells require nuclear reprogramming, also known as epigenetic reprogramming. This process involves erasing the epigenetic memory of the previous nucleus, thereby conferring the totipotent state on the newly formed embryos, and adding new epigenetic information. Despite embryos cloned by SCNT developing to term, the technology remains inefficient.

Various researchers point out that inadequate, failed, or incomplete epigenetic reprogramming and epigenetic aberrations during development are the primary causes leading to the abnormal development of cloned embryos at different points

before and after implantation (Behdarvandiyan et al., 2021; Li et al., 2022). Additionally, offspring of cloned animals often exhibit epigenetic anomalies, suggesting an epigenetic origin. However, SCNT-mediated nuclear reprogramming is still poorly understood, and the main factors determining the developmental potential of cloned embryos remain unclear (Wang et al., 2020).

4.1. Strategies to improve epigenetic reprogramming efficiency

Several researchers have clearly pointed out that, in donor cells, low levels of DNA methylation or high levels of histone acetylation facilitate reprogramming. However, SCNT typically produces embryos with extremely high DNA methylation and lower levels of histone acetylation (Beaujean et al., 2004). This is because differentiated cells carry an epigenetic load that is challenging for the oocyte to reprogram, thus reducing the efficiency of this technology. Moreover, somatic cells are known to exhibit more extensive DNA methylation patterns than embryonic or pluripotent cells, making the genome of the latter more easily reprogrammed and potentially more efficient as nuclear donors in somatic cell cloning (Long et al., 2014). Therefore, since the production of the first cloned sheep using differentiated adult cells, several efforts have been made to improve the efficiency of epigenetic reprogramming in somatic cells. These efforts include interventions before and/or shortly after transfer to increase epigenetic reprogramming and the speed of reprogramming.

Numerous chemical compounds have been reported to aid in cellular reprogramming, acting as histone deacetylase inhibitors (HDACi), histone methyltransferase inhibitors (HMTi), DNA methyltransferase inhibitors (DNMTi). Examples include trichostatin-A (TSA) (Akagi & Matsukawa, 2022; Ding et al., 2008; Hosseini et al., 2016), scriptaid (Rissi et al., 2019; Wang et al., 2011), sodium crotonate (NaCr) (Li et al., 2022), Trans-2-phenylcyclopropylamine (2-PCPA) (Mao et al., 2018), UNC0638 (Wang et al., 2017), and 5-aza-2′-deoxycytidine (5-aza-dC) (Ding et al., 2008) (Figure 4). However, despite an apparent improvement in rates, they remain low for early development and further reaching terms compared to conventional *in vitro* fertilization.

One of the most widely used compounds is TSA, which increases the number of acetylated histones by inhibiting the histone deacetylase enzyme, leading to chromatin relaxation. This relaxed state promotes an open chromatin configuration, potentially facilitating access to regions involved in pluripotency and making them more accessible to reprogramming factors present in oocytes after transfer (Hosseini et al., 2016). Ding et al. (2008) found that pre-treatment with TSA before transfer helps increase blastocyst formation rates, and when combined with 5-aza-dC, it further enhances these rates. Additionally, TSA has been shown to increase histone acetylation and decrease DNA methylation in 2-cell embryos. TSA has also been tested in post-activation pseudozygotes, leading to changes in the epigenetic state of embryos produced by SCNT, improving blastocyst formation competence, pregnancy rates, and reaching term (Sawai et al., 2012). Furthermore, treatment of donor cells with TSA improved the pre-implantation development of embryos reconstructed with treated cells in buffalo (Luo et al., 2013). However, the study conducted by Akagi & Matsukawa (2022) showed only a slight improvement in blastocyst rates, with no significant differences compared to the control group. Furthermore, in sheep, TSA appears to be ineffective as blastocyst rates were lower than the control (Al-Ghadi et al., 2020). In Murrah buffalo, using adult male and female fibroblastic cells with a treatment of 50 nM TSA and 7.5 nM 5-aza-dC, the blastocyst rate of treated male and female embryos was significantly lower than the control group (Sandhu et al., 2023).

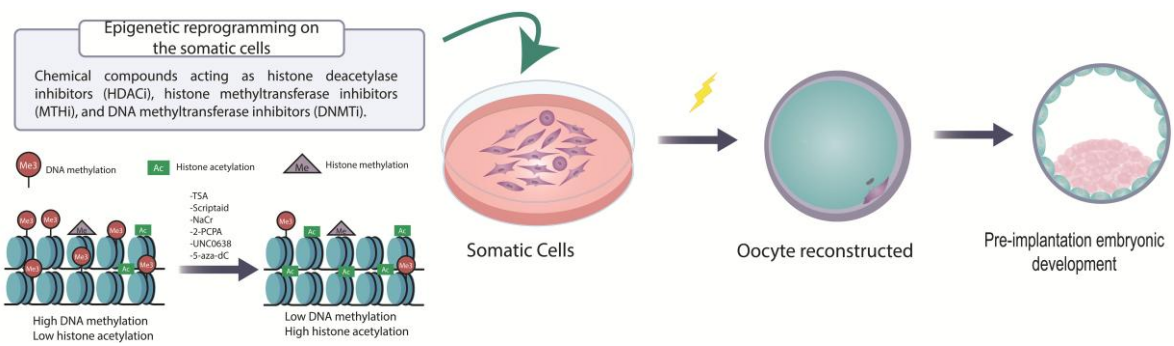

Figure 4. Chemical compounds for epigenetic reprograming.

Likewise, SCNT bovine embryos treated with TSA showed better development before implantation (Akagi & Matsukawa, 2022), but no improvement in the offspring rate (Sawai et al., 2012).

Scriptaid can increase cleavage rates, blastocyst formation, and blastocyst hatching rates of embryos produced by SCNT after post-activation treatment. This compound increases the average intensity of H3K9 acetylation and Oct4 and IFN-t transcripts while decreasing the average intensity of H3K9 methylation, suggesting its beneficial effects on the development, nuclear reprogramming, preimplantation, and implantation of SCNT embryos (Rissi et al., 2019; Wang et al., 2011). In sheep SCNT embryos, Scriptaid supported development to the blastocyst stage, whereas embryos treated with trichostatin A (TSA) did not reach this stage, possibly due to species differences or TSA toxicity at high concentrations (Al-Ghadi et al., 2020).

Another compound that could be explored in future research on SCNT to enhance epigenetic reprogramming is NaCr. NaCr has been noted for its role in gene expression regulation and maintenance of stem cell pluripotency; furthermore, it enhances cell cycle synchronization rates (Zhao et al., 2024). It may promote reprogramming of somatic cells and improve cloned embryo development (Li et al., 2022). Recently, Zhao et al. demonstrated that NaCr treatment in bovine fibroblasts increased blastocyst development, with rates significantly higher than those in the control group (Zhao et al., 2024). Further studies are needed to fully understand its potential benefits in SCNT and its effects on epigenetic modifications during the reprogramming process.

2-PCPA is a specific inhibitor of lysine-specific demethylase 1 (LSD1), which can improve the aberrant development of cloned embryos by increasing the level of H3K4 dimethylation (H3K4me2). This intervention mainly affects the transcriptional activation of some genes. On the other hand, H3K4me2 demethylation is primarily carried out with the help of LSD1, which inhibits gene expression. Cloned embryos often exhibit aberrant levels of H3K4me2 modification. Moreover, the low levels of H3K4me2 in cloned embryos suggest that increasing H3K4me2 levels by adding H3K4 demethylation inhibitors may be beneficial for early embryonic development (Mao et al., 2018). Another aberrant increase observed in cloned embryos is H3K9me2, characterized by a higher level of histone H3K9 dimethylation (H3K9me2). This increase may serve as a barrier for somatic nucleus reprogramming in cloned embryos.

UNC0638 is a type of small molecule that can specifically inhibit the enzymatic activity of histone methyltransferase EHMT2 and reduce the levels of H3K9 dimethylation (H3K9me2) in cells (Fu et al., 2014). It is suggested that UNC0638 may have a beneficial effect in terms of modifying the abnormal state of H3K9me2 and gene expression in cloned embryos. However, embryonic development may not necessarily improve despite these modifications (Fu et al., 2014; Wang et al., 2017). On the other hand, Sampaio and colleagues indicated that this compound also affects other epigenetic marks such as H3K9me3, 5mC, and 5hmC. Furthermore, despite reducing the levels of H3K9me2 and H3K9me3 in SCNT-cloned blastocysts, it does not improve their preimplantation embryonic development. The authors also note that specific reduction of H3K9me2 by inhibiting EHMT1/2 during nuclear reprogramming affects the levels of H3K9me3, 5mC, and 5hmC in preimplanted bovine embryos (Sampaio et al., 2020).

Epigenetic reprogramming via DNMTi can be achieved using 5-aza-dC. This compound reduces DNA methylation by inhibiting the enzyme DNA methyltransferase (Ding et al., 2008). This compound reduces DNA methylation by inhibiting the enzyme DNA methyltransferase (Ding et al., 2008; Enright et al., 2005). Although it generates a slight increase in blastocyst rates, its benefits are not apparent (Ding et al., 2008). However, the reduction of DNA methylation in donor cells is expected to improve the efficiency of cloned transferred embryos by providing donor cells with epigenetic characteristics similar to *in vivo* embryos (Enright et al., 2005).

5. Abnormalities in clones

Embryonic development and birth rates remain low, ranging from 5 to 15% (Keefer, 2015; Long et al., 2014; Wells et al., 2003). For example, in sheep, the birth of three offspring was reported, of which one was apparently normal (Loi et al., 2001), and two died shortly after birth (Hajian et al., 2011); while in goats they also died after birth (Folch et al., 2009). In cattle, where two offspring were produced, only one survived for a long period (Janssen et al., 2003; Selokar et al., 2025; Su et al., 2025)

The reduction in birth rate and survival after birth is main due to placental and fetal abnormalities, which induce spontaneous abortions or death after birth, and is speculated to be due to aberrant epigenetic reprogramming (Figure 5). Epigenetic problems are key to the pathologies of cloned animals because reduced or insufficient reprogram-

ming of the donor nucleus results in abnormal gene expression, leading to abnormal development of the placenta and fetus. Additionally, death can occur during embryonic development after implantation, in the perinatal period (immediately after birth or in the first hours), and during the first 6 months (Chavatte-Palmer et al., 2004).

The reduction in birth rate and survival after birth is mainly due to placental and fetal abnormalities, which induce spontaneous abortions or death after birth. Epigenetic problems are related to pathologies of cloned animals reflecting the failure of donor nucleus reprogramming, leading to abnormal development of the placenta and fetus. In this context, in ruminants, a post-implantation silenced imprinted gene called Mash2 has been shown to be expressed specifically in the placenta and in greater abundance during the period of trophoblast proliferation immediately before implantation (Arnold et al., 2006). Thus, in the work carried out by Arnold et al. (2006), 75% of SCNT embryos did not survive until day 40, perhaps because insufficient numbers of specialized binucleated/giant trophoblast cells (TGC) were present after this time to allow implantation and cotyledon development.

Hill et al. (2000) provide information on possible reasons for developmental failures related to placental phenotypic abnormalities, such as reduced number (~half) of macroscopically visible cotyledons, hemorrhagic areas in the cotyledons and chorioallantois, variations in the flat cuboidal chorionic epithelium with marked decrease in vascularization, and rudimentary cotyledonary areas revealing an apparently normal epithelium with villi formation. Other effects are associated with placental abnormalities, such as placentomegaly, reduced vascularization, hypoplasia of the trophoblastic epithelium, and alteration of the basement membrane. Other effects have been reported in cattle and sheep associated with placental abnormalities, such as placentomegaly, reduced vascularization, hypoplasia of the trophoblastic epithelium, and alteration of the basement membrane, among other reported abnormalities, such as enlarged umbilical cord, placental hypertrophy, and abnormal size of placental cotyledons (Palmieri et al., 2008). Additional placental abnormalities include enlarged umbilical vessels, hydroallantois, placentomegaly, and elongated placentomes (Chavatte-Palmer et al., 2012; Constant et al., 2006; Heyman et al., 2002; Hill et al., 2000; Kohan-Ghadr et al., 2008). These abnormalities negatively affect the mother-embryo/fetus interaction directly or indirectly, compromising the nutrition and normal development of the developing clone.

Whereas, in fetal abnormalities, it has been observed that clones suffer from large/abnormal animal syndrome (LOS) (Constant et al., 2006; Rissi et al., 2019), small fetuses for their developmental stage, delayed limb and head development, and liver abnormalities (Hill et al., 2000). Respiratory failure due to lung problems (Chavatte-Palmer et al., 2004; Hill et al., 1999) and abnormal kidney development (Chavatte-Palmer et al., 2004) are also common in cloned animals, in addition to cardiovascular pathologies (Hill et al., 1999), and various other complications, including liver (Chavatte-Palmer et al., 2004) and cardiopulmonary diseases (Hill et al., 1999; Hill et al., 2000). Regarding parturition, clones may present delayed parturition by up to 30 days (Gao et al., 2019).

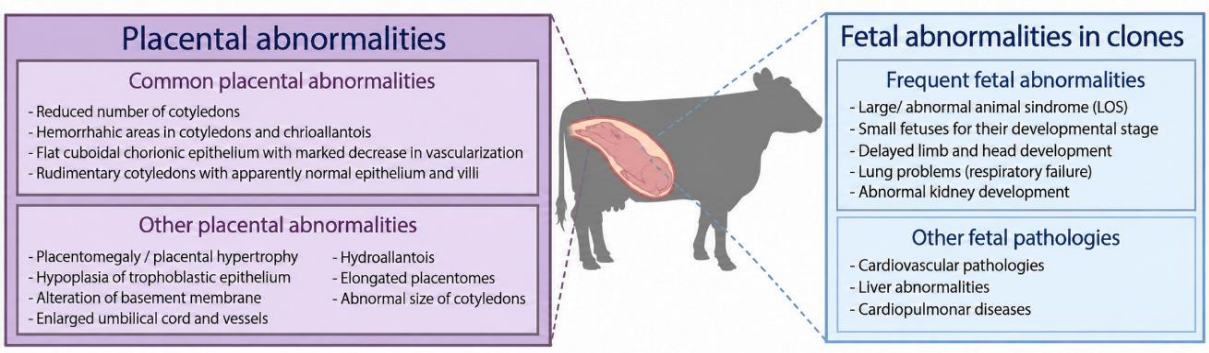

Figure 5. Embryonic, placental, and fetal abnormalities associated with aberrant epigenetic reprogramming in cloned ruminants.

6. Current challenges and future perspectives

SCNT and iSCNT continue to face biological constraints that limit their practical application in producing cloned domestic and wild ruminants. Despite three decades of progress, blastocyst formation, term pregnancy, and live-birth rates remain low, and the resulting offspring frequently display placental, metabolic, and epigenetic abnormalities (Adams et al., 2024; Pankammoon et al., 2025). These limitations do not stem from a single factor but from the cumulative and interdependent interaction of several molecular barriers acting throughout nuclear reprogramming.

One of the main obstacles reported in ruminant cloning is the persistence of epigenetic marks inherited from the somatic donor cell, which prevents complete erasure of transcriptional memory and compromises embryonic genome activation (EGA) during the maternal-to-zygotic transition (Ogura et al., 2021; Zhou et al., 2020).

In bovine and ovine species, aberrant DNA methylation patterns and histone modifications such as H3K9me3 and H3K4me3 delay or block the chromatin remodeling required to reach a functional totipotent state (Li et al., 2025). Recent murine models have shown that combining overexpression of histone demethylases (Kdm4d and Kdm5b) with histone deacetylase inhibitors such as trichostatin A (TSA) can simultaneously overcome pre-implantation epigenetic barriers and post-implantation non-canonical imprinting mediated by H3K27me3 (Li et al., 2025). In ruminants, however, these treatments have shown inconsistent outcomes depending on the donor–recipient pairing; TSA improved blastocyst formation in gaur–bovine embryos but had no significant effect in other interspecies models, demonstrating that the efficacy of epigenetic modifiers is highly species-specific (Srirattana et al., 2012; Zuo et al., 2014).

When embryo reconstruction combines the somatic nucleus of a wild species with the enucleated cytoplast of a closely related domestic species as in gaur–bovine, Przewalski's gazelle–bovine, or Bactrian camel dromedary pairings additional layers of incompatibility arise (Lanza et al., 2000; Wani et al., 2017; Zuo et al., 2014). Phylogenetic divergence between donor and recipient species negatively affects nuclear pore complex assembly and the transport of maternal transcription factors, impairing proper embryonic genome activation (Adams et al., 2024; Pankammoon et al., 2025). Compounding this, mitonuclear incompatibility arises from the mismatch between donor nuclear DNA and the mitochondrial DNA inherited predominantly from the recipient oocyte, disrupting electron transport chain assembly and oxidative phosphorylation and thereby compromising the reconstructed embryo's energy supply (Ma et al., 2016; Jiang et al., 2011). The resulting mitochondrial heteroplasmy can further interfere with tricarboxylic acid cycle intermediates such as acetyl-CoA and α-ketoglutarate, molecules that directly regulate DNA methylation and histone acetylation, establishing a functional link between mitochondrial metabolism and nuclear epigenetic regulation (Matilainen et al., 2017). This interdependence explains why targeting epigenetic reprogramming in isolation, without addressing mitonuclear and nucleocytoplasmic compatibility, has proven insufficient to consistently improve iSCNT efficiency in ruminants.

The quality and cell-cycle status of the donor somatic cell are additional determinants of cloning efficiency in ruminants. Cell-cycle synchronization through confluence, serum starvation, or roscovitine treatment in bovine fetal fibroblasts has been shown to alter the proportion of cells in the G0/G1 phase, a condition generally considered more favorable for nuclear reprogramming after transfer (Cho et al., 2005). Complementing this, optimizing embryo culture media according to species-specific metabolic requirements, together with antioxidant supplementation (e.g., vitamin C) and mitoprotective molecules such as coenzyme Q10 or resveratrol, helps reduce the oxidative stress generated during oocyte manipulation and embryo reconstruction (Pankammoon et al., 2025). Although these strategies do not directly resolve genomic incompatibility, they reduce cumulative cellular damage and favor a more supportive environment for reprogramming.

The development of next-generation sequencing (NGS) technologies, including single-cell RNA sequencing (scRNA-seq), chromatin immunoprecipitation sequencing (ChIP-seq), and the assay for transposase-accessible chromatin using sequencing (ATAC-seq), has enabled more precise characterization of the gene networks and chromatin dynamics governing embryonic genome activation across different donor–recipient combinations (Pankammoon et al., 2025). These tools facilitate the identification of candidate genes for targeted intervention and the rational selection of the most suitable recipient species for a given wild donor. In parallel, CRISPR/Cas9-based gene editing is emerging as a promising strategy for correcting nuclear genes encoding mitochondrial proteins, thereby improving mitonuclear compatibility (Ma et al., 2016). Nevertheless, its direct application to the mi-

tochondrial genome remains limited by the scarce DNA repair machinery of mitochondria and by technical difficulties in precisely delivering editing components into the mitochondrial matrix, which calls for careful evaluation of potential off-target effects before routine use in conservation programs.

Despite these technical limitations, iSCNT remains one of the few biotechnological tools capable of generating viable offspring from the genetic material of critically endangered or functionally extinct wild species. The cases of the gaur (Lanza et al., 2000), the banteng (Sansinena et al., 2005), the Bactrian camel (Wani et al., 2017), and the wild buffalo (Priya et al., 2014) illustrate how interspecies cloning can complement germplasm banks and so-called "frozen zoos," helping preserve genetic variability in populations with few reproductively viable individuals (Borges & Pereira, 2019). However, the low efficiency observed evidenced by high neonatal mortality, as occurred in the first cloned gaur underscores that cloning should not be regarded as a substitute for conventional *in situ* conservation strategies, but rather as a complementary tool within an integrated approach that includes population management, conventional assisted reproduction, and habitat preservation (Rola et al., 2021). Moving forward, progress in SCNT and iSCNT for domestic and wild ruminants will depend on integrating genomics, epigenomics, and mitochondrial biology within protocols tailored to each specific donor–recipient pairing (Pankammoon et al., 2025). Rather than targeting a single mechanism in isolation, future research should simultaneously address nucleocytoplasmic compatibility, mitonuclear coordination, and epigenetic reprogramming, recognizing their interconnected nature. The incorporation of advanced imaging techniques, such as super-resolution microscopy and fluorescence recovery after photobleaching (FRAP), could allow real-time visualization of nuclear pore complex assembly and maternal factor transport in reconstructed ruminant embryos (Pankammoon et al., 2025).

Likewise, standardizing protocols for somatic cell cryopreservation and for recovering viable nuclei from post-mortem tissue will expand the range of options for species with limited donor availability (Loi et al., 2001). Taken together, these technological advances, combined with a rational selection of donor–recipient pairings based on phylogenetic distance (Meredith et al., 2011), will progressively translate experimental findings into practical applications for producing cloned ruminants and for the effective conservation of threatened wild species' biodiversity.

7. Conclusions

The conservation of animal biodiversity has become one of the most critical issues for researchers around the world. In this context, SCNT has emerged as a biotechnology aimed at safeguarding wild animals that are in danger of extinction. However, SCNT faces limitations such as low success rates in pre- and post-implantation development, as well as clone abnormalities, which reduce birth and survival rates to adulthood. Additionally, the need for high specialization in cloning, high costs, and ethical and legal issues, among other factors, further limit this technique.

To increase success rates in animal cloning, efforts have been focused on optimizing the technique, such as selecting the donor cell and synchronizing cells. Furthermore, over the years, various strategies have been implemented, such as using chemical compounds that aid in cell cycle synchronization and epigenetic modifications. However, success rates in obtaining ruminant animal clones remain low compared to other species, such as mice.

Through this review, we have shown the common abnormalities observed in cloned animals, deficiencies in cloning, the importance of donor cells and cell cycle synchronization, as well as strategies to improve cell cycle synchronization and epigenetic reprogramming. Therefore, focusing on these points could help improve cloning efficiency and develop new approaches that increase success rates in producing clones via SCNT in ruminant species, both domestic and wild.

Author contributions

IMLA and VJFF contributed to initiation of the study; **IMLA, DKBQ, MSC, CAPO, MCH, NABN, FKPS, and LMM** wrote sections of the manuscript and revised it for content. All authors contributed to manuscript revision and read and approved the submitted version.

Funding: The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES, Brasília, Brazil) for the first author's scholarship.

Conflict of interest: The authors have no conflict of interest to declare.

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