

Effects of sperm DNA methylation on domesticated animal performance and perspectives on cross-species epigenetics in animal breeding

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Implications

- Identification of sperm-specific DNA methylation markers at different time points is important for the performance improvement of domestic animals.
- DNA methylation can regulate sire fertility and semen quality and influence offspring phenotypes. The effects of inter-/trans-generational inherited DNA methylation information induced by paternal experience and environment stimuli should not be underestimated in farm animals.
- Cross-species mapping of epigenetic information from the well-studied species (e.g., human and mouse) could help dissect the genetic mechanisms underlying complex traits in farm animals (e.g., cattle and pigs).
- In the post-genome-wide association study era, the analysis of relationships between genetic variations and epigenetic modifications will help researchers dissect biological processes underlying complex traits and design advanced selection strategies in animal breeding.

Key words: DNA methylation, domesticated animals, inter-/trans-generational inheritance, sperm

Introduction

In domestic animals, the initiation and implementation of the FAANG (Functional Annotation of Animal Genomes) and FarmGTEx (Farm Animal Genotype-Tissue Expression)

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projects symbolize the imminent arrival of the post-genome era (Liu et al., 2020a). With the popularization of artificial insemination technology, the semen quality of excellent breeding bulls, breeding boars, and other male animals have become the focus of research. In addition to DNA sequence information, the heritable information within the semen consists of genome-associated non-DNA sequence information (epigenome). DNA methylation is the most stable epigenetic modification, and fully exploring the influencing factors of DNA methylation in inter-individual heterogeneity is an important approach for improving the accuracy of livestock genomic selection. An increasing amount of evidence showed that non-DNA sequence information may be as important as DNA sequence information in animal breeding schemes in the nearest future.

This review aimed to focus on the following (Figure 1): 1) the heterogeneity and conservation between sperm and other tissues; 2) the influences of environment on the phenotypes of individual and its offspring through sperm DNA methylation; 3) the genetic basis of DNA methylation; 4) the significance of comparing epigenomic information among species; 5) the research status of sperm DNA methylation in domestic animals.

Variations in DNA methylation across tissues and time points

Characteristics of DNA methylation in sperm

Epigenomic information plays a vital role in the differentiation of tissues during embryonic development and is involved in the formation of complex traits. Many single nucleotide polymorphisms (SNPs) identified by genome-wide association study (GWAS) are enriched in tissue-specific regulatory regions, such as tissue-specific promoters marked by H3K4me3 (Liu et al., 2020b). The establishment of an epigenetic reference map is important for the analysis of complex traits and animal breeding. In humans, the Roadmap epigenomics consortium had established 111 reference epigenomes using 2,624 genome-wide data sets, including

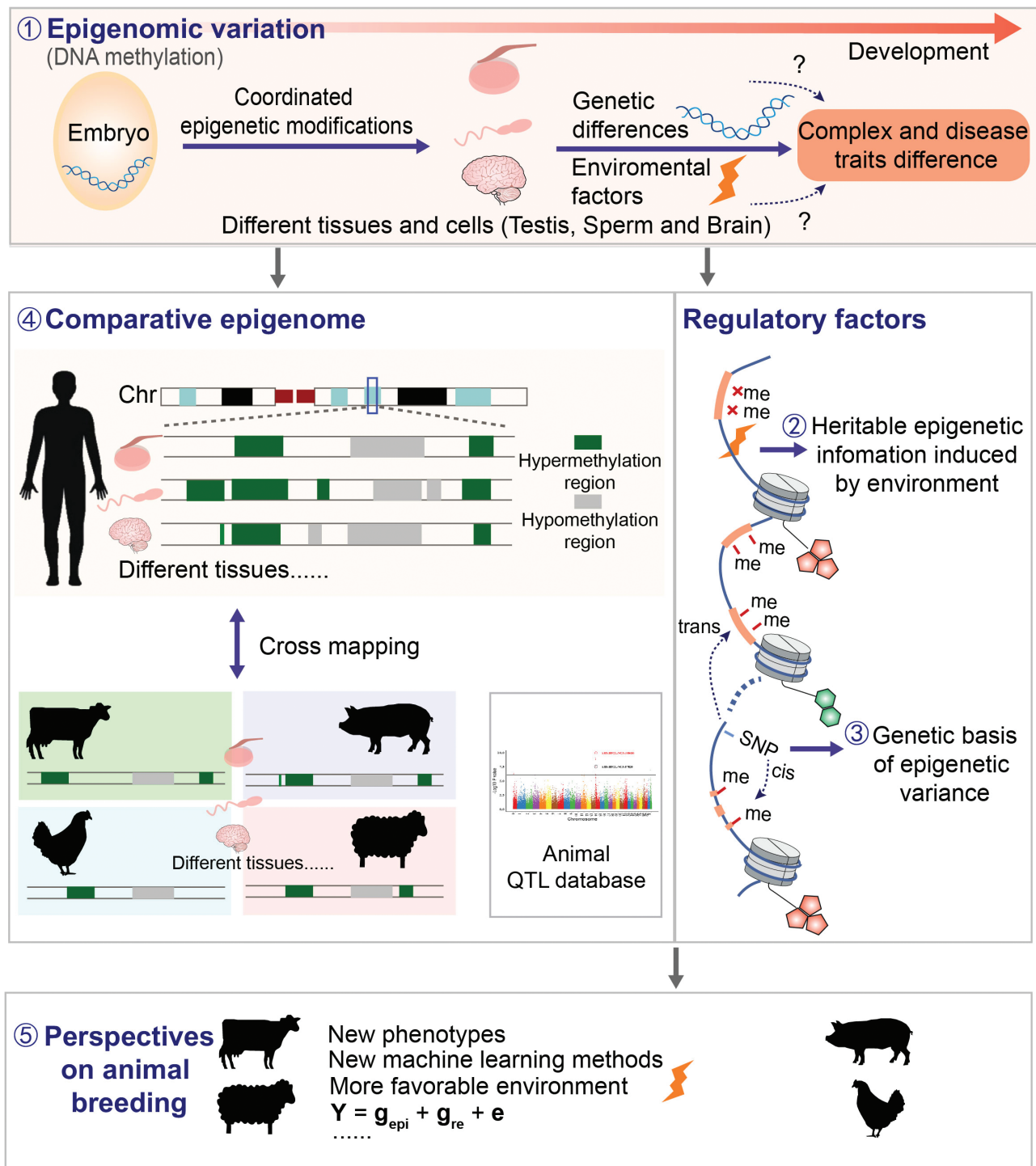


Figure 1. Understanding and applying epigenetic information in animal breeding. First part: DNA methylation variation across tissues and times. Second and third parts: the influences of environment and genome on DNA methylation. Fourth part: the comparisons of DNA methylation information across species. Last part: the research progress and perspectives of sperm DNA methylation in domestic animals. Symbols of the formula in the last part, Y : the vector of phenotypes for complex traits, g_{epi} : the vector of genetic values corresponding to genomic variants in epigenome functional elements, g_{re} : the vector of genetic values corresponding to the rest of genome, and e : the vector of residual effects.

166 RNA-seq datasets, 277 DNA methylation datasets, 360 DNA accessibility datasets and 1,821 histone modification datasets (Kundaje et al., 2015). It helps us understand the genetic and biological mechanisms of human disease traits. In livestock, the FAANG consortium has started to identify

regulatory elements and to build epigenomic reference, which greatly promotes the molecular basis of the dissection for phenotypic divergence and animal breeding. Sperm acts as a carrier of genetic material, and its DNA methylome affects maternal pregnancy rate and offspring phenotype

(Liu et al., 2019a). Moreover, sperm plays an important role in the phenotypic divergence during species evolution. The DNA methylation pattern in sperm significantly differs from that in other somatic cells and tissues and undergoes two re-programming processes. Most of the histones in sperm are replaced by protamine. Identifying specific methylation regions in sperm is important in improving reproductive and semen quality traits in livestock with low heritability. A previous study had identified the partially sperm-specific methylated domains and hypomethylated regions in livestock. These regions are enriched in meiosis- and spermatogenesis-related genes, as determined by comparing sperm with somatic tissues (brain, mammary gland, and blood) (Zhou et al., 2018). However, these studies only used limited tissues, thereby limiting our understanding of the patterns of sperm methylation and dissection of complex traits in livestock.

Changes of DNA methylation at different developmental and life stages

DNA methylation level changes with the different developmental and life stages and corresponds to the physiological characteristics of specific developmental or life stages. Comparing the DNA methylation changes of bull spermatozoa across 10 months (early puberty), 12 months (late puberty), and 16 months (pubertal; considered as adult) and in differently methylated regions (DMRs) across stages are associated with spermatozoon motility and early embryonic development (Lambert et al., 2018). In addition, some differential methylation regions that are highly correlated with age have been identified. The methylation status of such regions can be used as a marker to predict age. A previous study identified 353 differentially methylated CpG sites and built a widely applicable “Epigenetic Clock” for cells using the DNA methylation datasets of 51 tissues and cell types of different ages (Horvath, 2013). These loci have high reliability in predicting individual biological age. Moreover, improving the service life of the production herds (e.g., longevity of cows) is of great significance to the development of animal husbandry. Thus, the age-related epigenetic markers are important as molecular markers of animal longevity traits and can be added to statistical models (e.g., GFBLUP and BayesRC) to improve the accuracy of genomic prediction and to accelerate genetic progress.

Epigenetic markers vary during development, and are also affected by different environmental factors. The identification of these influencing factors and the degree of variation is the significant key to the improvement of livestock and poultry phenotypes. Moreover, the interpretation of the genetic basis of complex traits by integrating epigenome information with the vast number of quantitative trait locus (QTL) in animals needs to be considered.

Paternal experience induces the inherited changed DNA methylation of sperm

Increasing evidence shows that the changes of genome-associated non-DNA information (DNA methylation,

non-coding RNA, and histone modification) can pass from parent to their offspring and influence the offspring’s development, fertility, behavior, production, and health. In the rat, this epigenetic information can be passed on for three generations through sperm (Skinner et al., 2018). However, in domestic animals, in which epigenetic information is involved in intergenerational and trans-generational transmission, how this information influences the parents’ and offspring’s phenotype is far less elucidated. Numerous inter-/trans-generational studies have been conducted in humans and model organisms, and their results provide valuable insights into the epigenetic inheritance of farm animals. As the most stable epigenetic modification, DNA methylation can be a promising marker in animal breeding. DNA methylation of sperm could be changed by various kinds of stimulus and could influence offspring’s development and performance (Siddeek et al., 2018). In general, the potential effects of stressors, food intake, disease susceptibility, and heat stress on the sperm DNA methylation of farm animals are discussed in this section.

Life stress

For animals commonly subjected to intensive pasture in intensive farming, maternal separation of offspring (cow and chicken), the use of limiting bar (pig), and high-density culture (chicken), natural behavior is restricted, which potentially has a persistent effect across generations. A rodent study has indicated that males (F1) subjected to maternal separation after birth showed decreased DNA methylation in sperm during adulthood, and its male offspring (F2) exhibited slow behavioral response under adverse conditions (Gapp et al., 2016). However, exposure of the father to early moderate stress stimuli is good for physical health (Gapp et al., 2014), and finding the optimum level of stimulation in farm animals will help produce more healthy animals.

Food intake

Increasing evidence has shown that paternal diet influences offspring metabolism, development, and health through the epigenetic information in the sperm. Folate is involved with one carbon metabolism and directly influences DNA methylation. Numerous studies on the effect of folate on parents and their offspring have been conducted. In a rodent study, taking 15% of the recommended folate altered sperm DNA methylation, and induced birth defects (craniofacial and musculoskeletal malformations) in the offspring (Lambrot et al., 2013). In addition, environmental toxicants damaged the DNA methylation of sperm across generations (Skinner et al., 2018). The abovementioned results indicated that differential food intake could induce long-lasting and inter-/trans-generational effects.

Disease susceptibility

In domestic animals, elucidating the mechanism of disease resistance and susceptibility traits is difficult due to low heritability. Studies in the mouse have shown that the risk of tumor incidence can be transmitted across generations through aberrant DNA methylation and histone modification (Lesch et al., 2019). Thus,

in farm animals, the exploration of the inheritable epigenetic information in relation to disease susceptibility traits contributes to the understanding of the “missing” heritability observed in GWAS of disease traits.

Heat stress

With escalating global warming, heat stress seriously influences the performance and welfare of domestic animals. In the late gestation of dairy cows, the negative effect of heat stress could pass on at least two generations, and cause more than 300 million economic losses per year in the United States (Laporta et al., 2020). How epigenetic modifications respond to heat stress and whether the “immediate” epigenetic information changes can be transmitted across generation in domestic animals are still unknown. A study involving wild guinea pig males indicated that heat stress induces DNA methylation changes in the testis and liver of male offspring (Weyrich et al., 2016), and these epigenetic changes potentially reflect the adaptational ability of the species in response to heat stress. In the future, heat stress will become an increasingly conspicuous environmental factor. Thus, researchers should focus on analyzing and preventing its negative effects on the epigenetic modifications of farm animals and their offspring.

Non-negligible seminal plasma

Seminal plasma is conventionally regarded as a means of sperm transport, and many studies have shown its vital role in modulating the immunological responses of maternal reproductive tract and in influencing offspring health. Rodent studies showed that poor paternal diet alters offspring metabolism, development, and gut bacterial profiles through changed sperm or seminal plasma. Bovine seminal plasma reportedly initiates the inflammatory response in the maternal endometrium (Ibrahim et al., 2019). However, in livestock, the procedures of diluting semen during artificial insemination and in vitro fertilization are common, especially in cattle, and these procedures may reduce the physiological effect of seminal plasma on the maternal reproductive tract and even influence offspring development. Extracellular vesicles in seminal plasma may become a popular topic among researchers who study the inter-/trans-generational transmission of paternal life experience.

The genetic basis of DNA methylation

Methylome can be partially explained by genetic variants

With the appearance of the first GWAS on domestic animals, many genetic variants associated with various complex traits and disease susceptibility and resistance were identified. However, most of the identified SNPs lie in non-protein-coding regions, and these SNPs’ biological function and influence on phenotypes need to be elucidated. Epigenetic information could bridge the gap between genome and phenotypes.

Generally, the level of DNA methylation or other epigenetic modification on each locus can be regarded as a quantitative

trait. These DNA methylation loci may have their own DNA methylation QTL (meQTL) and heritability (van Dongen et al., 2016). Studies about the genetic component of DNA methylome were widely carried out due to the early application of Human Methylation 450K BeadChip Kit (Illumina). In humans, dissecting the additive genetic, common environmental, and remaining variance of these loci can be performed by using the classical twin design (monozygotic and dizygotic twins). The result showed approximately 0.19 heritability of DNA methylation (van Dongen et al., 2016). Notably, more than 35% of the tested CpG sites harbored at least one *cis* or *trans* meQTL, and the downstream effects of identified SNPs associated with disease were effectively explained by using these meQTLs (Bonder et al., 2017).

Influences of sex, age, and tissue on meQTLs

Remarkably, sex, development stages, and tissue types can influence the DNA methylome. The effects of genetic variation on the DNA methylome are highly stable in development (Gaunt et al., 2016), thereby indicating that in domestic animals, most of the identified meQTLs at one time point could be represented throughout the animal’s life. In addition, DNA methylation varies in different tissues, but a modest proportion of shared meQTLs (45%–73%) has been found between various tissues (Lin et al., 2018), which may provide a chance to apply epigenomic information from easily obtained sperm and blood samples to tissue-specific complex traits, e.g., schizophrenia in human brain and mastitis in dairy mammary gland. Single nucleotide variants may result in various molecular changes, including chromatin accessibility, DNA methylation, histone modifications, and gene expression. However, the internal coordination among these molecular features remains unclear. With the improvement of algorithm and further experimental validation, the causal relationship between these molecular data can be well evaluated.

With the reduced cost of sequencing and the recent concerted effort of the FAANG and FarmGTEx projects to explain genome and epigenome in farm animals, the number of discovered epigenome QTLs will increase. The accurate molecular mechanisms of complex traits can be well elucidated through this epigenetic information. Domesticated animal selection that involves DNA methylation may occur in the future (Figure 2). To better apply the combination of genetic and epigenetic information to domestic animals, more molecular data, optimized sequencing technologies, and computational algorithms are needed. Coordinated development of genetic and epigenetic information will ensure better accuracy in breeding and stock selection programs.

Cross-species mapping from human and model organisms to livestock and poultry

The plentiful epigenetic data in human and model organisms

Identifying epigenetic regulatory elements and building-up “one-to-one” annotation, e.g., epigenetic marker (DNA

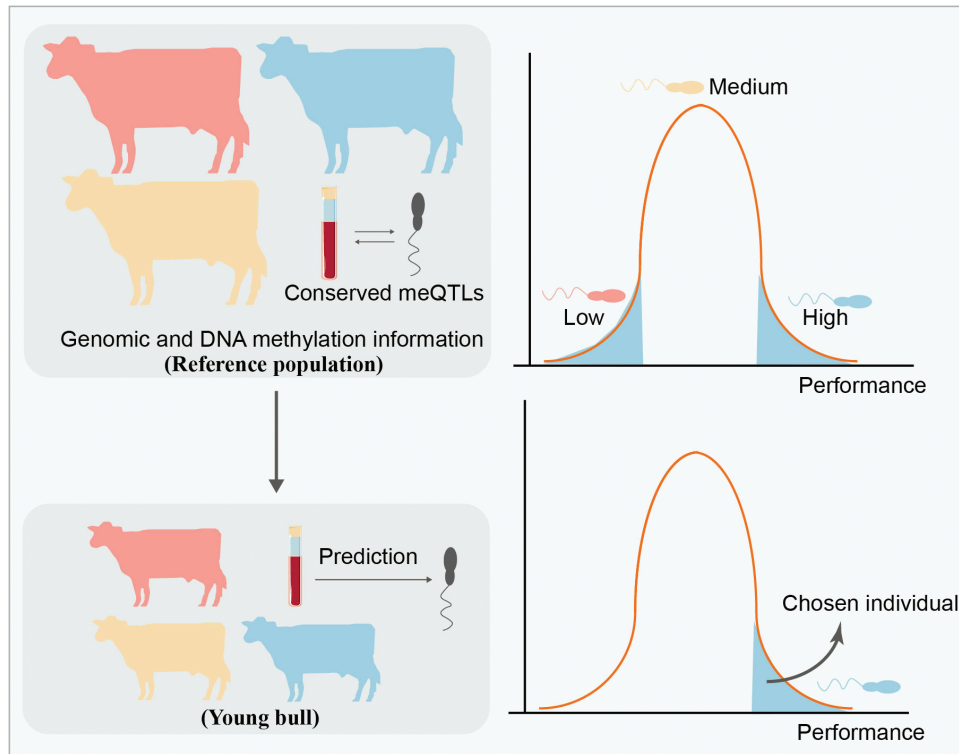


Figure 2. Combining genomic information and conserved meQTL information between sperm and other tissues contribute to selection.

methylation and histone modification marker) vs. complex traits across tissue and developmental stages are important, because these will lead to a better understanding of complex biological processes resulting in the achievement of more reliable therapies and accurate selection strategies (Figure 1). However, because of costly sequencing, establishing “one-to-one” relationship in livestock is extremely difficult. Compared with livestock and poultry, with the establishment and implementation of international projects, such as ENCODE, Roadmap Epigenomes Project, and GTEx, more epigenetic data are produced in human and model organisms through approaches, such as WGBS, ChIP-seq, ATAC-seq, and RNA-seq (Figure 3). We wondered whether the existing epigenetic data from human and mice can be applied to the improvement of animal selection. Abundant evidences from comparative analysis among species indicated that regulatory elements are highly conserved across species, and these regulatory elements play an important role in tissue-specific gene expression (Blake et al., 2020). Therefore, cross-species mapping is a new method to identify regulatory elements for domesticated animals by referring human functional genomics. A previous study in cattle identified tissue-specific histone markers and investigated the relationship between important traits and corresponding tissues by cross-species mapping with human, thereby providing a reference for future studies on relevant tissues for dairy cow complex traits (Liu et al., 2020b). Although many breeds of livestock exist, the population for some breeds is small, such as Jersey cattle, thereby making genetic analysis difficult. In this case, comparative analysis across species could help identify markers

and expound complex genetic traits. In sheep, functional annotation reveals that proximal regulatory elements enriched in selective sweeps referring human data contribute to understand the evolution of modern breeds (Naval-Sanchez et al., 2018).

Significance of cross-species comparative DNA methylation

In humans, sequencing data are many, but obtaining tissue samples can be difficult due to ethical issues. Thus, the knowledge on the biological mechanisms underlying some complex traits is limited. Compared with humans, collecting a large number of tissue samples and phenotypic records from livestock can be easy. Moreover, pedigree records in livestock are complete. Livestock species existed in the same environment and are subjected to consistent diet and management due to intensive farming, thereby reducing the confounding factors to a certain extent in an epigenetic study. Therefore, using livestock as an animal model of complex human traits may promote the rapid development of human medical research. By comparing the differences in sperm DNA methylation patterns between cattle and human, causal variations associated with body type traits and immune traits were found in the conserved methylation regions of both species (Fang et al., 2019a). These results imply that the cow may serve as a model animal for human immune traits and body conformation owing to complete pedigree and phenotypic records and large populations.

Although we can identify some conserved functional elements between species by cross-species comparisons, it is not

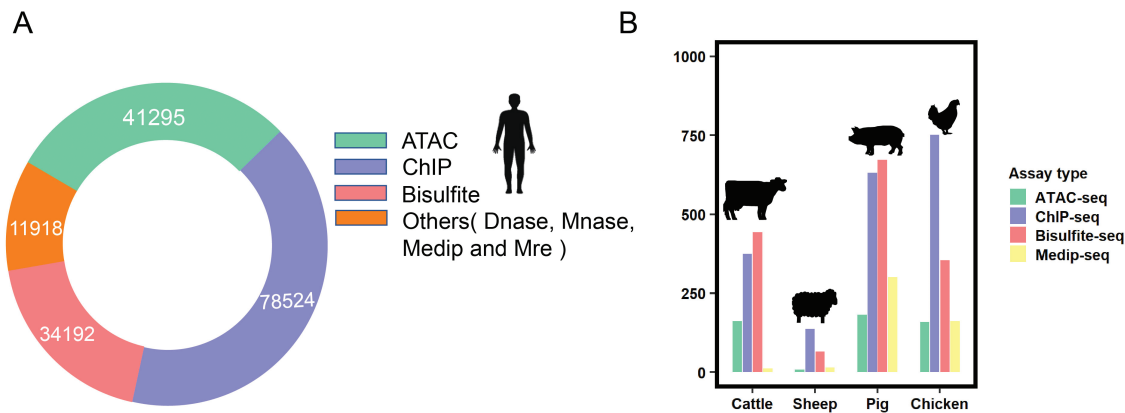


Figure 3. Reported epigenetic data in human (A) and domestic animals (B) in the Sequence Read Archive database. The numbers in the figure represent the number of reported epigenetic data.

possible to identify breed-specific functional regions. It is imperative to establish a reference map of the functional elements in livestock and poultry. Moreover, the use of these reference maps to elucidate the genetic mechanism of complex traits is an important issue that needs to be addressed in the future.

Effects of sperm DNA methylation of domestic animals on phenotype and its application perspective

Sperm DNA methylation variance in different breeds

Domestic animals, such as Holstein (dairy cow) and Hereford (beef), have evolved into different breeds with unique economic traits. They undergo strong long-term selection in modern agriculture, which leads to differentially retained genetic and epigenetic information among different breeds. Thus, measuring and assessing the epigenome among different breeds is essential for understanding the formation mechanism of livestock and poultry economic traits. A recent study comparing the DNA methylation characteristics of testicles and *longissimus dorsi* of Bamaxiang and Large White pigs found that the DNA methylomes differed among different breeds but were stable within a single breed (Zheng et al., 2020). Sire sperm carries stable DNA methylome, and similar to blood, it can be easily and repeatedly collected. Using sperm to predict the epigenome pattern of other tissues would be promising. Sperm is a suitable sample to annotate the function of DNA methylome among different breeds. Within a single breed, a DNA methylation clock constructed based on sperm can characterize the biological age of individuals and predict productive life.

Conserved DNA methylation regions across breeds may be used to mine conserved breeds' meQTLs and to improve cross-breeding animal genetic selection because of the unique linkage disequilibrium among the different breeds. The DMRs identified in the sperm of different breeds can be used to study breed-specific phenotypes, such as high disease resistance and high

fertility. Conserved and specific DNA methylation information among different breeds can be used for animal breeding.

Sperm DNA methylation regulates sperm-related traits

As a proverb in China states, "If a female animal is good, a litter of young animals will be good. If a male animal is good, all the young animals on the side of a hill will be good." The sires of livestock and poultry are extremely valuable in the breeding industry. The fertility of excellent bulls, boars, and other male animals has become a focus of research due to the popularization of artificial insemination technology. Different from the human, sire animals have long-term and detailed reproductive records, which make male animals ideal as specific biological models to study male fertility. In actual production, sire conception rate and sperm quality can be used to evaluate the fertility of livestock and poultry. However, sperm-related traits are usually low-heritability quantitative traits that are susceptible to environmental influence (Yin et al., 2019). Epigenetics bridges the genome and the environment. Thus, epigenetic markers may be regarded as effective information that excludes low fertility sires from breeding programs. Sire's sperm DNA aberrant hypermethylation is associated with low fertility traits (Fang et al., 2019b; Table 1), which is similar to human sterility and possesses potential application value (Pacheco et al., 2011).

Monozygous (MZ) twins have the same genetic background and are excellent materials for studying epigenetics. We used sperm from MZ twin bulls to analyze sperm-related traits and DNA methylome variants at the individual level. The semen quality and the breeding value of the offspring daughter's reproductive traits are remarkably different, which may be due to the stable DMRs of the sperm across the life (Liu et al., 2019a). We found that the highly variably methylated regions of Holstein bull sperm are related to reproductive traits of the offspring at the population level (Liu et al., 2019b). The epigenome of sperm can be transmitted to the oocyte together with the haploid genome to promote

Table 1. Study of DNA methylation regulating sperm-related traits and offspring phenotypes

Species/breed	Period	Accepted treatment/ physiological status	Sample size	Regulated phenotype	Influenced offspring phenotype	Reference
Holstein bull	Adult	Healthy	2	Sperm quality	Daughters' reproductive traits	(Liu et al., 2019a)
Holstein bull	Adult	Healthy	6	Sire conception rate (SCR)	NA	(Fang et al., 2019b)
Holstein bull	1–2 years old	Healthy	28	Sperm quality	Daughters' reproductive traits	(Liu et al., 2019b)
Norwegian Red bull	413–542 days	Healthy	16	Fertility	NA	(Narud et al., 2021)
Japanese Black bull	12–144 months	Healthy	28	Sire conception rate (SCR)	NA	(Takeda et al., 2021)
<i>Sus scrofa</i>	1–2 years old	Healthy	7	Fertile	NA	(Pétille et al., 2021)
Large White pig	Days 35 of age	Methyl donor diet	16 F0; 60 F2	NA	Carcass Traits	(Braunschweig et al., 2012)
Polypay rams	10.7–13.6 weeks	Supplemental methionine	20 F0; 194 F1	NA	Weight and scrotal circumference	(Gross et al., 2020)

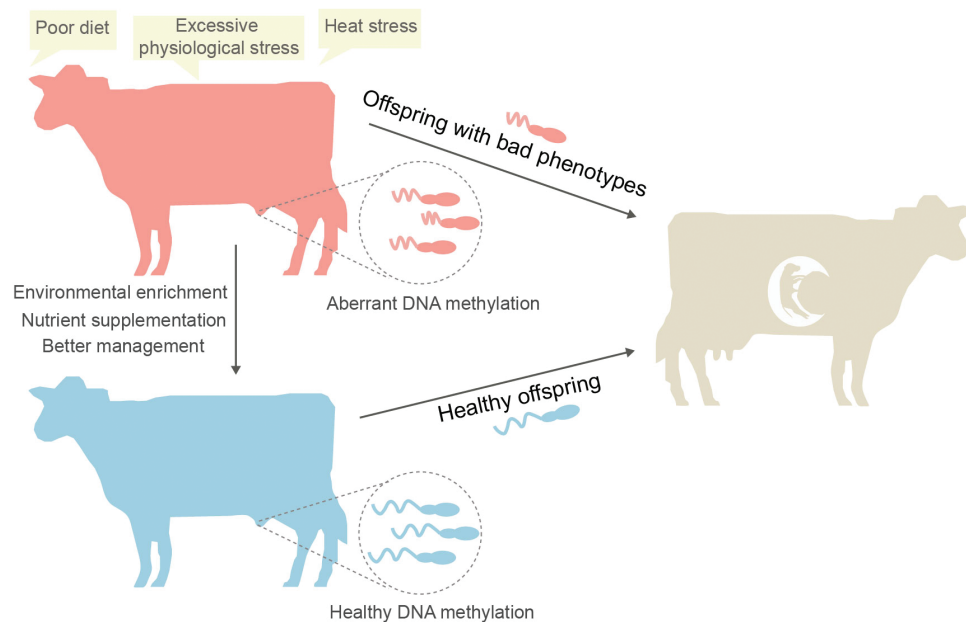


Figure 4. The effects of environment on the changes of bovine sperm DNA methylation.

embryogenesis and affects the phenotype of the offspring (Table 1). These epigenetic “codes” can predict the phenotype of the offspring and even enter genomic selection as bioinformatics information. In addition, we could adjust some external factors, such as fodder nutrition, breeding environment to improve sires’ sperm quality, and offspring performance, by regulating sperm DNA methylation (Figure 4). A nutritional epigenetics study found that adding micronutrient supplements into the diet of F0 sire pigs and sheep may affect the weight and fat deposition of the offspring (Braunschweig et al., 2012; Gross et al., 2020). Breeding of sires is often characterized by high selection intensity and large offspring, and this livestock system

will amplify the influence of sire sperm DNA methylome on the future performance of many offspring. Thus, the effects of DNA methylation on offspring phenotype production in livestock and poultry should be considered important.

Sperm DNA methylome contributes to breed-specific phenotype, regulates sperm-related phenotypes, and even has potential regulatory effects on offspring traits. However, our knowledge of the different periods of DNA methylation regulation during spermatogenesis and embryogenesis in domestic animals is incomplete. The mechanisms underlying sperm DNA methylation’s regulation of complex traits in livestock and poultry need to be further explored.

Conclusions

Epigenetic modification serves as the “gold bridge” between genotypes and phenotypes. Epigenetic variation can arise due to tissue type, developmental stages, individual characteristics, and environmental effects. Although the genetic basis of sperm epigenomic variations has been reviewed in livestock and poultry, including the effects of epigenetics on complex traits and the phenotype of the offspring, it is still challenging to add epigenomic information to animal breeding and genomic selection. With the increase in the amount of sequencing data in farm animals and cross-species mapping from well-studied organisms, the epigenomic marker is an important marker that can be applied to the interpretation of the genetic basis of complex traits and the improvement of animal breeding.

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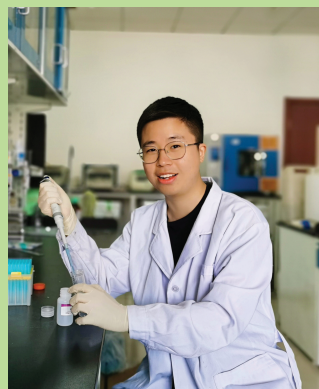
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Conflict of interest statement. The authors declare that they have no conflict of interest.

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He initiated and is currently leading the Farm animal Genotype-Tissue Expression (FarmGTEx) project. This is an international collaborative endeavor (30+ institutions worldwide already committed to join) to provide a comprehensive atlas of tissue-specific gene expression and genetic regulation for over 50 primary tissues in livestock species.

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