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Impact of Epigenetic Modifications on Developmental Success in Cloned Buffalo Embryos

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ABSTRACT

Cloning through somatic cell nuclear transfer (SCNT) has been a hopeful method of cloning superior buffaloes, but its effectiveness is still very low due to the failure of epigenetic reprogramming. Epigenetic marks such as DNA methylation, histone modifications, and non-coding RNA regulation are significant factors in the correct activation and expression of genes responsible for embryonic development. In cloned embryos, aberrant epigenetic reprogramming frequently leads to developmental arrest, failure of implantation, and abnormalities decreasing overall viability.

This article discusses the complex role of epigenetic alterations in guiding the developmental competency of cloned buffalo embryos. In particular, it emphasizes the problem presented by aberrant patterns of DNA methylation, dysregulated histone modifications, and dysregulation of non-coding RNA. In addition, it presents novel developments in epigenetic remedies to enhance cloning efficiency through the utilization of DNA methyltransferase inhibitors, histone deacetylase inhibitors, and RNA interference methods to better reprogram the nucleus.

Through the examination of existing research and developments in epigenetic modification, this work offers an overview of how its optimization may bring about increased rates of survival and overall efficiency in buffalo cloning. Future research implies that CRISPR-mediated epigenetic editing, single-cell epigenomic analysis, and targeted transcriptome analysis may open up new lines of research towards overcoming existing technical limitations in SCNT technology. The results of this research emphasize the need to incorporate epigenetic interventions to further develop cloning methods and maximize genetically valuable buffalo populations.

Keyword: *Epigenetics, Buffalo Cloning, DNA Methylation, Histone Modification, Somatic Cell Nuclear Transfer (SCNT), Developmental Success.*

1. INTRODUCTION

Cloning, specifically by somatic cell nuclear transfer (SCNT), has been extensively researched in livestock production as a means to conserve elite genetics, improve production characteristics, and aid in conserving biodiversity. Buffalo (Bubalus bubalis) cloning is of high agricultural and economic value because the species is utilized in milk, meat, and draught animal production. Despite the progress achieved in SCNT, buffaloes' cloning success rate remains significantly low mainly because of errors in proper epigenetic reprogramming.

Epigenetics is defined as heritable gene expression changes that are not associated with alterations in the DNA sequence. Such modifications, which encompass DNA methylation, histone modification, and non-coding RNA regulation, are critical in regulating cellular differentiation and early embryonic development. In embryos fertilized naturally, these epigenetic marks are dynamically reprogrammed to set up totipotency. Nonetheless, in cloned embryos produced from somatic donor cells, the epigenetic state tends not to reset properly, resulting in incorrect gene expression, developmental failure, failure to implant, and postnatal abnormalities. The failure to fully reprogram epigenetically is one of the main causes of SCNT inefficiency in buffaloes.

Importance of Epigenetics in Buffalo Cloned Embryos

Embryonic development success in cloned buffaloes is greatly reliant on accurate epigenetic reprogramming. The most important epigenetic changes affecting SCNT success are:

DNA Methylation: This chemical alteration, where methyl groups are added to cytosine residues, controls gene expression through silencing or activation of genes. Donor somatic cells in cloned embryos tend to keep their initial DNA methylation marks, hindering proper embryonic gene activation.

Histone Modifications: Post-translational histone protein modifications like acetylation, methylation, phosphorylation, and ubiquitination impact chromatin structure and transcriptional activity. Erroneous histone modifications in cloned embryos result in aberrant gene expression patterns, impacting developmental competence.

Non-coding RNAs (ncRNAs): Short regulatory RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are important for post-transcriptional gene regulation. Aberrant expression of ncRNAs in cloned buffalo embryos may lead to impaired gene regulation and developmental failure.

Challenges in Cloning Efficiency

Although much work has been done, the efficiency of buffalo cloning using SCNT is low, with a cloned embryo successfully developing into viable offspring in only 5-10%. The main reasons for this inefficiency are:

Incomplete reprogramming of epigenetics: The somatic nucleus donor is not fully reprogrammed, and hence, there is residual somatic memory.

Aberrant gene expression: Aberrant regulation of key developmental genes causes early embryonic death or fetal malformations.

Low implantation and survival rates: Even if cloned embryos develop to the blastocyst stage, there is still high failure in implantation and high mortality rates.

Scope of This Study

This article will seek to give a thorough overview of how epigenetic changes affect the developmental outcome of cloned buffalo embryos. The mechanisms of DNA methylation, histone regulation, and non-coding RNA, their roles in gene expression, and epigenetic reprogramming strategies are discussed. In addition, novel epigenetic interventions, such as the utilization of DNA methyltransferase inhibitors, histone deacetylase inhibitors, and RNA interference methods, are presented to hypothesize solutions that can increase SCNT efficiency.

Future Directions

With the rapid advancement of epigenetic studies and genome editing tools, upcoming strategies like CRISPR-based epigenetic editing, single-cell epigenomics, and transcriptomic profiling can shed new light on enhancing cloning success. Overcoming epigenetic obstacles in buffalo SCNT has the potential to revolutionize cloning efficiency and play a crucial role in livestock improvement, genetic conservation, and biomedical uses.

Sheep cloning was successfully achieved through nuclear transfer from a cultured cell line, demonstrating a breakthrough in genetic engineering (Campbell et al., 1996). Further research explored the impact of histone deacetylase inhibitors on nuclear reprogramming in cloned embryos, emphasizing epigenetic modifications as a key factor (Gao et al., 2018). Additionally, non-coding RNAs play a significant role in regulating cloned embryo development, influencing epigenetic mechanisms (Shi et al., 2019).

CRISPR technology has also been applied to enhance somatic cell nuclear transfer (SCNT) efficiency, allowing precise genome and epigenome modifications (Zhang et al., 2016). However, nuclear transfer techniques often result in epigenetic abnormalities, requiring corrective interventions (Loi et al., 2006). In the case of buffalo cloning, advancements have led to improved epigenetic reprogramming, addressing key developmental challenges (Singh et al., 2021).

This research highlights the importance of epigenetic regulation in cloned buffalo embryos and emphasizes new trends in overcoming present limitations in SCNT technology. By optimizing epigenetic manipulation, scientists are able to improve the success rate of buffalo cloning, which opens the door for more effective reproductive and genetic conservation methods in the future.

2. Epigenetic Mechanisms in Cloned Embryos

Epigenetic processes are instrumental in controlling gene expression, ensuring normal embryonic development, and reprogramming somatic cells into a totipotent state. During natural fertilization, such changes are dynamically erased to define the proper patterns of gene expression necessary for normal development. Such is not the case in somatic cell nuclear transfer (SCNT), wherein the epigenetic profile of the donor nucleus is usually carried over, causing aberrant gene activation, dysfunctional embryonic development, and poor cloning efficiency.

This part delves into the three main epigenetic processes influencing cloned buffalo embryos:
DNA Methylation
Histone Modifiers
Non-coding RNA Regulation

2.1 DNA Methylation

DNA methylation is an essential epigenetic modification where a methyl group (-CH_3) is added to the cytosine bases in CpG dinucleotides, which in turn results in gene silencing or activation. Accurate patterns of DNA methylation are needed for controlling gene expression, inactivation of the X-chromosome, and genomic imprinting.

DNA Methylation in SCNT Cloned Embryos

In natural fertilization, the paternal genome is actively demethylated, whereas the maternal genome is passively demethylated during cell divisions to reach a totipotent embryonic stage. But in SCNT-derived embryos:

The donor somatic cell nucleus tends to maintain its initial methylation status, thus preventing activation of embryonic genes.
Inadequate DNA demethylation results in aberrant gene expression and inability to activate crucial developmental genes.
Imprinted genes controlling fetal development and placental activity can remain mis-methylated, causing developmental abnormalities.

Methods to Correct DNA Methylation Mistakes in Cloned Embryos

In order to enhance DNA methylation reprogramming of cloned buffalo embryos, scientists have explored:

DNA Methyltransferase Inhibitors (DNMTi): Chemicals like 5-aza-2'-deoxycytidine correct methylation mistakes by minimizing hypermethylation of essential genes.

Optimized Donor Cell Selection: The use of low methylation variation donor cells may improve nuclear reprogramming.

Epigenetic Editing Tools: CRISPR-based epigenetic editing can accurately modify DNA methylation patterns for better cloning success.

2.2 Histone Modifications

Histone proteins are involved in the center of chromatin structure and gene expression. Histone post-translational modifications, including acetylation, methylation, phosphorylation, and ubiquitination, control the accessibility of transcription factors to DNA.
Histone Dysregulation in Cloned Embryos
Reprogramming of histone modifications in SCNT-derived buffalo embryos is usually incomplete and results in:
Retention of somatic histone marks, inhibiting proper chromatin remodeling.
Defective histone acetylation, resulting in tightly condensed chromatin and gene silencing.
Inappropriate histone methylation, impacting crucial developmental pathways.
Major Histone Changes Impacting SCNT Effectiveness
Histone Acetylation (H3K9ac, H4K16ac): Typically activates transcription by opening up chromatin. SCNT embryos commonly exhibit diminished histone acetylation, restricting gene activation.
Histone Methylation (H3K27me3, H3K9me3): Such modifications silence genes. In SCNT, abnormal histone methylation may suppress vital developmental genes.

Strategies to Enhance Histone Reprogramming in Cloned Embryos

To improve histone modification patterns, scientists have utilized:

Histone Deacetylase Inhibitors (HDACi): Trichostatin A (TSA) and Valproic Acid (VPA) induce histone acetylation, enabling improved chromatin relaxation and transcriptional activity.

Chromatin Remodeling Enzymes: These enzymes assist in the removal of abnormal histone marks, enhancing gene expression patterns.

Microinjection of Modified Histones: Preloading donor cells with corrected histone modifications can enhance nuclear reprogramming.

2.3 Non-coding RNA Regulation

Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are pivotal in post-transcriptional regulation of genes and embryonic development.

Dysregulation of Non-coding RNAs in Cloned Buffalo Embryos
SCNT tends to cause:
Misexpression of miRNAs, disrupting developmental pathways.
Aberrant lncRNA expression, interfering with chromatin remodeling and gene activation.
Defective RNA-based signaling, perturbing early embryo growth and cell differentiation.

Strategies to Correct Non-coding RNA Defects

RNA Interference (RNAi): Modulating miRNA expression restores normal gene regulation.
LncRNA-Based Therapeutics: Regulation of lncRNA levels can be used to promote nuclear reprogramming.
Transcriptome Analysis: Detection of crucial ncRNA regulators can be utilized to refine cloning approaches.

2.4 Integrative Role of Epigenetic Modifications in Cloning Success

The three principal epigenetic processes—DNA methylation, histone modification, and non-coding RNA regulation—are interlinked and together contribute to nuclear reprogramming. Interference in one pathway may induce cascading effects on gene expression, diminishing the developmental potential of cloned buffalo embryos.

Through the use of targeted epigenetic interventions, scientists can effectively enhance the reprogramming of somatic nuclei, thus improving embryonic development and cloning efficiency in buffaloes. The following section will discuss existing challenges and possible solutions in overcoming epigenetic barriers in SCNT cloning.

3. Challenges in Epigenetic Reprogramming in Cloned Buffalo Embryos

Somatic cell nuclear transfer (SCNT)-based cloning has been promising for buffalo reproduction, but its efficacy is low as a result of epigenetic reprogramming failures. The nucleus of the donor somatic cell tends to have epigenetic memory, making it difficult for proper gene expression necessary for embryo development. It results in reduced blastocyst formation, increased embryonic death, and postnatal abnormalities. This section addresses the major problems influencing epigenetic reprogramming in cloned buffalo embryos.

3.1 Incomplete DNA Methylation Reprogramming

DNA methylation plays a crucial role in reprogramming gene expression patterns in embryos at an early stage. During SCNT, the donor nucleus tends to maintain its somatic methylation marks, interfering with normal development.

Problems in DNA Methylation Reprogramming

Abnormal methylation of imprinted genes: Insufficient reset of imprinted genes causes fetal growth and placental defects.

Hyper- or hypomethylation of crucial developmental genes: Inappropriate methylation patterns hinder the activation of necessary genes at the appropriate time.

Maintenance of somatic cell methylation: In contrast to fertilized embryos, which are subject to regulated methylation reprogramming, cloned embryos are unable to erase and re-initiate proper methylation patterns.

Consequences

Embryonic arrest during early cleavage stages.
Aberrant pluripotency gene expression (OCT4, SOX2, NANOG).
Low implantation and pregnancy rates.

3.2 Aberrant Histone Modifications

Histone modifications control chromatin accessibility and gene expression. In cloned buffalo embryos, the histone landscape of the donor nucleus is generally not remodeled adequately, impacting transcriptional activity.

Shared Histone Modification Problems in SCNT

Sustained somatic histone marks (H3K9me3, H3K27me3) repress early embryonic genes.
Decreased histone acetylation (H3K9ac, H4K16ac) results in compacted chromatin and restricted gene activation.
Unreliable histone modifications retard zygotic genome activation (ZGA), essential for embryonic development.

Consequences

Impaired embryonic gene activation.
Enhanced apoptosis in early embryos.
Abnormalities in the development of cloned calves.

3.3 Dysregulated X-Chromosome Inactivation (XCI) in Female Embryos

During regular embryogenesis, X-chromosome inactivation (XCI) maintains gene expression balance between males (XY) and females (XX). During cloned buffalo embryos, XCI is usually abnormally or incompletely regulated, resulting in developmental abnormalities.

Problems in XCI in SCNT Cloning

Unpredictable inactivation/reactivation of the X chromosome interferes with gene dosage equilibrium.
Retained somatic X-inactivation patterns compromise female embryo viability.

Consequences

Extreme embryonic lethality in female buffalo clones.
Enhanced frequency of placental defects and postnatal abnormalities.

3.4 Abnormal Non-Coding RNA Expression

Non-coding RNAs (ncRNAs), specifically microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), control gene expression during early development. During SCNT, their expression is usually disrupted, causing aberrant embryonic development.

Issues with Common Non-Coding RNA Regulation

Misexpression of miRNAs represses critical developmental pathways.
Aberrant expression of lncRNAs influences chromatin remodeling and transcriptional regulation.

Consequences

Inability to form pluripotency in cloned embryos.
Increased risk of developmental failure.

3.5 Zygotic Genome Activation (ZGA) Defects

ZGA is an important process during which the embryonic genome gains control over development. During SCNT embryos, ZGA tends to be delayed or inefficient, resulting in abnormal development.

Challenges in Activating ZGA

Maintenance of somatic chromatin structure prevents transcription.

Insufficient transcription factor (OCT4, SOX2, NANOG) activation needed for ZGA.

Consequences

Enhanced fragmentation and apoptosis in embryos in the early stages.
Decreased development of healthy blastocysts.

3.6 Postnatal Health Complications

Although SCNT buffalo embryos can reach the term, a large number of clones suffer from health complications due to ongoing epigenetic malformations.

Prevalent Postnatal Abnormalities in Cloned Buffalo Calves

Large Offspring Syndrome (LOS): Cloned calves tend to be abnormally large at birth as a result of incorrect methylation of imprinted genes.

Immunological deficiencies: Incorrect activation of genes influences the function of the immune system and renders clones susceptible to disease.

Shortened life expectancy: Epigenetic faults induce metabolic impairments and malformations in organs.

3.7 Maternal and Environmental Impacts on Epigenetic Reprogramming

Although nuclear reprogramming can be partially achieved, maternal factors and environmental circumstances further complicate epigenetic restructuring.

Maternal Cytoplasm and Culture Condition Influences

Inadequate oocyte-derived factors: SCNT embryos depend on maternal cytoplasm for epigenetic reprogramming, yet in most cases the process is not as efficient as in fertilized embryos.

Composition of the culture medium: Inferior culture conditions may disturb DNA methylation and histone regulation, making cloning less successful.

Consequences

Increased epigenetic diversity between clones.
Lower implantation and survival rates.

3.8 Potential Solutions to Overcoming Epigenetic Defects

Some potential solutions in improving epigenetic reprogramming in SCNT buffalo embryos are:

Epigenetic Modulators

DNA methylation inhibitors (e.g., 5-aza-2'-deoxycytidine) correct aberrant methylation patterns.

Histone deacetylase inhibitors (HDACi) such as Trichostatin A (TSA) increase chromatin remodeling and gene activation.

Optimized Donor Cell Selection

Employing young, pluripotent-like cells enhances nuclear reprogramming.

Pre-treatment of donor cells with epigenetic reprogramming factors increases cloning efficiency.

Transcriptome-Based Reprogramming

RNA sequencing (RNA-seq) of successful SCNT embryos can inform reprogramming method improvements.

Advanced Culture Systems

Improved embryo culture media with epigenetic regulators can increase blastocyst formation and implantation rates.

4. Strategies to Enhance Epigenetic Reprogramming

Enhancing the efficiency of somatic cell nuclear transfer (SCNT) in buffalo involves surmounting epigenetic reprogramming failure. Epigenetic marks like DNA methylation, histone adjustment, and regulation by non-coding RNA have to be set right to resume embryonic gene expression. Advanced approaches to facilitating epigenetic reprogramming and developmental success in cloned buffalo embryos are discussed here.

4.1 Application of Epigenetic Modifiers

Epigenetic reprogramming may be improved with chemical compounds that target DNA methylation and histone modifications. These small molecule compounds facilitate the reset of somatic cell memory and the activation of important embryonic genes.

4.1.1 DNA Methylation Inhibitors

5-Aza-2′-deoxycytidine (5-Aza-dC): An inhibitor of DNA methyltransferase (DNMT) that decreases aberrant DNA methylation and induces proper embryonic gene activation.

RG108: A non-toxic DNMT inhibitor that increases the reprogramming efficiency of donor nuclei.

Advantages:

✓ Improves genome-wide demethylation in cloned embryos.

✓ Improves activation of pluripotency genes (OCT4, SOX2, NANOG).

4.1.2 Histone Deacetylase Inhibitors (HDACi)

HDAC inhibitors increase histone acetylation, which results in more open chromatin and improved gene expression.

Trichostatin A (TSA): Most potent HDAC inhibitor, greatly enhances nuclear reprogramming and blastocyst formation.

Scriptaid: Less toxic HDAC inhibitor that improves the development of cloned embryos by enhancing the pattern of histone acetylation.

Advantages:

✓ Reprograms cloned embryos to have proper chromatin structure.

✓ Facilitates Zygotic Genome Activation (ZGA).

✓ Increases embryo survival and implantation rates.

4.2 Choice of Best Donor Cells

The donor somatic cells should be selected depending on their nuclear reprogramming characteristics. The plasticity of the epigenetic state of donor cells enhances the efficiency of cloning.

4.2.1 Pluripotent or Fetal Cell Usage

Fibroblasts from fetal tissue are more epigenetically plastic, which is why they are the best option for SCNT.

Induced pluripotent stem cells (iPSCs) from somatic cells have greater reprogramming efficiency.

4.2.2 Pre-Treatment of Donor Cells

Low-serum short-term culture enhances epigenetic plasticity.

Reprogramming factor (OCT4, SOX2, KLF4, c-MYC) application increases nuclear plasticity prior to SCNT.

Advantages:

✓ Prevents epigenetic memory retention from somatic cells.

✓ Enhances the possibility of correct gene activation in cloned embryos.

4.3 Somatic Cell Reprogramming via Nuclear Transfer Optimization

SCNT procedures can be optimized to provide improved nuclear remodeling and chromatin restructuring.

4.3.1 Extended Nuclear Transfer Activation

Delayed nuclear transfer activation enables:

Improved DNA demethylation prior to first cell division.

Improved chromatin remodeling and histone modifications.

4.3.2 Embryo Culture Modifications

Supplementation of culture media with epigenetic regulators (e.g., TSA, 5-Aza-dC) facilitates improved reprogramming.

Co-culture with oviductal cells facilitates natural epigenetic modifications.

Advantages:

✓ Facilitates improved chromatin relaxation and reprogramming.

✓ Improves early embryonic gene expression and viability.

4.4 Modulation with Non-Coding RNA

No-coding RNAs (ncRNAs) control gene expression and epigenetic modifications. Modulating their levels can improve SCNT efficiency.

4.4.1 miRNA Modulation

Certain miRNAs (e.g., miR-125b, miR-302) enhance gene activation by cloned embryos as well as pluripotency.

4.4.2 Long Non-Coding RNA (lncRNA) Modulation

LncRNAs like Xist (for X-chromosome inactivation) can be modulated to block gene dosage imbalances in female embryos.

Advantages:

✓ Reduces correct gene regulatory networks.

✓ Minimizes developmental defects in cloned buffalo calves.

4.5 Maternal Cytoplasmic Environment Improvement

Oocyte cytoplasm is the key to somatic nucleus reprogramming. Enhancement of maternal components can increase the efficiency of SCNT.

4.5.1 Oocyte Selection

Oocytes from younger buffaloes contain more reprogramming factors.

Maturation of oocytes in optimal conditions (e.g., antioxidant supplementation) increases epigenetic changes.

4.5.2 Supplementing Oocyte Cytoplasm with Reprogramming Factors

Supplementation of oocyte cytoplasm with mRNAs or proteins derived from normal fertilized zygotes accelerates nuclear reprogramming.

Advantages:

✓ Increases reprogramming efficiency.

✓ Enhances blastocyst formation efficiencies.

4.6 Genome Editing Strategies for Epigenetic Reprogramming

New CRISPR-based strategies enable precise management of epigenetic changes and enhance gene activation in cloned embryos.

4.6.1 CRISPR/dCas9-Based Epigenetic Editing

CRISPR-dCas9 combined with DNMT inhibitors is used to demethylate developmentally important genes.

CRISPR-dCas9 combined with histone acetyltransferases induces histone modifications for increased gene activation.

4.6.2 RNA-Based Epigenetic Editing

Small interfering RNAs (siRNAs) or antisense oligonucleotides are employed to adjust gene silencing in SCNT embryos.

Benefits:

✓ Facilitates targeted correction of epigenetic mistakes.

✓ Enhances developmental competence of cloned buffalo embryos.

4.7 Optimizing In Vivo Embryo Transfer Conditions

Optimization of embryo transfer processes enhances the probability of cloned embryos surviving implantation and full-term gestation.

4.7.1 Synchronization of Recipient Buffalo

Hormonal cycle synchronization enhances uterine receptivity.

4.7.2 Uterine Environment Optimization

Adjusting maternal diet and supplementation enhances epigenetic stability in embryos.

Advantages:

✓ Enhances implantation rates.

✓ Decreases pregnancy loss in cloned buffalo calves.

5. Conclusion

Epigenetic modifications play a critical role in determining the developmental potential of cloned buffalo embryos. Understanding and manipulating these mechanisms can lead to improved cloning efficiency and animal production. Ongoing research into targeted epigenetic interventions holds promise for overcoming current limitations in SCNT technology.

REFERENCES

1. Campbell, K. H., McWhir, J., Ritchie, W. A., & Wilmut, I. (1996). "Sheep cloned by nuclear transfer from a cultured cell line." Nature, 380(6569), 64-66.
2. Gao, R., Wang, C., & Gao, Y. (2018). "Effects of histone deacetylase inhibitors on nuclear reprogramming and development of cloned embryos." Biology of Reproduction, 98(5), 645-655.
3. Shi, L. H., Miao, Y. L., & Ouyang, Y. C. (2019). "The role of non-coding RNAs in epigenetic regulation of cloned embryo development." Stem Cell Research, 40(1), 100533.
4. Zhang, Y., Ma, H., & Zhang, Z. (2016). "CRISPR-based genome and epigenome editing for improving SCNT efficiency." Cellular Reprogramming, 18(2), 111-120.
5. Loi, P., Ptak, G., & Modlinski, J. A. (2006). "Nuclear transfer: Epigenetic abnormalities and their potential correction." Cell and Tissue Research, 323(3), 247-254.
6. Singh, M., Palta, P., & Manik, R. S. (2021). "Advances in buffalo cloning and epigenetic reprogramming." Indian Journal of Animal Sciences, 91(6), 492-500.