

Research Article

Epigenomics of Livestock Animals: From Epigenetic Reprogramming in Clones to Environmentally Induced Epigenetic Variation and Its Breeding Implications

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Abstract

Epigenetics — heritable changes in gene expression that occur without alteration of the underlying DNA sequence — has emerged as a critically important dimension of livestock biology with far-reaching implications for reproductive biotechnology, genetic improvement, developmental programming, and the emerging field of epigenomic breeding. In livestock, epigenetic phenomena underlie the fundamental challenge of somatic cell nuclear transfer (SCNT), where incomplete reprogramming of donor cell epigenomes remains the primary cause of the low efficiency and high developmental failure rates that have constrained practical cloning applications for three decades. The same epigenetic machinery — DNA methylation, histone modification, chromatin remodelling, and non-coding RNA regulation — mediates the developmental programming effects of maternal nutrition, thermal stress, social environment, and management practices on offspring phenotype, with consequences that may persist across multiple generations through the germline. This review comprehensively examines the epigenetic marks and enzymatic machinery relevant to livestock species, the mechanisms and limitations of epigenetic reprogramming during SCNT and iPSC generation, the growing evidence for environmentally-induced epigenetic variation and its potential contribution to phenotypic plasticity and non-Mendelian inheritance in cattle, pigs, sheep, and poultry, and the current state of epigenome-wide association studies (EWAS) and their integration with genomic selection models. The FAANG initiative's systematic functional epigenomic annotation of livestock genomes is highlighted as an essential resource for functional interpretation of QTL and GWAS signals. Finally, the review examines the prospects for epigenetic editing — targeted modification of specific epigenetic marks using dCas9-based epi-tools — as a next-generation precision tool for livestock biotechnology.

Keywords: Epigenomics, DNA methylation, Histone modification, SCNT reprogramming, Livestock, iPSC, FAANG, Developmental programming, EWAS, Epigenetic editing

1. Introduction

The central dogma of molecular biology — DNA → RNA → protein — provides the mechanistic framework for understanding how genetic information is encoded and expressed. However, the observation that genetically identical organisms (monozygotic twins, cloned animals) can exhibit markedly different phenotypes depending on their developmental history, nutritional environment, and social experience requires an additional layer of explanation: epigenetic regulation. Epigenetic mechanisms — including DNA methylation, covalent histone modifications, chromatin remodelling by ATP-dependent complexes, and regulatory non-coding RNAs — modulate gene expression without altering the primary DNA sequence, creating cell-type-specific patterns of gene activity that define the distinctive transcriptome of each tissue despite sharing a common genome.

In livestock, epigenetics intersects with applied animal biotechnology in several critical ways. The epigenetic reprogramming challenge in SCNT — the requirement to erase and rewrite the differentiation-specific epigenetic state of a somatic donor nucleus to a totipotent embryonic state — is now recognised as the central barrier to efficient livestock cloning, with aberrant epigenetic reprogramming responsible for the majority of developmental failures that result in the characteristically low live birth rates of 1–5% typically achieved in bovine SCNT (Zakhartchenko et al., 2001). Understanding and manipulating epigenetic reprogramming in SCNT and iPSC generation is therefore a major research priority for improving the efficiency of these reproductive biotechnologies.

Simultaneously, growing evidence suggests that environmentally-induced epigenetic modifications — particularly DNA methylation changes in response to maternal nutrition, heat stress, microbial colonisation,

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and management practices — contribute to phenotypic variation in livestock production traits in ways that are not captured by conventional genetic models. If epigenetic variation is heritable through the germline (transgenerational epigenetic inheritance), it potentially represents a source of rapid phenotypic adaptation that could complement genomic selection. This review examines both dimensions of livestock epigenomics — the mechanistic underpinnings of reprogramming and the applied implications of epigenetic variation — in the context of current knowledge as of 2022.

2. Epigenetic Machinery in Livestock

2.1 DNA Methylation

DNA methylation in livestock, as in all mammals, occurs predominantly at the 5-position of cytosine (5-methylcytosine, 5mC) in CpG dinucleotide contexts, catalysed by the DNMT family of methyltransferases. DNMT3A and DNMT3B establish de novo methylation patterns during gametogenesis and early development, while DNMT1 (the maintenance methyltransferase) copies existing methylation patterns to the newly synthesised strand after each DNA replication. The ten-eleven translocation (TET) enzymes (TET1, TET2, TET3) oxidise 5mC to 5-hydroxymethylcytosine (5hmC) and further oxidation products, providing a pathway for active DNA demethylation that is critical for epigenetic reprogramming in early bovine embryos. Table 1 provides a systematic overview of the major epigenetic marks, their writer and eraser enzymes, reader domain proteins, and their relevance to livestock biology.

Table 1. Major epigenetic marks, their enzymatic writers, erasers, and readers, with specific relevance to livestock species

Epigenetic Mark	Writer Enzyme	Eraser Enzyme	Reader Domain	Livestock Relevance
DNA methylation (5mC)	DNMT1, DNMT3A/B	TET1, TET2, TET3	MBD proteins, MeCP2	Gene silencing; imprinting; SCNT reprogramming
H3K4me3 (active promoter)	MLL1-4; SET1A/B	KDM5A/B/C/D	PHD fingers; TFIID TAF3	Marks active gene promoters; tissue-specific TFs
H3K27me3 (silencing)	EZH1/2 (PRC2)	KDM6A/B (UTX/JMJD3)	Cbx proteins (PRC1)	Developmental gene silencing; X-inactivation
H3K36me3 (transcribed gene)	NSD1-3; SETD2	KDM2A/B; NO66	PWWP domain proteins	Elongation mark; exon definition; RNA splicing
H3K9me3 (heterochromatin)	EHMT1/2; SETDB1	KDM3A/B; KDM4A-D	HP1 $\alpha/\beta/\gamma$	Constitutive heterochromatin; transposon silencing
Histone acetylation (H3K27ac)	p300/CBP; PCAF	HDAC1-11; SIRT1-7	Bromodomain proteins	Enhancer activity; super-enhancers in lactation
Non-CG methylation (5hmC)	TET enzymes (oxidation of 5mC)	Passive dilution	Specific readers TBC	Oocyte maturation; early bovine embryo

5mC = 5-methylcytosine; 5hmC = 5-hydroxymethylcytosine; TF = Transcription factor; PRC = Polycomb Repressive Complex; HDAC = Histone deacetylase; HPA = Hypothalamic-pituitary-adrenal.

2.2 Histone Modifications

The nucleosome — 147 bp of DNA wrapped around an octamer of histone proteins (H2A, H2B, H3, H4) — is the fundamental unit of chromatin packaging in eukaryotes. The N-terminal tails of histones are subject to a remarkable variety of post-translational modifications (PTMs) including methylation, acetylation, phosphorylation, ubiquitination, and SUMOylation at specific lysine and arginine residues, collectively constituting the 'histone code'. Key combinatorial patterns include H3K4me3 marking active gene promoters, H3K27ac marking active enhancers, H3K27me3 marking Polycomb-repressed developmental genes, and H3K9me3 marking constitutive heterochromatin. The FAANG initiative has generated systematic histone ChIP-seq maps for cattle,

pig, sheep, goat, horse, and chicken across multiple tissues, providing a reference epigenome atlas for functional annotation of livestock genomes.

3. Epigenetic Reprogramming in SCNT and iPSC

The central dogma of SCNT is that somatic cell differentiation — which is epigenetically established and maintained by tissue-specific patterns of DNA methylation and histone modification — must be erased and rewritten to an embryonic state by the cytoplasm of the enucleated oocyte. The efficiency of this process determines SCNT developmental rates. Table 2 summarises published blastocyst rates and full-term efficiencies for SCNT and iPSC reprogramming across major livestock species, including interventions aimed at improving reprogramming.

Table 2. Epigenetic reprogramming efficiency for SCNT and iPSC generation in livestock species, with chemical and molecular interventions

Species / Cell Type	Technique	Blastocyst Rate (%)	Full-term Efficiency (%)	Key Reference
Bovine fibroblast	SCNT (conventional)	20–35	1–5	Vajta & Gjerris, 2006
Bovine fibroblast	SCNT + H3K9me2/3 demethylase (KDM4D)	35–48	4–8	Matoba et al, 2014
Bovine fibroblast	SCNT + 5-azacytidine treatment	28–42	3–7	Enright et al., 2003
Porcine fibroblast	SCNT (conventional)	15–28	0.5–2	Vajta et al., 2003
Bovine somatic	iPSC reprogramming (OSKM)	iPS colony efficiency <0.01%	Not established	Koh et al., 2016
Porcine somatic	iPSC (OSKM + LIF)	iPS colony efficiency 0.002–0.01%	Not established	Ezashi et al., 2009
Bovine mammary epithelial	SCNT from elite cow	25–38	1–4	Heyman et al., 2002

OSKM = Oct4/Sox2/Klf4/c-Myc Yamanaka factors; 5-aza = 5-azacytidine (DNMT inhibitor); KDM4D = H3K9me2/3 demethylase.

The most impactful single-factor improvement in SCNT efficiency to date has been the co-injection of mRNA encoding the H3K9me3 demethylase KDM4D into bovine SCNT-reconstructed embryos, which was shown by Matoba et al. (2014) to increase blastocyst rates and full-term development by 1.5–2-fold by removing the H3K9me3 'heterochromatin barrier' that resists reprogramming in somatic nuclei. Subsequent work has identified additional epigenetic barriers including aberrant DNA methylation at imprinted loci, failure to reactivate totipotency genes (ZSCAN4, ZSCAN5), and persistence of somatic-specific super-enhancer landscapes. Combinatorial approaches targeting multiple epigenetic barriers simultaneously — using HDAC inhibitors (scriptaid, TSA), DNMT inhibitors (5-azacytidine), and specific demethylase mRNA injection — represent the current frontier of SCNT efficiency improvement. Figure 1. The epigenome–phenome axis in cattle: showing how environmental inputs (nutrition, stress, toxins, management) impinge on the epigenome through epigenetic machinery, and how the resulting epigenetic states regulate transcription to produce phenotypic outputs relevant to animal biotechnology.

HATs/HDACs | KDMs ↓ EPIGENOME (DNA methylation | H3K4/27/36/9 methylation | H3K27ac enhancers | 5hmC dynamics) ↓ TRANSCRIPTOME (Tissue-specific gene expression; developmental programming; stress response genes) ↓ PHENOME (Milk production | Fertility | Disease resistance | Feed efficiency | Welfare indicators) ↓ Transgenerational inheritance via germline epigenetic marks (imprinting; sperm/oocyte methylation)

Figure 1. Conceptual framework of the epigenome–phenome axis in livestock, illustrating pathways from environmental inputs through epigenetic machinery to phenotypic outcomes relevant to animal production and biotechnology

4. Environmentally Induced Epigenetic Variation

The developmental origins of health and disease (DOHaD) hypothesis, first formulated by Barker and colleagues from epidemiological observations in humans, proposes that nutritional, hormonal, and environmental challenges during critical developmental windows program permanent epigenetic states that alter offspring metabolism, growth, disease susceptibility, and reproductive performance. Table 3 summarises the major environmentally-induced epigenetic effects documented in livestock species and their production or health consequences.

ENVIRONMENTAL INPUTS → (Nutrition | Thermal stress | Maternal behaviour | Microbial colonisation | Management) ↓ via Epigenetic Machinery (DNMT1/3A/B | TET1-3 | PRC2/EZH2 |

Table 3. Environmentally induced epigenetic modifications and their consequences in livestock species

Environmental Factor	Species / Tissue	Epigenetic Change	Production / Health Consequence
Periconceptual undernutrition	Sheep (fetal liver)	↑ RXRA promoter methylation	Altered glucocorticoid metabolism; metabolic disease risk
Heat stress (chronic)	Dairy cow (blood)	Hypomethylation of HSP70 promoter	Constitutive HSP70 expression; fertility impairment
High-grain diet (SARA)	Cattle (rumen)	Altered H3K4me3 at	Leaky gut; systemic inflammation; laminitis

Environmental Factor	Species / Tissue	Epigenetic Change	Production / Health Consequence
	epithelium)	epithelial barrier genes	
Early-life methyl donor supplementation	Piglet (liver, muscle)	↑ global DNA methylation; ↑ IGFBP3 methylation	Improved growth trajectory; feed efficiency
Maternal social stress (gestation)	Pig offspring (brain)	↓ NR3C1 (glucocorticoid receptor) methylation	HPA axis dysregulation; fearfulness
Paternal diet (high fat)	Mouse → cattle parallel	↑ sperm DNA methylation at metabolic loci	Transgenerational metabolic phenotype in offspring

SARA = Subacute ruminal acidosis; HSP = Heat shock protein; HPA = Hypothalamic-pituitary-adrenal axis; IGFBP3 = Insulin-like growth factor binding protein 3.

The periconceptual period — spanning the final weeks of oocyte growth, fertilisation, and the first two weeks of embryonic development — is characterised by genome-wide epigenetic reprogramming (demethylation followed by de novo remethylation) that is particularly sensitive to nutrient availability. Methyl groups for DNA and histone methylation are derived from one-carbon metabolism (folate cycle and methionine cycle), making methylation reactions sensitive to dietary supply of methyl donors (methionine, choline, betaine, folate, vitamin B12). Supplementation of periconceptual methyl donors in sheep and cattle has been associated with lasting changes in offspring DNA methylation patterns and phenotypic outcomes including altered growth rates, fat deposition, and immune responsiveness, consistent with epigenetic programming mechanisms.

5. EWAS and Epigenomic Breeding

Epigenome-wide association studies (EWAS) — the systematic comparison of genome-wide DNA methylation (or histone modification) patterns between animals with extreme phenotypes for production or health traits — offer a complementary approach to GWAS for identifying loci at which epigenetic variation is associated with phenotypic variation. Unlike GWAS, which detects fixed genetic variants, EWAS can in principle identify epigenetic loci influenced by environmental, developmental, or stochastic factors, providing mechanistic insights into non-genetic components of phenotypic variation. EWAS in dairy cattle have identified differentially methylated regions (DMRs) associated with milk yield, somatic cell count, and metabolic disease susceptibility, with some DMRs co-localising with GWAS QTL, suggesting that the genetic effect is mediated through epigenetic modification of cis-regulatory elements.

6. Epigenetic Editing

The development of catalytically inactive Cas9 (dCas9) fused to epigenetic effector domains has created a new class of tools — epigenetic editors — capable of

depositing or removing specific epigenetic marks at targeted genomic loci without inducing DNA double-strand breaks. dCas9-DNMT3A fusions enable targeted CpG methylation, dCas9-TET1 enables targeted demethylation, and dCas9-p300 enables targeted histone acetylation. In livestock, the potential applications include silencing specific imprinted genes to improve SCNT efficiency, activating silenced resistance genes to confer disease protection without sequence alteration, and programming specific epigenetic states in embryo-derived cells to improve iPSC reprogramming. Regulatory ambiguity — whether targeted epigenetic editing constitutes genetic modification — remains a key challenge for the translation of epigenetic editors to commercial livestock biotechnology applications.

7. Conclusions

Epigenomics has matured from a niche research area into a central discipline at the intersection of livestock developmental biology, reproductive biotechnology, and applied breeding. The identification of H3K9me3 as a major SCNT barrier and the demonstration that its removal substantially improves bovine cloning efficiency represent a mechanistic breakthrough with direct practical implications. The growing evidence for environmentally-induced epigenetic programming of production and health traits opens new avenues for management interventions — particularly during the periconceptual period and early postnatal life — that could deliver economically meaningful improvements in livestock productivity and resilience without genetic modification. The integration of EWAS with GWAS, functional epigenomics from FAANG, and targeted epigenetic editing tools defines a rich research frontier that will occupy livestock epigenomicists for decades.

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