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Role of Trichostatin A as Reprogramming Enhancer on *In Vitro* Development of Cloned Embryos: A Review

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A B S T R A C T

In cloning during reconstruction of embryo, the oocyte cytoplast reprograms the DNA of a donor nucleus, changing its status from a somatic state to an embryonic state. This remarkable change is due to the unique combination of reprogramming factors (DNA-associating factors) present in the oocyte. The reprogramming factors in the oocyte are not suitably designed to handle the epigenetic status of the somatic cells which often results in an incomplete transition due to inadequate remodelling of the donor nucleus. Recently various reprogramming enhancers have been used either on the developing cloned embryos or on donor cells which check the epigenetic errors by altering histone acetylation levels and increase the developmental competence of cloned embryos. Trichostatin A (TSA), a histone deacetylase inhibitor (HDACi) holding promise for increasing the developmental competence of cloned embryos produced either by somatic cell nuclear transfer (SCNT) or by hand-made cloning (HMC) technique in different species.

Introduction

Trichostatin A is histone deacetylase inhibitor (HDACi) (Turner, 2000), which is known to be a critical factor for successful reprogramming during SCNT (Armstrong *et al.*, 2006). Histone acetylation decreases the affinity of histone proteins to DNA sequences by neutralizing the positive charge of the histone tails (Hong *et al.*, 1993) which in turn, facilitates transcriptional processes (Horn and Peterson, 2002) because a more extended and open chromosomal structure provides more access to transcriptional regulatory proteins to their target sequences (Vettese-Dadey *et al.*, 1996). Histone acetylation also facilitates better genome imprinting and there by increases the developmental competence of embryos (Turner, 2000). The efficiency of cloning in animals in terms of the birth rates of offspring can be affected by a number of biological and technical factors. However, in cloning, there are inevitably considerable individual differences in the quality of recipient oocytes, donor cells and recipient females, so it is usually very difficult to determine the decisive factors statistically (Ogura *et al.*, 2013). Since cloning process is not very efficient. Only few of transferred embryos routinely develop to full term. This is because the reprogramming factors in the oocyte are not suitably designed to handle the

epigenetic status of the somatic cells. Therefore, the reprogramming of donor DNA by the oocytes often results in an incomplete transition due to inadequate remodelling of the donor nucleus and may result in errors at epigenetic level (Santos *et al.,* 2003). Several epigenetic remodelling agents have been used to improve the developmental competence of cloned embryos and trichostatin A is most widely studied among them.

Effect of trichostatin A on developmental competence of cloned embryos

Ianger *et al.,* (2008) studied the effect of trichostatin A, on development and histone acetylation of cloned bovine pre-implantation embryos. The reconstructed SCNT embryos were activated with 50 nM TSA for 13 hours, produced eight-celled embryos with levels of acetylation of histone H4 at lysine 5 (AcH4K5) similar to fertilized counterparts and was found to be significantly ($p<0.005$) greater than that of control NT embryos. Further, TSA treatment resulted in SCNT embryos with pre-implantation developmental potential similar to fertilized counterparts, in terms of cleavage and blastocyst rates or blastocyst total cell number ($p<0.05$).

Tsuji *et al.,* (2009) studied the effect of TSA treatment duration on the developmental potential of mouse SCNT oocytes.

To determine the effects of TSA treatment duration, nuclear-transferred (NT) oocytes were cultured for 0 to 26 hours with 100 nM TSA. The SCNT oocytes treated with TSA for 8 to 12 hours had the highest rate of development to blastocysts and full-term foetuses were obtained after treatment for 8 to 12 hours.

When oocytes were treated for 14 hours and 26 hours, blastocyst development rate was significantly decreased and foetuses were not obtained. The findings demonstrated that long-term TSA treatment of SCNT mouse oocytes inhibit the potential to develop into blastocysts and foetuses after transfer.

Beebe *et al.,* (2009) studied the effect of 50 nM TSA on embryo reconstructs for 24 h after electrical activation resulted in a threefold increase in blastocyst rate (64%) and also resulted in an increase in the average blastocyst cell number (63%).

Cervera *et al.,* (2009) studied the effects of the trichostatin A on pre-implantation development, histone acetylation and gene expression pattern of nucleus transferred porcine embryos. Treatment with 5 nM TSA for 26 hours after reconstitution resulted in embryos that reached the blastocyst stage at a higher level (48.1%) and increased number of cells (105.0) than that of the control embryos in which blastocyst rate were20.2% and blastocyst cell number 75.3.

Himaki *et al.,* (2010) studied the effects of trichstatin A on *in vitro* development and transgene function in somatic cell nuclear transfer embryos derived from transgenic Clawn miniature pig cells. The SCNT embryos were incubated with or without TSA (50 or 100 nmol/L) after activation, cultured *in vitro* and assessed for cleavage, blastocyst formation and transgene function. The rate of blastocyst formation was found to be significantly ($P < 0.05$) higher in SCNT embryos treated with 50 nmol/L TSA than that in control whereas, the rate of cleavage and cell number of blastocyst did not differ.

Cui *et al.,* (2011) studied embryo viability and gene expression of cloned bovine pre-implant embryos in the presence and absence of TSA compared to embryos produced by *in vitro* fertilization or parthenogenetic activation. The treated embryos were found to be more viable with abated epigenetic errors.

Kishigami *et al.,* (2006) studied the optimum concentration, timing and period of trichostatin A treatment for cloned mouse embryos were found to be significant. Eventually, this method led to a greater than fivefold increase in the success rate of mouse cloning.

Wang *et al.,* (2011) found significant improvement in the *in vitro* and full-term development of nuclear transferred (NT) bovine embryos with the trichostatin A.

The epigenetic reprogramming of somatic cell nuclei were studied by comparing the extent of DNA methylation, chromatin structure and development-related genes in *in vitro* fertilized (IVF) group, NT group, TSA treated NT group single blastocyst using quantitative real-time PCR.

Hu *et al.,* (2012) studied trichstatin A on *in vitro* development of sheep SCNT embryos. Treatment of cloned sheep embryos with 50nM TSA for 24 hours after activation significantly ($p<0.05$) improved blastocyst formation rate 23.3% compared to control 16.7%. Moreover, TSA treatment significantly ($p<0.005$) increased total cell number per blastocyst (69.6±9.7) compared with control group (64.1±8.6). Furthermore, TSA treatment increased expression of the development related genes-OCT4 and SOX2 in SCNT blastocysts.

Jeong *et al.,* (2013) studied the effect of TSA on *in vitro* development of porcine SCNT embryos and found that TSA treatment (50 nM) for 24 hours following oocyte activation improved blastocyst formation rates (22.0%) compared with 8.9% in the non-treatment group. The total cell number of the blastocysts also increased significantly.

Saini *et al.,* (2014) studied the effects of trichostatin A on the buffalo skin fibroblast donor cells and embryos produced by hand-made cloning. Treatment of donor cells with TSA (50 nM) resulted in higher *in vitro* development to the blastocyst stage, reduction of the apoptotic index. Transfer of cloned embryos produced with donor cells treated with TSA led to the birth of a calf that survived for 21 days.

Trichostatin A has been found to exhibit positive effect on developmental competence of cloned embryos in different species when used on donor cells, embryos or both. Not only this but cloned embryos which have been produced using reprogramming enhancers result in low birth defects and increase birth rates when transferred in synchronised animals. Thus using trichostatin A on cloned embryos has a great advantage in future for correcting their epigenetic errors and also on reduction in birth defects that arise due to their untreated transfer in cloning protocols.

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