Metabolic and environmental effects on embryo epigenome

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Abstract

Extensive epigenetic changes occur during early embryonic development, which are necessary to reset the gametic gene expression program to an embryonic totipotent state and ensure full developmental potential is achieved. Epigenetic changes are catalized by enzymes, many of which use products of intermediate cell metabolism as cofactors or substrates. Early development occurs in a fluid environment provided by various compartments as gametes/embryos develop and whose composition is sensitive to metabolic and physiological changes. Therefore, nutritional and environmental factors can potentially alter the embryonic epigenome, influencing the trajectory of development, with long term effects on healthspan. In this manuscript, we review connections between metabolism and epigenetics and how environmental factors can affect gametes and embryos, with potential effects on the animal.

Keywords: Epigenetics, preimplantation development, metabolism, healthspan, DNA methylation, histone modifications

Introduction

Epigenetics (above genetics) refers to changes in phenotypes not caused by changes in the genetic code. At the molecular level, epigenetics is mediated by modifications of DNA and chromatin that do not involve changes in the DNA code. These modifications are mostly represented by DNA methylation, posttranslational histone modifications (e.g. acetylation and methylation), and small noncoding RNAs.[1]

There are many phenomena controlled by epigenetic mechanisms including, among others, cellular differentiation, genomic imprinting, and X chromosome inactivation (XCI).[2] In multicellular organisms, almost all cells contain the same DNA sequence, but can vary widely in shapes and functions, due to differences in epigenetic information.[3] Genetic imprinting is a phenomenon in mammals and some other species, in which only 1 copy (allele) of certain genes is expressed in a parent of origin basis, irrespective of the actual allele sequence. For example, in female cells, an entire X chromosome is inactivated by epigenetic mechanisms to compensate for gene dosage differences with males carrying only 1 X chromosome.[4] Generally, epigenetic changes are inherited across cell divisions, so that once a differentiated cell divides, it gives rise to a similarly differentiated cell (i.e. skin cell give rise to skin cell); or in the case of genomic imprinting, the identity of the parental alleles is maintained in all cells of the individual; or for the case of X chromosome inactivation, the same X chromosome is silenced in all progeny that originated from the cell undergoing the initial XCI event.[5]

Although epigenetic marks are inherited during cell division, they are not permanent and are actively modified in situations such as cellular differentiation, allowing a single cell precursor to give rise to multiple types of differentiated cells; reactivation of X chromosomes during development; or erasure and re-establishment of imprinting in the germline to confer parental origin specificity to the next generation.[6] One of the most dramatic changes in epigenetic information is observed during early development, as 2 highly differentiated cells, sperm and egg, are reprogrammed to the totipotent naïve state of early blastomeres (embryo cells).[7]

Recently, there has been considerable interest in whether environmental factors (e.g. diet, temperature) modulate establishment and maintenance of epigenetic modifications and could thereby influence gene expression and phenotype in organisms.[1] Several recent studies documented that chemical pollutants, dietary components, temperature changes and other external stresses can have long-lasting effects on development, metabolism, health and production in livestock.[8,9]

Given that 1) epigenetic changes can be affected by the environment, 2) there are dynamic changes in epigenetic information during early development, and 3) epigenetic states can be inherited during cell division, it is reasonable to expect that alterations to embryos or gametes, may compromise their epigenomes, with long-term consequences for the ensuing animal. Current livestock production systems often expose animals to adverse metabolic and environmental stresses that could

hamper optimal healthspan and reproductive capacity. In addition, use of artificial reproductive technologies exposes gametes and/or embryos to suboptimal conditions that can affect embryo metabolism and animal health/productivity.[10]

The objective of this manuscript is to survey the connection between various environmental stressors and the livestock embryonic epigenome.

Early embryonic development and epigenetic reprogramming

Embryonic development starts with fusion of the spermatozoon with an oocyte. Fertilization triggers a complex cellular programme that transforms 2 highly specialized germ cells, oocyte and sperm, into a totipotent zygote.[6] The transition from 2 highly specialized cells containing unusual chromatin states to an early embryo state is among the most dramatic and complex reprogramming events in biology.[11] Detailed molecular changes in the epigenome of livestock embryos have been comprehensively reviewed elsewhere,[12-14] so we provide a very general overview.

After the sperm nucleus enters the oocyte cytoplasm, genome wide epigenetic remodeling occurs. The paternal genome exchanges protamines for histones, undergoes an active process of DNA demethylation, which requires the activity of ten-eleven translocation (TET) enzymes and acquires a new set of histone modifications, necessary for proper gene expression and embryo development. Concomitantly, maternal DNA is passively demethylated. After zygotic demethylation, the embryonic genome continues to be demethylated during the following few cleavage divisions until the blastocyst stage. Similarily, extensive changes in histone modifications ensue after fertilization, leading to an overall increase in histone acetylation and loss of repressive histone marks (e.g. H3K9me3 and H3K27me3). This loss of repressive DNA methylation and histone modifications is thought to facilitate reactivation of the embryonic genome.[15] Also, global epigenetic remodeling during preimplantation development ensures that gametic genomes return to pluripotency.[16] For example, loss of methylation in promoters of master regulators of pluripotency genes such as OCT4 and NANOG is required for establishment and maintenance of the inner cell mass lineage in the blastocyst.

Periconceptional period and its importance to proper embryonic development, animal growth and healthspan

Correct embryonic development is fundamental for animals to have a healthy life, grow properly and reproduce. Among all events occurring during animal ontogenesis, the periconceptional period is critical.[17] The periconceptional period usually comprises all events taking place during gamete maturation, fertilization and early embryonic development. In a manner somewhat arbitrary, we can also consider implantation and early stages of placenta formation part of the periconception period.

It is now clear that early embryo development in mammals can be affected by parental diet, as well as environmental stress factors, including factors affecting either parent in the months and weeks that precede fertilization.[18] Gamete maturation and early embryonic development occur in a fluidic medium (i.e. epididymal, follicular and oviductal fluids) which is susceptible to alterations by animal diet and environment. During the periconceptional period, exposure to altered diets or environmental stressors can result in epigenetic alterations with potential effects on subsequent animal development and performance.[17]

There are epidemiological evidences and studies in laboratory animals that an inadequate or deficient diet, exposure to chemical toxicants and environmental stressors during the periconceptional period can affect embryo metabolism, development and even behavior of offspring.[19] Some classical examples demonstrating high sensitivity of maturing gametes and developing embryos to environmental insults include folic acid deficiency causing neural tube defects in humans [20] or altered coat color in mice,[21] dysmelia caused by thalidomide exposure in humans [22] and reduced fertility of heat stressed dairy cows.[23] However, for these and other observations, molecular mechanisms connecting stressors to altered phenotypes are not fully understood.

Connection between metabolites and alterations to the epigenome

A model by which nutrients can regulate gene expression was elegantly proposed by Francois Jacob and Jacques Monod in 1960;[24] however, precise molecular mechanisms linking nutrient availability to appropriate gene expression response remain poorly understood. Recently, it has been demonstrated that virtually all components from intermediary metabolism (e.g. acetyl-CoA, α-ketoglutarate, β-hydroxybutyrate, butyrate, lactate and succinate) act as cofactors or cosubstrates of chromatin-modifying enzymes.[25] Curiously, the epigenetic regulatory machinery is highly responsive to metabolic cues. For example, metabolites (e.g. S-Adenosyl methionine (SAM), acetyl-coA) are substrates for enzymes that catalyze deposition of covalent modifications on histones, DNA and recently discovered to be present on RNAs. These modifications, referred to as posttranslational modifications (PTMs) of chromatin, have a major role in activation or repression of gene transcription. PTMs include acetylation, methylation, phosphorylation, butyrilation, and crotonylation of histone proteins and methylation of DNA. Some of these chromatin modifications are involved in maintenance of stable patterns of gene expression (e.g. DNA methylation and H3K9me3), usually referred to as epigenetic regulation.[26]

Since intermediate metabolite availability is required for activity of histone-modifying enzymes, nutritional deficiencies or exesses by consumption of specific types of food potentially disrupt chromatin homeostasis and consequently alter gene expression patterns.[27] Epigenetic marks are controlled by 3 types of enzymes: "writers" add epigenetic modifications, which are recognized by "readers" (effector proteins). When a specific epigenetic mark is no longer necessary, or the cell needs to change the pattern of gene expression, these marks are removed by "erasers".[28] Here we will exemplify the most common chromatin modifying enzymes and their cofactors or substrates (comprehensive reviews are available).[25,26]

Histone acetyltransferases (HATs) use acetyl-CoA as an acetyl donor. Histone acetylation is associated with active chromatin and is removed by histone deacetylases (HDACs) that are part of transcriptional repressor complexes. Most HDACs require the micronutrient Zn^{2+} as a cofactor.[29]

Curiously, the ketone body beta-hydroxybutyrate was discovered to be an endogenous regulator of HDACs.[30] Because ketone bodies are an integral part of ruminant biology, connections between ketone bodies and epigenetic remodeling are crucial to understand reproduction and physiology in cattle, especially dairy. Sirtuins are a special group among all HDACs, classified as class III HDACs and require NAD+ as a cosubstrate; they are a potential connection between low-calorie situations and gene silencing.[31] Histone and DNA methylation are mediated by lysine methyltransferases (KMTs) and DNA methyltransferases (DNMTs), respectively. Both classes of enzymes use S-adenosylmethionine (SAM) as methyl donor. SAM is the second most common enzymatic cofactor after ATP and has a major role in epigenetics and embryo development. SAM is part of 1-carbon metabolism, which has another crucial player, folate. Because animals cannot synthesize folate, they must obtain it by dietary intake.[32] Paternal or maternal deficiency in folate causes severe fetal abnormalities, ranging from neural tube defects to abnormal spermatogenesis,[33] emphasizing importance of understanding connections among diet, epigenetics and development.

Removal of methyl groups from lysine residues in histones involves 2 classes of lysine demethylases (KDMs). Demethylation by LSD1 (lysine-specific demethylase 1) requires reduction of Flavin adenine dinucleotide (FAD). However, the Jumonji C (JmjC) domain-containing KDMs catalyze a different demethylation reaction that requires α-ketoglutarate (αKG), oxygen and Fe(II). Hydroxylation of 5meC in DNA by TET enzymes, a crucial step for reprogramming parental genomes after fertilization, involves a similar reaction. Collectively, these demethylases are known as αKG-dependent dioxygenases.[34]

Fluctuation in oxygen concentrations can affect activity of KMDs and TET enzymes, which can result in altered epigenetic remodeling and embryonic development. This is supported by the observation that increased oxygen during in vitro embryo culture can affect capacity of embryos to establish pregnancies and develop to term.[35]

Lipids are also dietary components with regulatory activities on chromatin modifying enzymes. Metabolites such as SAM (described above) connect lipid metabolism to histone methylation. Butyrate is produced in the rumen and has strong HDAC inhibitory activity. Other fatty acids produced in the rumen (e.g. propionate) or ruminal wall (e.g. beta-hydroxybutyrate) have regulatory and epigenetic roles. Since their concentrations can be manipulated by ruminant diets

(i.e. concentrates versus forages), they provide an avenue to understand the connection between nutrition and epigenetic regulation in livestock.[36]

Epigenetic maturation of gametes

Based on the observation that after fertilization there is a massive erasure of DNA methylation and remodeling of histones and their modifications brought by gametes, mechanisms by which epigenetic information can be passed from parent to offspring through the germline remain difficult to rationalize.[37] Surprisingly, there are compelling evidences that some epigenetic marks can escape this reprogramming.[38] In addition, it has been demonstrated that gametes undergo epigenetic remodeling during their maturation process.[39] Gametes gain cargos (i.e. small noncoding RNAs, lipids, proteins) and acquire specific epigenetic marks during their final stages of maturation; these may contribute to epigenetic inheritance of parental environment to the offspring.[40]

Epididymis

In mammals, final maturation of sperm occurs during its transit through the the epididymis, a long and specialized convoluted tube that exchanges secretory factors required for sperm to gain motility and fertility.[41]

Composition of epididymal luminal fluid is distinctly different from that of blood plasma, indicating potential mechanisms to protect sperm from undesired molecules. The blood epididymis barrier, responsible for this process, carefully controls the lumicrine microenvironment so that sperm are bathed in an appropriate fluid milieu at each stage of maturation as they travel through each segment of the epididymis (i.e. caput, corpus and cauda).[42] Highly specialized and region specific microenvironments are created along the epididymal lumen by active secretion and absorption of water, ions, organic solutes, and proteins. For instance, some molecules can be concentrated 10 - 100 fold (i.e. inositol, carnitine) in the lumen of the caput epididymidis, whereas others, such as glucose and albumin, are effectively excluded. Additionally, there are variations in physical parameters, such as osmolarity, oxygen tension and extracellular pH (i.e. becomes acidic in the cauda region).[42]

In the past few years, there has been accumulating evidence that parents can transmit dietary and environmental information to their offspring via epididimal transport to sperm.[40] From an evolutionary perspective, if parents can "inform" their progeny about prevailing environmental conditions, offspring would have a higher chance of surviving or coping with potential stressors.[43]

The paternal model of epigenetic inheritance is attractive for study, as it can be cleanly tested. Using IVF or ICSI, it was possible to demonstrate that environmental information was carried in the spermatozoon.[40,44,45] On the maternal side, the uterus can affect the offspring phenotype, bringing a confounding factor.[19] Howewer, as discussed above, a plausible mechanism explaining epigenetic inheritance of phenotypic traits is still lacking and difficult to reconcile. Difficulty arises from some observations: 1) large part of sperm DNA methylation is erased following fertilization, and 2) sperm is largely devoid of nucleosomal histones.[37] Supporting the idea that sperm DNA methylation is probably not a carrier of epigenetic information, mice eating several diets (control, low protein, high fat) had no consistent effects on sperm cytosine methylation.[46]

Regarding histones, mammalian sperm exhibit a highly unusual chromatin state that is markedly different from other somatic cells,[47] as the vast majority of histone proteins are lost during spermatogenesis. Histones are first replaced by transition proteins and later by small basic proteins termed protamines; however, not all histones are lost, with murine sperm retaining up to ~ 8% of their histones.[47] Recent studies of histone retention in human and mouse sperm suggest a bias for sperm histone retention as promoters of genes expressed during early development.[48] Conversely, in other studies, the majority of sperm histone retention preferentially occurred in gene-poor genomic regions, with only a small subset of nucleosomes being retained over promoters of developmental regulators.[49] The genomic location of nucleosome retention is still a matter of debate, and it seems that protocols used to digest chromatin can affect results.[50] Currently there is some level of consensus that retained histones may carry epigenetic information, which could affect gene expression in early embryos and contribute to intergenerational epigenetic inheritance. Indeed, using mass spectrometry, it was possible to identify multiple PTMs on histones and protamines in adult mouse sperm.[51] Since PTMs are responsive to the environment, they can be modified during spermatogenesis or epididymal maturation and contribute to transfer of epigenetic information from sire to offspring.

Another potential mechanism by which the lumicrine microenvironment from the epididymis can affect histone marks on sperm is changes in pH. Recently it was demonstrated that cellular histone acetylation levels globally decrease as pH decreases.[52] Along the epididymis, luminal pH decreases and the cauda region has an acidic extracellular pH,[53] which could be important for controlling histone acelytlation in sperm retained histones.

Among all potential carriers of epigenetic information (i.e. DNA methylation, histone marks and small noncoding RNAs) small RNAs are gaining considerable interest as the main player. Small RNAs comprises several small molecules (i.e. < 40 nucleotides), including well-studied microRNAs, siRNAs, piRNAs, as well as less understood molecules such as enhancer-derived RNAs (eRNAs) and tRNA fragments (tRFs).[54] These molecules are implicated in various transgenerational epigenetic inheritance paradigms such as: 1) RNA interference in *Caenorhabditis elegans*; 2) paramutation in maize; 3) epigenetic silencing in Arabidopsis and fission yeast; and 4) silencing of transposons in the Drosophila germline. However, most of these cases occur in organisms whose genome encodes an RNA-dependent RNA polymerase, providing a mechanism by which an initiating signal can be maintained over multiple organismal generations. On the contrary, mammals lack these key enzymes, which makes small RNA epigenetic inheritance far less robust and subtle.[55] That said, a plausible mechanisms in mammals could be represented by small RNAs delivered to the embryo at fertilization that could alter zygotic gene expression.[43] Since embryonic development progresses as a cascade of events, altering events at the beginning of development can affect cell differentiation or allocation, eventually causing placental problems. Placental malfunction can lead to fetal growth restriction and thrifty phenotype in adulthood.[19] Supporting this view, in a model of chronic paternal stress, it was possible to identify 9 miRNAs differentially expressed in sperm of stressed animals. Injection of those microRNAs to unexposed zygotes caused a remarkable recapitulation of the offspring stress dysregulation phenotype.[56] In another example, causing traumatic stress in early life altered mouse microRNA (miRNA) expression, behavioral and metabolic responses in progeny. Injection of sperm RNAs from traumatized males into fertilized wild-type oocytes reproduced behavioral and metabolic alterations in resulting offspring.[44] In 2 independent studies, male mice submitted to low-protein or chronic high-fat diets had different populations of tRFs in mature sperm. Injection of purified tRFs fragments from males kept on diets into normal oocytes caused metabolic disorders and concomitant alteration of genes in metabolic pathways in the progeny.[40,45] Curiously, these tRFs were gained during epididymal transit.[40]

Extracellular vesicles secreted by the epididymal cells, also termed epididymosomes, shaped the sperm epigenome after testicular maturation.[57] This is a remarkable example of soma to germline relationships in mammals. Furthermore, the cargo secreted by the epididymal cells changes based on animal diet, offering some potential mechanisms to explain how dietary information can be transmitted from father to offspring.

All of these observations and mechanisms of epigenetic inheritance gathered from laboratory animals open avenues to improve animal production in livestock. In the near future, it may be possible to screen for specific microRNAs or other small noncoding RNA entities in semen from boars, bulls, bucks, rams and stallions to identify candidates conferring higher fertility or desirable phenotypic characteristics.

Ovarian follicle

The ovarian follicle is the basic unit of the female reproductive system. Each follicle contains 1 oocyte and companion cells, which periodically grow to release a mature and competent oocyte. The oocyte growth phase is a protracted process in mammals, taking ~ 3 wk in mice and several months in humans and livestock.[58] During growth, the oocyte increases > 100 fold in volume as it accumulates organelles and an enormous supply of molecules (e.g. messenger RNAs and proteins), that are necessary to acquire meiotic competence and support early stages of embryogenesis. Oocyte meiotic and developmental competence is gained in a gradual and sequential manner during folliculogenesis.[59] Epigenetic marks are also acquired gradually during this process. Oocytes acquire DNA methylation in a size-dependent manner.[60] Several histone modifications (e.g. H3K9ac, H4K12ac) increase steadily during oocyte growth and become globally deacetylated by the time of germinal vesicle breakdown.[61]

During its growth, the oocyte interacts with its companion somatic cells. Communication between oocyte and the granulosa is vital for oocyte development and granulosa cell differentiation.[62] Additionally, oocytes depend on cumulus cells to gain metabolites and regulatory signals, which are fundamental for nuclear and cytoplasmic maturation.[63]

Cumulus-oocyte complexes are surrounded by follicular fluid, composed of secretions from follicular cells and exudates from plasma.[64] Because oocyte growth and maturation are lengthy processes, oocytes are potentially exposed to changing environmental conditions that might induce epigenetic modifications affecting embryo development.[65] Some epigenetic modifications acquired during oocyte maturation are retained during early embryonic development. Failure to establish the correct epigenetic pattern during oogenesis may disrupt embryonic development. Given the crucial role of specific posttranslational modifications of histones in regulating gene activity, they also represent a potential mechanism for intergenerational transmission of epigenetically induced states.[65] Fine-tuning of the process of histone acetylation/deacetylation is crucial for regulation of gene expression during folliculogenesis and chromosome condensation during meiosis progression.[61] Deletion of the histone deacetylase genes, Hdac1 and Hdac2, leads to an arrest of oocyte growth and development, as well as a decrease in overall transcriptional activity. The ketone body beta-hydroxybutyrate (BHB) was discovered to be an endogenous regulator of HDACs activity.[30] Pioneer studies demonstrated that the follicular fluid mirrors plasma BHB concentrations.[66] That said, this may be a mechanism by which folliculogenesis can be affected in early post-partum cows, linking a dietary-altered molecule (BHB) with a chromatin-modifying enzyme (HDAC).

In vitro models of oocyte exposure to BHB concentration in cows with subclinical/clinical ketosis failed to demonstrate a detrimental developmental effect or global effects on histone acetylation when oocytes were treated with BHB alone.[67] However, when other molecules were also altered in the culture medium (e.g. glucose), some detrimental effects were observed.[68] Follicular fluid is a complex biochemical environment, making it difficult to mimic in vitro. Additionally, every dietary component may cause only a minor affect on a specific pathway, although the sum of multiple alterations could undermine oocyte competence.[69]

Several well-documented examples of diet causing intergenerational epigenetic effects on offspring have been described, mainly in mice, with a recent report of this epigenetic phenomenon in monkeys.[70] In livestock, there is compelling epidemiological evidence that parental diets affect offspring metabolism.[8,71]

Exposing females to various dietary regimens (i.e. high fat, low protein, micronutrient deficient) causes widespread effects on their offspring's health.[72] These outcomes are observed even when embryos at the blastocyst stage are transferred to pseudopregnant recipients, ruling out altered uterine milieu as the causative mechnism.

Several of the reported studies usually fed mice altered diets for various intervals. Surprisingly, effects were observed after feeding a high-fat diet for as little as 4 days before mating. These experiments target oocytes during the final stages of growth and during meiotic maturation and demonstrate that this is a critical period during the lengthy process of oogenesis.[65] Interestingly, harmful effects of metabolism can be mitigated by pharmacological interventions. Treatment with endoplasmic reticulum stress inhibitors for 4 days alleviated oocyte ER stress and improved oocyte quality in genetically obese mice.[73]

A number of defects in oocytes have been identified that could underlie alterations in offspring health. For example: 1) mitochondria with reduced activity and altered ultrastructure; 2) excessive accumulation of lipids in the cytoplasm; and 3) meiotic spindle abnormalities, are features commonly observed on animals exposed to altered diets.[72] Granulosa cells of diabetic mice or from mice fed high-fat diets have impaired glucose uptake and increased apoptosis.[65] Similarly, cows in massive lipolysis caused by energy imbalance have elevated NEFA concentrations in follicular fluid, which also causes apoptosis of follicular cells.[74]

Communication between oocytes and surrounding granulosa cells is fundamental for acquisition of some regulatory RNAs (e.g. microRNAs) and various metabolites.[75,76] These molecules can be transported to oocytes by trans-zonal projections and through gap junctions or can be packed into exosomes and released into the perivitelline space for oocyte uptake.[39] As discussed above, since multiple metabolites can affect chromatin modifying enzymes activity,[25,26] alterations to granulosa cells could impact the oocyte epigenome. While the mentioned studies and observations indicate

direct influence of maternal nutritional state on multiple parameters of oocyte physiology and potential connections with epigenetic machinery, mechanisms by which diet or disease can influence quality of livestock oocytes remain to be fully elucidated.

Oviduct

To creat a new organism from 2 completely distinct cells (i.e. oocyte and spermatozoon), massive reprogramming of the gametes' epigenome is necessary. This reprogramming occurs shortly after fertilization, as the embryo transits through the oviduct.[11] The mammalian oviduct is a complex conduit responsible for final maturation of oocyte and spermatozoon, providing an adequate environment for fertilization and nourishing the early embryo. Embryos spend the first few days of development in the oviduct before they reach the uterus to undergo implantation. During this interval, the oviductal environment has an opportunity to modulate the embryonic epigenome and affect the trajectory of embryonic development.[77]

To support early embryonic development, the oviduct secretes molecules such as glycosaminoglycans, proteoglycans, carbohydrates, ions, hormones, growth factors, cytokines and microvesicles loaded with miRNAs and proteins. Also, the oviduct controls physiological parameters such as O_2 and CO_2 concentrations, pH, temperature, osmotic pressure, water content, electrolyte concentration and viscosity.[78] However, the exact oviductal medium composition and its modulation by the environmental, remain ill defined for most livestock species.

Early embryos progress quickly through the cell cycle, with chromatin-modifying enzymes working intensively during embryo transit through the oviduct. That said, these processess are likely sensitive to signals received from the oviduct, which can arrive via variousways of communication.[79] On this basis, there are several mechanisms by which the oviduct can shape the embryo epigenome. For example: 1) fluctuations in oxygen concentration can alter activity of dioxygenases such as TETs that oxidize 5-mC in the paternal pronuclei allowing its removal; 2) epigenetic remodeling enzymes can also be affected by variations in oviductal pH or alterations in luminal secreted substrate concentration, some of which are necessary for enzymatic activity; and 3) variations in ions such as Ca^{2+}, which may affect exocytotic processes involving secretion of extracellular vesicles (i.e. oviductosomes), were recently reported to contain small RNAs that can be uptaken by embryos.[77]

Recently, it was reported that embryos induce changes in oviductal gene expression and consequently modulate their own environment, demonstrating that communication is bidirectional.[80]

In vitro models are helping to elucidate roles of various oviductal constituents on embryos. Exposing bovine oviduct epithelial cell (BOEC) to elevated non-esterified fatty acids (NEFA) in vitro affected their metabolism and their barrier function, substantiating the idea that the oviduct acts as a gatekeeper and may actively alter early embryonic developmental conditions.[81] Using in vitro embryo culture systems, it was demonstrated that high oxygen concentrations deregulate epigenetic writers and erasers enzymes, affecting global levels of embryonic DNA methylation.[82] Although full consequences of these observations needs to be determined, embryos cultured in high oxygen concentrations generally have more reactive oxygen species and lower developmental potential.

Two recent studies have shown direct correlations between presence of oviduct factors during embryo culture and DNA methylation marks in the pig and cow.[83,84] Porcine blastocysts produced in vitro with oviduct fluids as additives in culture medium had global DNA methylation and gene expression patterns closer to those of the in vivo-produced blastocysts. In addition, embryos produced in absence of reproductive fluids had methylation changes in critical genes controlling embryonic development, such as insulin-like growth factor 2 receptor *IGF2R* and Neuronatin *NNAT*.[85] Bovine embryos cultured with oviductal fluid had higher methylation in CpG island of LINE-1 retrotransposons, suggesting that oviductal fluid components can protect against harmful retrotransposition, by maintaining retroelements silenced.[86] These studies supported the view that the oviductal environment can modulate the epigenetic landscape of the embryo.

The oviduct also secretes regulatory molecules known as embryokines, growth factors that modulate embryonic development by targeting various signaling pathways. Bovine embryos treated with the embryokine colony stimulating factor-2 (CSF-2) during in vitro culture resulted in animals with similar birthweight to controls but increased growth rate,[87] supporting the notion that the preimplantation period is not only important for embryo survival, but also for programming healthspan of the resulting animal.

Conclusion

Although mammalian gametes and embryos develop under a highly controlled internal medium, alterations to their environment are possible as a consequences of environmental, dietary, metabolic and physiological conditions.[8,88,89] Understanding interactions of the embryo with its environment and effects on changes of the embryonic epigenome on further development are fundamental to ensure that health of embryos/offpring is not compromised by production system interventions. Also, improved understanding of effects of the epigenome on offspring health could be harnessed to introduce directed epigenome changes to the embryo with predictable results. Epigenome editing in livestock using small RNAs packed in extracellular vesicles,[39,57] addition of molecules (i.e. metabolites, embryokines) to the culture system,[67,87] or using CRISPR-dCas9 systems,[90] could become a common practice, with potential to revolutionize the livestock industry.

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Conflict of interest

Authors declare no conflicts of interest.

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