

Somatic Cell Nuclear Transfer in Mammals: Reprogramming Mechanism and Factors affecting Success

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Abstract

During the natural event of fertilization highly differentiated spermatozoa may revert to the totipotency of early embryonic stages. Such cells have the capacity to develop into a whole organism or to differentiate into any of its cells or tissues. Reversion is driven by the cytoplasm of the mature oocyte which is capable of reprogramming the donor nucleus. It is likely that the idea of using the laboratory technique of somatic cell nuclear transfer (SCNT) for creating an ovum with a donor nucleus stemmed from this observation. Many attempts have been made to determine the underlying mechanism of reprogramming and to improve nuclear transfer procedures in order to optimize the outcomes of SCNT. Although numerous reports have demonstrated the proof of principle of using SCNT technology to clone fertile offspring in several different mammalian species, success rates remain low. Most failures appear to result from incomplete reprogramming of the donor nucleus in an enucleated oocyte. The pressing need to improve the efficiency of SCNT may require a modified or entirely novel methodology to be established. This brief review discusses the mechanism responsible for reprogramming and considers several areas for research into SCNT in mammals that could yield beneficial outcomes.

Keywords: Somatic cell nuclear transfer; reprogramming; clone; oocyte; mammal

Introduction

It is apparent that advancements in reproductive technologies such as artificial insemination, embryo transfer and cloning by somatic cell nuclear transfer (SCNT) have played important roles in both human fertility clinics and the livestock industry. For the latter, nuclear transfer has been used to produce a large number of genetically identical valuable offspring. Hence, this technology has been proven to enhance breeding of domesticated animals [1]. Consequently, the production of cloned mammals by SCNT is a field of study attractive to many researchers. Dolly the sheep, born in 1997, represented the first successful cloned offspring produced using adult mammary somatic cells [2]. Since then, it has also proved possible to produce newborn young from SCNT-derived embryos in a wide range of mammal species, for example mice [3], rabbits [4,5], goats [6], pigs [7,8], water buffalo [9], cattle [10,11] and camels [12]. Moreover, in order to facilitate routine production of live offspring, the capacity has developed to freeze nuclear transfer embryos long-term by the vitrification method [13].

Despite these noted accomplishments, however, the pace of progress of nuclear transfer in mammals has appeared slow, mainly because of extremely low success rates with regard to SCNT embryo production and pregnancy. Furthermore, a number of abnormalities occur with SCNT offspring such as increased birth weight [14,15] and respiratory problems [16]. This may also be explained by inaccuracies in the reprogramming of the nucleus in enucleated oocytes. However, Mizutani et al. [17] claim that the failure of reconstructed embryos to

develop to term is a result of cytogenetic abnormalities that occur during the early cleavage stages of those embryos.

Nuclear transfer is based on the unique ability of metaphase II-arrested oocyte cytoplasts to convert transplanted somatic cell nuclei by electrofusion to become capable of completely reversing from differentiated stages to totipotency [18]. Interestingly, a recent study by Guan et al. demonstrated that goat oocytes can reprogram human skin cells [19]; although there was a high level of abnormal chromosomes, this provides proof of principle that reconstructed embryos from interspecies sources can develop to blastocyst stages.

To date, the mechanism of reprogramming, or how genomic reprogramming occurs, remains largely elusive [3,18]. However, recent reviews have identified in part the underlying mechanism of reprogramming in host egg cells [20-22]. Although the efficiency of SCNT has improved, efforts to improve the success rate further are continuing in an attempt to make this technology of increased commercial interest to livestock breeders. Nuclear transfer is a technically demanding task, with practitioners only improving their skills through experimentation and experience [3]. Development of nuclear transfer has advanced through the availability of protocols including simplified manipulation of recipient oocytes and nucleation using handmade cloning methods [8,23-25], and modification of the in vitro culture system [26].

The published literature covers many aspects of the application of SCNT in mammals. This review focuses on the process of reprogramming of the donated somatic nucleus in the recipient enucleated oocyte. In addition to this underlying mechanism, the importance of certain factors which affect the success of nuclear transfer is discussed.

Reprogramming

Definition of Reprogramming of Donor Nucleus

There are several different definitions of nuclear reprogramming. Dean et al. [27] contended that successful cloning by use of somatic cells requires the reprogramming of a somatic nucleus to a stage of restored totipotency. Niemann et al. [22] stated that nuclear reprogramming is a process in which a differentiated somatic nucleus reverts back to a totipotent stage. While agreeing with these views, Nguyen et al. [28] proposed that both completely differentiated cells and partially undifferentiated cells can be returned to totipotency. At the molecular level, Gurdon and Melton [20] defined nuclear reprogramming as “a switch in gene expression of one kind of cell to that of another unrelated to cell type”. In addition, Han and Sidhu [21] maintained that nuclear reprogramming or epigenetic modification is regarded as the alternation in gene expression pattern but not in DNA sequences. However, Whitworth and Prather [29] claimed that it is important to distinguish between reprogramming and remodeling. According to these authors, when transferred into the cytoplasm of a mature enucleated oocyte, the donor nucleus is remodeled to resemble the nucleus of a zygote, leading to changes in chromatin structure, and reprogramming is a result of this change.

Reprogramming Mechanism

Gurdon and Melton [20] showed that reprogramming somatic cells to pluripotent stages may take place in each of three ways: involving SCNT; nuclear transfer to an amphibian oocyte; and cell fusion. In SCNT technology, Jullien et al. [30] pointed out that this may include egg-NT and oocyte-NT. Egg-NT is the transplantation of an individual somatic nucleus to an unfertilized enucleated oocyte, resulting in cell division. In contrast, oocyte-NT is the transfer of multiple somatic nuclei into the germinal vesicle of an immature amphibian oocyte, and there is no cell division.

Abnormalities may potentially result from incomplete reprogramming of the donor nucleus in enucleated oocytes. In addition, Dean et al. [27] asserted that successful cloning by SCNT is dependent on reprogramming of a somatic nucleus to the totipotent stage, a mechanism that is recognized to be very complicated. Consistent with this idea, Han and Sidhu [21] argued that the mechanism of reprogramming somatic cells remains unclear due to the nature of the complex cellular changes involved. Nevertheless, understanding of the reprogramming process has recently improved. Of note, Gurdon and Melton [20] described general principles relating to the mechanism of reprogramming in somatic cells which involve three successive steps: the increase in volume of nuclei; the removal of differentiated marks; and the exchange of chromatin. Moreover, Jullien et al. [30] added that this process occurs in an ordered manner, not randomly. This is a consequence of epigenetic modifications that are considered as alterations in gene expression patterns but not in DNA sequences, including genomic imprinting, DNA methylation and histone modification [21]. The efficiency of reprogramming is dependent on the type of cell providing the donor nucleus [18]. The success of nuclear reprogramming increases when the embryo nucleus is used as the nucleus donor rather than differentiated cells, such as adult somatic cells [20]. Furthermore, Nguyen et al. [28], in attempting to construct giant mouse oocytes by the electrofusion of enucleated oocytes, showed that these oocytes do not promote the development of cloned mice. Hence, it is important to recognize that an enhanced

understanding of epigenetic reprogramming is a key factor for the future improved outcomes of SCNT technology.

Factors affecting Success of SCNT

Several factors contribute to the success of SCNT technology. These include the intrinsic features of donor and recipient cells, cell cycle synchrony, and artificial interventions such as oocyte enucleation methods and the enhancement of in vitro culture media for SCNT embryos. Each of these factors is discussed below.

Donor Cells

It is clear that the type of cell donating the nucleus which is used in nuclear transfer has a critical influence on the level of success. Gurdon and Melton [20] maintained that the efficiency of SCNT is strongly affected by the donor cell type. A decade previously, Stice et al. [31] had also noted that both the identity of donor cells and the synchrony of the cell cycle greatly influence nuclear reprogramming. Zakhartchenko et al. [32], who evaluated the efficiency of nuclear transfer when using morulae, early stage embryos and blastocysts to provide donated nuclei, concluded that there is a decrease in the development in vitro of embryos derived from blastocyst donor cells. Takano et al. [33], on the other hand, stated that only 1-6% of blastomeres that originate from 8-16 stage embryos develop to blastocysts.

It is interesting to note that if nuclear transplanted embryos serve as the source of donor cells, unlimited genetically identical embryos may be produced [31]. However, only one cloned embryo can be re-cloned to produce more embryos. This assertion is supported by Ectors et al. [34], who performed a trial using in vitro-derived morulae after one or two cycles of nuclear transfer and successfully obtained offspring from those embryos. However, their results varied in the blastocyst percentages between the first and the second cycle of nuclear transfer. It is reasonable to claim that the next generation of cloned embryos is likely to have decreased developmental potential due to extended exposure to improper conditions such as low temperature and microsurgery [31,35].

In accordance with the utilization of embryonic donor cells in nuclear transfer, somatic cells are also used but success rates vary. For example, Yin et al. [36], using cumulus cells from rabbits as donor cells, failed to generate offspring. In contrast, Wakayama et al. [37] succeeded in producing live young using murine cumulus cells. In a prior study, Du et al. [38] used porcine fetal fibroblasts but obtained a low blastocyst yield of only 3%. Zakhartchenko et al. [39] noted that nuclei from bovine mammary gland cells are able to enhance the early development of nuclear transferred embryos to the blastocyst stage. Additionally, Wells et al. [11] showed that using mural granulosa cells as the cell donor can save endangered breeds of cattle, especially when the animal is so old that ovum pick-up and IVF obtain poor quality eggs. Furthermore, Shiga et al. [40] reported that nuclear transplantation of somatic cells from aged, infertile bulls led to the birth and development of normal fertile bulls. Wakayama and Yanagimachi [3] noted that Sertoli cell and neuron nuclei from mice can develop well in vitro, but they do not develop to term. In short, the success rate of cloned embryos is greatly dependent on donor cell types; using non-specialized cells such as embryonic cells as donor cells achieves combined higher yields and better outcomes than the utilization of differentiated cells like somatic cells.

Recipient Oocytes

The quality of recipient oocytes may contribute to the success of SCNT. The unfertilized oocytes used for SCNT may originate from *in vivo* or *in vitro* sources. However, reviewing many reports, Whitworth and Prather [29] stated that there is superior development of SCNT embryos derived *in vivo* than those matured *in vitro*. They further noted that oocytes from sexually mature animals are better than oocytes from immature ones. It is possible that *in vivo*-derived oocytes may supply essential elements that assist the somatic cell nucleus when the donor cell is transferred into enucleated cytoplasm. This concept is supported by Tang et al. [41] who found that pretreating donor cells with mature oocyte extracts enhances the potential development of cloned embryos *in vitro*. However, Nguyen et al. [28] claimed that a huge oocyte constructed experimentally does not improve the development of cloned embryos in mice.

In addition to the origin of enucleated oocytes, the age of these cells has a crucial role in the development of nuclear transfer embryos. Typically, oocytes collected either from slaughterhouses or from live cows via the ovum pick-up technique are cultured *in vitro* in M199 medium supplemented with 10% calf serum for between 22-24 hours. As a result, recipient oocytes used for enucleation are used after this period of maturation. The study of Takano et al. [33] showed that *in vitro* 22-24 h is the optimal maturation time for recipient oocytes. This also revealed that either shorter (12-14 h) or longer (28-30 h or 42-44 h) incubation of oocytes has a negative impact on the success levels of nuclear transfer. On the contrary, Wakayama et al. [42] utilized aged oocytes in mice to demonstrate that even use of fertilization-failure oocytes as recipients may produce offspring.

In summary, the intrinsic properties of recipient oocytes have positive effects on their ability to reprogram successfully the donor nucleus.

Cell Cycle Synchrony

It is worth noting that synchronization of the cycles of donor and recipient cells has a strong effect on the success of nuclear transfer in mammals. As discussed, Stice et al. [31] highlighted that nuclear reprogramming is influenced greatly by not only the type of donor cells but also synchrony of the cell division cycle. In support of this notion, Wells et al. [43] submitted that there is a need for coordination between donor and recipient cells. These authors further stated that nuclear transfer embryos, which receive nuclei cells at the gap (G)₁ stage, ready for DNA synthesis, develop to term dramatically in comparison to those in the quiescent G₀ phase. Similarly, Verma et al. [44], experimenting with nuclear transfer in pigs, indicated that the success rate using karyoplasts at G₁ is higher than that for similar cells in either metaphase (M) or synthesis (S) stages of the cell cycle. Furthermore, the results of Wakayama and Yanagimachi [3] in mice demonstrated that donor cells such as Sertoli and neuron nuclei at G₀ phase do not enable development to live offspring. However, Galat et al. [5] argued that in rabbits the nuclei donor at G₀ has more developmental competence than that of others stages. In general, it is reasonable to assume that recipient oocytes are mature; therefore, G₁ and M phase are synchronized. Also, somatic donor cells often proliferate at different speeds. Consequently, they must be at a resting stage in which quiescent somatic donor cells may be reprogrammed fully following nuclear transfer [45]. Ideta et al. [46] reported that although development to the blastocyst stage does not differentiate between G₁ and G₀/G₁ SCNT bovine embryos, the further

development *in vivo* of G₁ cloned blastocysts is higher than those at G₀/G₁ stage. This was exemplified by conversion rates of 83% and 33%, respectively, to day 50 of gestation.

Oocyte Enucleation

When introducing oocyte enucleation technology, Wakayama and Yanagimachi [3] stressed that this protocol is technically very demanding and requires considerable practice in order to achieve consistently good outcomes. These authors acknowledged that when using a piezo-micropipette-driving unit there is only a relatively small hole in the zona pellucida that may not affect the potential development of nuclear transfer embryos. However, Vajta [24] and Vajta and Callesen [25] highlighted that, in contrast, the handmade cloning technique is rather simple but very efficient. Moreover, they stated that the role of zona pellucidae is not important. This is supported by a previous study conducted by Westhusin et al. [35], who showed that removal of 1/2 or 1/20 cytoplasm during oocyte enucleation has no discernable effect on the timing of differentiation, so producing the same number of embryonic cells of the resulting blastocyst. Additionally, Lagutina et al. [23] reported that zona-free nuclear transfer could result in favorable outcomes, but they maintained that a disadvantage of handmade cloning is the requirement to culture nuclear transfer embryos individually to avoid cell aggregation *in vitro*. Working with rabbits, Yin et al. [36], on the other hand, emphasized the role of zona pellucidae to this species.

Enhancement of Media

A contribution to the success of SCNT involves improvements in culture media by supplementing with defined factors, as chemical treatment enhances the genomic reprogramming of somatic cell nuclei [28].

In IVF technology, the *in vitro* culture system for embryos has been optimized and is of commercial value [47]. For SCNT, this methodology has been modified in order to promote the development of cloned embryos. Kishigami et al. [26] reported that the addition of Trichostatin A (TSA) - an inhibitor of histone deacetylase - can increase significantly the yield of mouse cloned embryos. TSA alters gene expression by interfering with the removal of acetyl groups from histones and therefore affects the ability of DNA transcription factors to access DNA molecules inside chromatin. Treatment of cloned embryos in activation phase with TSA-containing culture medium increases 5-fold the success rate of mouse cloning from cumulus cells without abnormality [26]. In corroboration, Luo et al. [48] obtained high buffalo SCNT blastocyst yields after treatment with TSA. Furthermore, Ding et al. [49] showed the effectiveness of combinations of TSA and 5-aza-2'-deoxycytidine in boosting the early development of bovine cloned embryos. In accord with these findings, Diao et al. [50] demonstrated that high SCNT porcine blastocyst yields follow combined treatment with these two chemicals. Huang et al. [51] reported that the addition of 50µg/ml vitamin C 15 hours after activation promotes the development of pig cloned embryos both *in vivo* and *in vitro* by enhancing cellular reprogramming due to an increase in acetylation levels of histone H₄. In contrast, Lee et al. [52] argued that vitamin C does not enhance the reprogramming of nuclei cells. Furthermore, Bang et al. [7], experimenting with cloning in pigs by SCNT, suggested that artificial activation of the cell cycle, followed 5 hours later by supplementation with colcemid and cytochalasin B, which arrest cells in M phase, does not have an effect on the development rate of porcine reconstructed embryos.

Conclusion

It is evident that nuclear transfer technology in mammals has improved considerably since its introduction in the 1990s. Although the success rate remains appreciably low, many attempts have been made in a range of species to improve the number of cloned embryos and viable offspring. Resolution of the underlying mechanism of reprogramming of the somatic cell nucleus in enucleated mature oocytes is critical to escalating the success of SCNT in the future. In addition, determining factors affecting the success of nuclear transfer is necessary to promote the potential development of cloned embryos and viable offspring, leading to advancements in this technology. As a consequence of such experimentation, it is feasible that SCNT will be made available for widespread use in agriculture and clinical medicine in the not too distant future.

Conflict of Interests

The authors declare no conflict of interests.

Authors Contributions

VH Do and AW Taylor-Robinson each contributed to preparing the manuscript and both approved the final version.

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