Introduction

A multitude of factors, including undernutrition, overnutrition, uterine capacity, as well as heat and other environmental stressors, can strongly influence the uterine environment during gestation. A rapidly growing body of evidence is uncovering a correlation between a suboptimal uterine environment during gestation and the incidence of metabolic diseases in the adult offspring. A variety of animal models, including sheep, cattle, rats, mice, and non-human primates, have been developed for investigation of this link between in utero environment and development of disease in response to a variety of environmental stressors. Maternal malnutrition and uterine capacity are perhaps the two more common factors influencing in utero development in horses. However, there is still a lack of experimental evidence regarding the developmental origins of disease in the horse. Given the observed incidence of equine metabolic disorders in veterinary practices, it is probable that similar mechanisms of fetal programming exist in the horse. The aim of this review is to provide a broad understanding of how fetal and neonatal environmental factors affect fetal programming, which may be applicable across a variety of species, including the horse.

Keywords: Epigenetics, fetal programming, pregnancy, nutrition, equine

Fetal programming

During gestation, the fetus is said to be in a state of developmental plasticity, a phenomenon by which a single genotype (genetic sequence) can give rise to many different phenotypes (physiological states) based on the in utero environmental conditions. Fetal programming can be defined as the process by which the fetus adapts to its uterine environment. This adaptive developmental process is thought to be evolutionary and intended to be advantageous for the fetus to thrive in suboptimal environments. The “thrifty phenotype hypothesis” proposes that a poor uterine environment induces permanent changes so that offspring can “thrive or more accurately survive” in a suboptimal postnatal environment. Practical examples of this in horses and other livestock species are “easy-keepers” which demonstrate a greater ability to convert nutrient intake into fat stores. This is likely a response to metabolic programming in utero rather than an inherited trait. When matched appropriately, predictive adaptive responses allow a species to survive in a compromised environment. Conversely, the “mismatch concept” suggests that the degree of disparity between the developmental environment and the environment of adulthood influences the risk of disease.

Maternal undernutrition

It is not surprising that the prenatal growth trajectory is both directly and indirectly impacted by maternal nutrition during gestation. Various weather conditions make undernutrition during gestation a common occurrence in livestock production. Furthermore, twinning in species that are typically monotocous, such as the horse, requires the division of nutrients between two fetuses and causes reduced uterine space for adequate fetal development.

Birth weight is the principal indicator of prior nutrient availability in utero and predictor of postnatal viability utilized in livestock. However, it is important to note that not all offspring of nutrient restricted mothers are born at a low birth weight or as intrauterine growth restricted (IUGR). In cattle exposed to nutrient restriction during early to mid gestation, IUGR was induced in only a subset of offspring. A similar model in sheep induced IUGR-like classification at mid gestation but at birth these lambs were of an average birth weight.

In horses, both experimentally induced maternal undernutrition, as well as undernourishment induced by maternal illness, had no influence on birth weight. However, the degree of undernourishment and amount of weight lost in these mares are make comparisons of these results with...
those observed in other animal models difficult. Nevertheless, the strong correlation between low birth weight and metabolic diseases emphasize the importance of this simple measure on properly managing potential health risks.

Various livestock models of maternal nutrient restriction have shown a disruption in the pattern of fetal organ development that ultimately results in impaired organ function during postnatal life. Sheep models have shown that maternal undernutrition results in impaired cardiovascular and renal function, as well as increased whole-body obesity.\textsuperscript{16-18} In sheep and pigs, IUGR is associated with a decreased number of skeletal muscle fibers, an increase in connective tissue content, and increased adiposity.\textsuperscript{19-22} Reduced musculature and increased adiposity may substantially reduce growth performance and nutrient utilization. In horses, it has been hypothesized that a suboptimal uterine environment leads to reduced skeletal muscle mass, impaired bone and cartilage formation, and weakened pulmonary and vascular systems, all of which can negatively impact athletic performance.\textsuperscript{23}

To further complicate things, the effects of fetal programming have been shown to persist in multiple generations. For example, maternal protein restriction in rats results in vascular dysfunction and hypertension in the F1 generations that was also maintained in the F2 generation.\textsuperscript{24,25} Such heritable developmental adaptations do not alter the DNA sequence and therefore are not genetic alterations. Instead, they are “epigenetic” alterations or alterations in how the DNA is utilized. Many epigenetic alterations are lost or “reset” when sperm and oocytes are produced. However, some epigenetic changes may be passed on to the subsequent generations.

**Maternal overnutrition**

Like with undernutrition, data has shown that maternal overnutrition perturbs fetal growth in various species.\textsuperscript{26-28} Furthermore, overnutrition and obesity during gestation are strongly associated with an increased risk of metabolic disorders during the postnatal life.\textsuperscript{29,30} Of the livestock species, obesity is most often encountered in horses. This largely stems from a shift towards the utilization of the horse for recreation and companionship. Indeed, it has been shown that obesity in horses is associated with increased occurrence of disorders such as equine metabolic syndrome, insulin resistance, diabetes mellitus, laminitis and equine motor neuron disease.\textsuperscript{31-35,49,50}

A link between maternal obesity and metabolic perturbations in the offspring has been shown in a number of species. Feeding rats a high-fat diet during pregnancy or lactation can induce cardiovascular dysfunction and high systolic blood pressure.\textsuperscript{36} Interestingly, the response to a maternal high-fat diet differed based on sex of the offspring. With potential application to the horse, impairment in glucose homeostasis of the offspring, with elevated plasma insulin levels at 1 year of age, were also seen in response to a maternal high-fat diet.\textsuperscript{37} Maternal diets high in fat have also been shown to induce similar disorders in mice and have resulted in increased levels of circulating markers of systemic inflammation.\textsuperscript{38,39,40}

In sheep, maternal overnutrition results in increased fetal weight at mid-gestation, with numerous organ and tissue weights also showing a proportional increase.\textsuperscript{41} However, at parturition, the birth weights of lambs from overnourished and obese dams did not differ compared to normal fed ewes.\textsuperscript{42,43} Subsequent sheep studies have shown that maternal obesity impairs skeletal muscle and liver metabolic function through downregulation of genes associated with muscle and liver development in the fetus.\textsuperscript{44,45} Alterations in these genes also resulted in increased fetal adiposity.\textsuperscript{41,46} Consequently, even though an alteration in birth weight is not seen, body composition with less muscle and increased adiposity has the ability to substantially impact postnatal growth and performance.

Feeding a high starch diet in non-pregnant horses results in reduced insulin sensitivity and increased occurrence of obesity and laminitis.\textsuperscript{47} Feeding these same high starch diets to pregnant mares during late gestation reduced insulin sensitivity in foals from postnatal day 5 to 160, as well.\textsuperscript{47} In addition, maternal overnutrition late in gestation has been shown to decrease IgG content in the colostrum, but no change was detected in the colostrum fat or protein content.\textsuperscript{48} Diets for other livestock species are often higher in fat compared to equine diets. This illustrates the need for equine studies.
utilizing high caloric diets with high levels of starch to induce obesity during gestation and the consequences of this diet composition on offspring development.

Uterine capacity

It is well documented that maternal size has a profound influence on fetal growth in utero, primarily through placental size and function. A number of studies in the mare have illustrated that the uterine environment dictates fetal growth rates, irrespective of the embryo’s genetic makeup. The original study illustrating the dramatic impact of uterine size was conducted by Walton and Hammond utilizing Shire horse and Shetland pony crosses. Shire mares were artificially inseminated with Shetland pony semen and Shetland pony mares being artificially inseminated with semen from Shire stallions. The Shire mare and Shetland stallion foal was much taller and heavier at birth than the reciprocal cross foal, with this growth and size differential continuing at maturity. Various laboratories have employed the use of embryo transfer to investigate the impact of uterine capacity on fetal growth and development in the horse. In these studies, reciprocal embryo transfer between large framed horses and small framed ponies, along with within-breed embryo transfers were compared. Foals carried by large framed mares were considerably heavier and taller than their genetically similar siblings carried by pony recipient mares. In addition, birth weights of foals from between-breed transfers were intermediate to those of either within-breed transfer. Furthermore, these size disparities remained to adulthood. In one study, increased uterine capacity for the pony in Thoroughbred recipient (P-T) transfers also resulted in altered cardiovascular function and response to stress. These P-T foals further displayed elevated basal insulin levels. Conversely, Thoroughbred foals gestated in pony recipient mares exhibited an increased production of catecholamines following acute stress. Another study, utilizing pony, Saddlebred, and draft control and reciprocal crosses not only found that maternal size regulated fetal growth but also found altered concentrations of thyroid hormone and impaired glucose homeostasis. These data show that maternal size and the in utero environment plays a potentially greater role in fetal growth determination than genotype and that the effects of either a large or small uterine environment have significant postnatal ramifications for growth and health.

Amino acid nutrition and epigenetics

Amino acids serve many fundamental functions beyond the basic building blocks for protein synthesis including serving as precursors for nitric oxide, purine and pyrimidine nucleotides, neurotransmitters, and other essential molecules. Amino acids, particularly methionine, serve as methyl group donors and have a critical role in regulating and maintaining the epigenome. Studies in species such as the pig and sheep have begun to illustrate the importance of individual amino acids in supporting optimal fetal growth and development. As example, dietary restriction of methionine, essential B vitamins, and folate to ewes during the peri-conceptual period induced alterations in DNA methylation and resulted in the development of insulin resistance and elevated blood pressure of the lambs. Interestingly, the male offspring appeared to be more susceptible to this dietary restriction. In contrast, supplementation of pregnant sows with arginine increased litter size at parturition by two piglets. In sheep, administration of arginine during late gestation increased fetal peri-renal brown adipose tissue development (MC Satterfield, unpublished observation). An increased amount of brown adipose tissue will likely enhance neonatal survival by increasing the lamb’s ability to combat cold temperatures.

Equine nutritional management is the most varied among the domestic livestock species. In consequence, an encompassing standard of nutritional requirements or recommendations has yet to be composed. Foundational studies emphasizing functions and importance of amino acids and other micronutrient requirements, have not been established. Yet, there is an overwhelming availability of equine dietary supplements. Therefore, it is necessary to utilize caution in supplementing pregnant mares as administering an inappropriate supplement, dose, or combination of these could have permanent consequences on the developing fetus, persisting throughout postnatal life.
Behavioral programming

During the early neonatal and postnatal life offspring are still susceptible to alterations in their epigenome. More specifically, genes associated with behavioral traits are largely influenced by maternal behavior and glucocorticoid exposure. Thus, it is not surprising that behavioral programming in the horse may be profoundly impacted due to typical managerial practices allowing for increased interaction with humans. The benefits or consequences of this can profoundly impact a horse’s willingness to train or compete.

The process of “imprinting” foals during early neonatal life has shown that conditioning foals through tactile interaction at birth and postnatal day one reduced foals’ resistance to touching of their legs and hind feet at three months of age.\(^6^3\) Interestingly, it is known that orphan foals reared without the presence of a mare are difficult to train. Assisting with a foal’s first suckling interferes with the development of the maternal/fetal bond and can cause the foal to avoid human approach and physical contact at a few weeks of age.\(^6^4\) Moreover, forced handling of foals during early postnatal life did not improve human/foal interaction later in life but exposure to a motionless human did improve this interaction.\(^6^4\)

Studies have investigated the effects of early neonatal exposure to glucocorticoids in foals. Induction of parturition through the use of oxytocin approximately 24 to 48 hours prior to parturition altered pancreatic function.\(^6^5\) Cortisol levels were also increased in foals born by induced delivery. Induced delivery may alter pancreatic sensitivity to glucose and/or cause tissue insulin resistance, along with increased cortisol secretion.\(^6^5\) In another study, spontaneously delivered foals receiving long-acting ACTH endogenously during the neonatal period resulted in increased plasma cortisol concentrations at three but not 13 weeks age.\(^6^6\) This treatment also lead to impaired pancreatic function.\(^6^7\) It is possible that ACTH-induced affects may occur much later in life but this warrants further investigation.

Limited evidence suggests that the early neonatal life is critical for physiological and behavioral adaptations, which may be altered by environmental and biological stressors. Not surprisingly, this small amount of data fails to elucidate the long-term consequences of adaptations occurring during this time period, but justifies the need for controlled studies to evaluate the extent to which the physiology of the foal can be programmed by environmental stressors in the early postnatal period. Future studies in this area may provide valuable insight for development of new managerial and training practices that can be utilized to maximize genetic and physiological potential of foals.

Conclusions

The prenatal uterine milieu, as well as the early neonatal environment, may profoundly impact the health, metabolism, physiology, and behavior of livestock throughout life. Importantly, a scarcity of information regarding these mechanisms, adaptations, and responses exists. More specifically, there is a paucity of data elucidating the impact of nutritional supplementation during gestation and the importance that individual amino acids or other nutrients serve in the equine diet. Furthermore, both the mechanisms and consequences of human and foal interaction during the neonatal period remain unknown. Collectively, these early findings support the need for continued and more rigorous investigation into these areas.

References


29. Ford SP, Long NM: Evidence for similar changes in offspring phenotype following either maternal undernutrition or overnutrition: potential impact on fetal epigenetic mechanisms. Reprod Fertil Dev 2011;24:105-111.


