

The Enhancement of Scripted in the Development of Cloned Sheep Embryos

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Abstract: *The low success rate of animal cloning by somatic cell nuclear transfer (SCNT) is believed to be associated with epigenetic errors including abnormal DNA hypermethylation. Recent reports have demonstrated that scripted treatment after somatic cell nuclear transfer (NT) improves success rate of bovine embryo cloning. The objective of this study is to test the effect of scripted in the improvement of the developmental rate of cloned sheep embryos in vitro. Sheep ovaries (2113 ovary) and ear skin fibroblast were collected from the central slaughter house at Riyadh, Saudi Arabia. The fibroblasts (ear skin cell or cumulus cells) used as somatic donor. The total of (4206) oocytes with their cumulus cells were collected from the sheep ovary and only (3025) ova cultured in the in vitro maturation (IVM) media in a CO₂ incubator (38.5°C) for 22 hrs. For the cloning treatment the (IVM) oocytes (1495) were enucleated under the micromanipulator and the somatic cells were transferred to the enucleated mature oocytes (1015 ova). The transferred somatic cells were electrically fused with the enucleated mature oocytes (710 ova). The fused cells were activated with calcium ionophore and cycloheximide (494 oocytes). Subsequently, they were cultured in TCM199 medium with 0.5 or 0.0 micro M concentrations of scripted treated (244 oocytes) and control (250) oocytes. The embryos were cultured inside an incubating chamber supplemented with air and (5% CO₂ + 5 % O₂ + 90 % N₂) in the incubator at 38.5°C for 6-7 days. The results show that the development embryo of the cloned sheep embryos in vitro most of them were degenerate (301 ~ 61%) and 46 ~ 25% remain in one cell stage, while 17 embryo developed to two cell stage (3 Scripted, 14 control), 24 embryo reached the four cell stage (5 Scripted, 19 control), 51 embryo (26 control, 25 scripted) blocked in eight cell stage, while 12 embryo remain at 16 cell stage (8 control, 4 scripted), and 34 embryo developed to morula stage (27 scripted, 7 control). Finally 9 embryo reached the blastula stage (two from control and 7 from scripted group. About 60 developed embryos were transferred to 20 sheep surgically. Finally three pregnant females confirmed by the ultrasound, with 4 new borne one is twins. However, when both control or treated the pre-implantation development of cloned embryos was compared we get twice development with the scripted treated compared to control. So the scripted enhance the development of cloned sheep embryos.*

Keywords: *In vitro* maturation (IVM), somatic cell nuclear transfer, cloning, sheep, embryo, Scripted.

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1. Introduction

Although cloning by somatic cell nuclear transfer (SCNT) has been achieved in various mammalian species, its efficiency has been very low. Successful cloning requires conversion from differentiated donor nuclei to embryonic nuclei after transfer of the somatic nuclei into enucleated oocytes. The low success rate of animal cloning by somatic cell nuclear transfer (SCNT) is believed to be associated with epigenetic errors including abnormal DNA hypermethylation (Dean *et al.* 2001). Some reports have demonstrated that scripted, a histone-deacetylase inhibitor, treatment after somatic cell nuclear transfer (NT) improves success rate of bovine embryo cloning (Satoshi, *et al.* 2008) (Oliveira *et al.* 2010)

Wang *et al.* (2011). Scripted belongs to the class of hydroxamic acid-containing histone-deacetylase inhibitors used to enhance protein expression with lower toxicity than Trichostatin A (TSA) (Li *et al.* 2008. Su, *et al.* 2000). Van Thuan and his coworker (2009) improve that the histone deacetylase inhibitor Scripted enhances nascent mRNA production and rescues full-term development in cloned inbred mice. Also significant improvement in cloning efficiency of an inbred miniature

pig by scripted treatment after somatic cell nuclear transfer (Zhao, *et al.* 2009 and Mao 2012).

Aim

Thus the objective of this study is to test the effect of scripted in the improvement of the developmental rate of cloned sheep embryos *in vitro* then transfer the cloned sheep embryo to recipient mother.

2. Materials and Methods

Sheep ovaries (about 2113 ovary) and ear skin fibroblast were collected from the central slaughter house at Riyadh, Saudi Arabia. The fibroblasts (ear skin cell or cumulus cells) used as somatic donor cells, before transfer they were cultured *in vitro* under serum starvation (0.5 fetal calf serum for five days) to reach G₀ stage of cell cycle. The total of 4206 oocytes with their cumulus cells were collected from the sheep ovary and only (3025) ova cultured in the *in vitro* maturation (IVM) media in a CO₂ incubator (38.5°C) for 22 hr. (Table 1 and Fig 1a,b,c).

For the cloning treatment the (IVM) oocytes (1495) were enucleated under the micromanipulator and the somatic cells were transferred to the enucleated mature oocytes (1015 ova) (Fig 2a, b, c). The transferred somatic cells were electrically

fused with the enucleated mature oocytes (710 ova). The fused cells were activated with calcium ionophore and cycloheximide (494 oocytes) (Table 1). Subsequently, they were cultured in TCM199 medium with 0.5 or 0.0 micro M concentrations of scripted treated (244 oocytes) and control (250) oocytes). The embryos were cultured inside an incubating chamber supplemented with air (5% CO₂ +5 % O₂+ 90 % N₂) in the incubator at 38.5°C for 6-7days.

3. Results

The results show that most of the fused oocytes with somatic cell nuclear transfer were degenerate (301 ~ 61%) and 46~25%) remain in one cell stage. A total of 147 developed embryo reached different stage (71scripted

treated and 76 control embryos).Such as 17/147(~ 11%) cloned sheep embryo were developed to two cell stage, while 24/147(~ 16%) embryo reached the four cell stage and 31/147(~ 21%) embryo blocked in eight cell stage. About 12/147(~8%) embryo remain at 16 cell stage, and 34/147 (~ 23%) embryo developed tomorula stage fig3a.Finally we got 9/147(~6%) embryo reached the blastula stage (2/76 from the non treated (~ 2.6 %) and 7/71 (~ 9.9%) of the developed embryos from treated with scripted group.(table-2)(fig3b). Surgically about 60 developed embryos has been transferred to 20 sheep. At the first time we transfer any developed embryos that reached atmorula stage, but none of them get pregnant. Then we transfer only blastula stage embryos to recipient females. Finally we get three pregnant females confirmed by the ultrasound and we got 4 new borne one of them was twins.(Fig .3 c).

Table 1: Total ova collected, In vitro matured and cloned sheep embryo

Ovaries	Ova collected	IVM ova	Cut ova %	enucleated ova	Injected ova	Fusion ova	Activation	Culture ova
2113	4206	3025	2085	1495	1015	710	512	494
Rate	1.75	72%	69%	71%	68%	70%	72%	96%

Table 2: The comparison between the developments of the cloned sheep embryos, scripted treat group with control none treated.

Development/Treatment	Culture ova no.	Degenerating ova	I cell ova.	2Cell	4 cell	8 cell	16 cell	Morula stage	Blastula stage
Scrip	244	152	21	3	5	25	4	27	7
Rate	49.9%	62.3%	26.6%	1.9%	3%	16%	2.4%	8%	9.9%
Cont.	250	149	25	14	19	26	8	7	2
Rate	50.1%	59.6%	25%	14.4%	20%	26%	7%	7%	2.6%

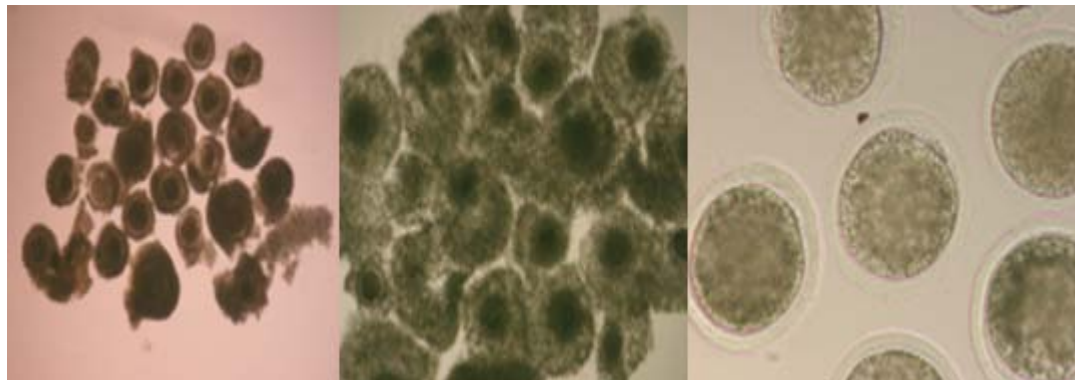


Figure (1a,b,c): Ova with follicle cells before and after *In vitro* maturation(IVM) of ova 22h.anova with polar bodies after 22hr(IVM).

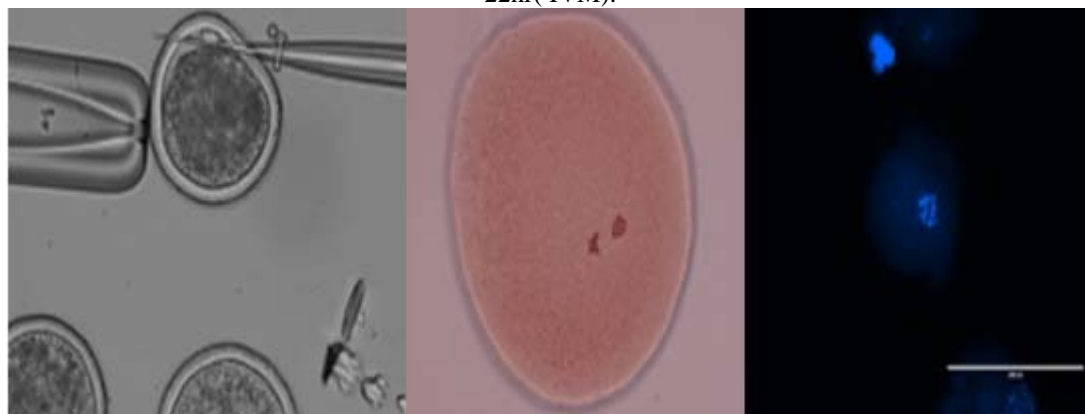


Figure (2a,b,c): Ova cutting and enucleating the removed nucleus stained and checked under fluorescent microscopy

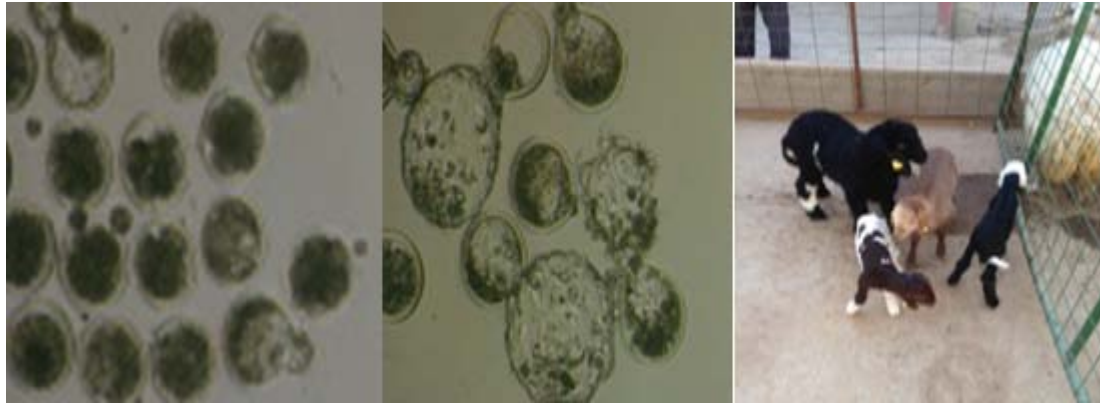


Figure (3a,b,c) Developed cloned sheep embryos at different stage with hatched blastula and four new born transferred sheep embryos.

4. Discussion

Scripted is a novel HDAC inhibitor with low toxicity. Scripted have been used to enhance development of cloned embryos and the treatment with .5nM of scripted significantly improved bovine blastocyst development. It was reported that Scripted has a beneficial effect on the efficiency of sheep cloning in post implantation development (Bordignon ,*et.al.*,2010).In the present study and in most cloning work, the higher ova degeneration rate, could be attributed either, to the type of somatic cell, because most of the donor cell were used was the skin or cumulus cells which might not on the G0, or it could be to electrical current used to fused the somatic cell with the oocytes that it might damage the cytoplasm of the ova . Also we inferred that there might be an interaction between the SCNT and the fusion treatment perhaps causes lower level of methylation and a higher level of histone acetylation changed the chromatin configuration, to facilitate the reprogramming of transferred nucleus, and subsequently to enhanced development of cloned embryos. Despite the higher ova degeneration rate, the(SCNT) oocytes treatment of 0.05 mM or 0.0 mM scripted has a higher development rate than that of the non-treated group on the *in vitro* cloned sheep embryo development to blastocyst formation rate. The current study confirmed the benefit and further defined an optimal concentration and duration of scripted treatment for improving development of cloned ovine embryos However, in mice and pigs, the use of a higher concentration of Scripted (1mmol/L) resulted in rates of blasto cyst formation and hatching that were numerically higher compared with the untreated group, indicating that there were no toxic effects at that concentration (Panda, *et.al.*, 2012 and Zhao,*et.al.*2010). Recently Wen-Bing and his co researcher 2013 concluded that Scripted may be more potent than TSA for supporting developmental competence of cloned ovine embryos. Although they note that blastocyst development was reduced when embryos were treated with 0.8 mmol/L Scripted for 24 hours or with 0.2 mmol/L Scripted for 72hours. they inferred that either a high concentration or prolonged Scripted treatment had negative effects on development of SCNT embryos, as previously described for TSA(Tsuji,*et.al.*,2009).However, there may be differences among species and donor cell types in the maximum effective Scripted concentration.

In conclusion, Scripted treatment was significantly more effective than control to enhance the developmental potential of cloned ovine embryos. In addition, Scripted modified the histone acetylation status of these embryos, which subsequently enhanced their nuclear reprogramming and development potential. So scripted enhance the development of cloned sheep embryos.

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