STUDYING THE EPIGENETIC SIGNALLING USING EPIGENETIC MODIFIER AND EFFECT ON DEVELOPMETAL COMPETENCE OF *IN-VITRO* CLONED MAMMALIAN EMBRYOS

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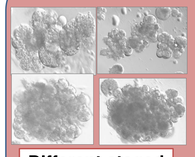
INTRODUCTION

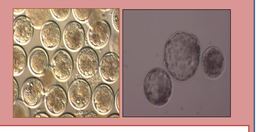
There is accumulating evidence that histone modifications signals genes to switch ON and OFF through promoter DNA methylation. Epigenetic regulation involves post translational histone modifications and DNA methylation. Histone modifications play key role during epigenetic reprogramming in mammalian embryo development guiding zygote to achieve pluripotent state and differentiation of cell lineages later. Aberrant epigenetic reprogramming leads to low developmental competence of cloned goat (Capra hircus) embryos in-vitro. Improper erasure of H3K9me2 epigenetic mark halts the cloned embryo growth. H3K9me2 hypermethylation associates with DNA hypermethylation in promoter regions of pluripotency genes i.e. POU5f1, SOX2 and NANOG which leads to inactive transcription of these genes resulting in low embryo division potential. In this study, an epigenetic modifier i.e. BIX01294, a quinazolinamine derivative is used to study epigenetic signalling known to decrease H3K9me2/3 levels in somatic cell reprogramming and increase the developmental competence of cloned mammalian embryos

RESULTS

CLONED and IVF EMBRYOS *in vitro* CULTURE



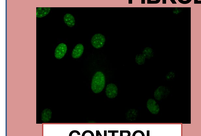
Different staged cloned embryos



IVF embryos and Blastocyst

Cloned and IVF embryos are produced by hand made cloning and in vitro sperm fertilization respectively after oocyte culture for 26 hours

IMMUNOSTAINING OF GOAT FETAL FIBROBLAST CELLS

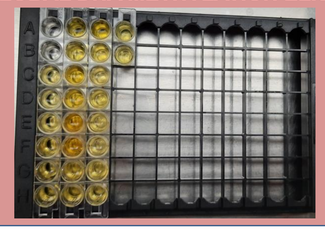


CONTROL

TREATMENT

Immunocytochemistry of fetal fibroblast cells was performed for H3K9me2 epigenetic mark with primary antibody against H3K9me2 and fluorochrome tagged secondary antibody Signal intensity was quantified with ImageJ software

GLOBAL DNA METHYLATION ELISA



Global DNA methylation ELISA was performed according to protocol

REAL TIME PCR

RNA isolation from morula stage cloned and IVF goat embryos

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cDNA preparation

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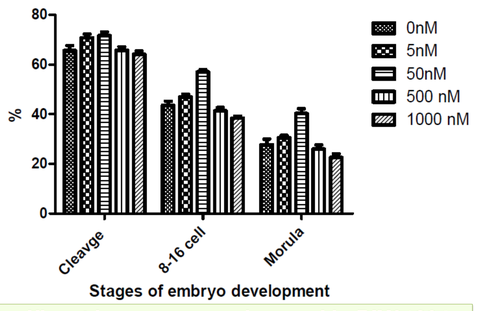
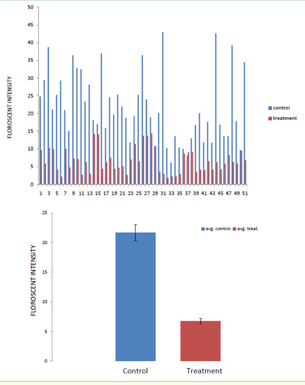
Real time PCR with pluripotency genes primers

BIX01294 EFFECT ON EMBRYO GROWTH



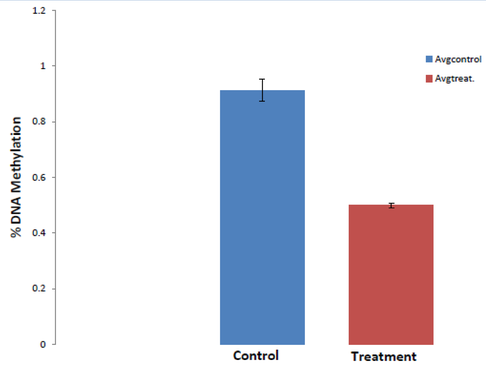
Significant increase was observed in BIX01294 treated cloned embryos (16.53%±0.2) compared to control group (6.53%±0.2) at zygotic genome activation and morula stage i.e. BIX treated (9.61%±0.33) compared to the control group (3.15 %±0.27)

FLOUROSCENT INTENSITY ANALYSIS



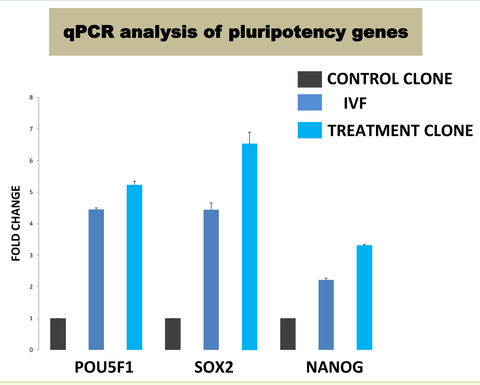
Significant reduction observed in signal intensity of BIX treated goat fetal fibroblasts cells (6.72±0.49) compared to control group (21.64±1.35).

GLOBAL DNA METHYLATION QUANTIFICATION



Significant reduction in global DNA methylation observed in BIX treated fetal fibroblasts cells to untreated cells i.e. 0.49% vs. 0.91% respectively

REAL-TIME PCR



Relative mRNA abundance of pluripotency genes i.e. Pou5f1 and Sox2 was significantly higher as compared to untreated and IVF embryos with $p<0.05$

CONCLUSION

H3K9me2 regulates pluripotency genes transcription through DNA methylation and decrease in H3K9me2 levels results in increase in developmental competence of cloned goat embryos

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