Proceedings of the 56th Annual Convention of the American Association of Equine Practitioners - AAEP -

December 4-8, 2010
Baltimore, Maryland, USA

Next Meeting:

Nov. 18-22, 2011 - San Antonio, Texas, USA

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Review of Fetal Programming: Implications to Horse Health

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Available evidence strongly suggests that the fetal and early neonatal periods of development are sensitive to environmental cues, which have long-lasting consequences to growth, health, and likely performance of the foal. The recent adoption of the horse as a companion animal has been associated with an increased incidence of obesity and a wide array of associated diseases including insulin resistance, Cushing’s disease, equine metabolic syndrome, diabetes mellitus, hyperlipaemia, laminitis, endotoxaemia, osteochondrosis dissecans, and equine motor neuron disease.1 The experiences of the mare during gestation and the foal immediately after birth may affect the foal’s behavior into adulthood. A paucity of information exists regarding this phenomenon in the horse, highlighting a need for extensive future research in this area. Authors’ address: 442 Kleberg MS2461, Department of Animal Science, Texas A&M University, College Station, Texas 77843; e-mail csatterfield@tamu.edu. © 2010 AAEP.

1. Introduction

An emerging body of evidence has established a link between the fetal environment in utero and subsequent adult health and disease. The foundation of this concept, sometimes referred to as the “Barker Hypothesis,” is rooted in two studies conducted by Dr. David Barker. The first study, conducted in Britain and Wales, established a link between low birth weight and development of coronary heart disease in adulthood.2,3 Indeed, the relative risk of death because of coronary heart disease is 50% greater when birth weight of the individual was less than 5.5 lbs compared with a birth weight of over 8.5 lbs.3 Poor maternal nutrition is a major contributor to low birth weight; armed with this knowledge, Dr. Barker studied people born immediately before or during the Dutch famine of World War II to test his hypothesis that a poor fetal environment results in susceptibility to disease later in life. The Dutch Famine occurred near the end of World War II, spanning the winter of 1944–1945, because a food embargo and destruction of communications stopped the supply of food to areas of the Netherlands under German occupation. It ended abruptly with the end of the war. The relatively short, defined period of famine coupled with impeccable birth and medical records provided a large and unique population to study. These studies suggested that not only reduced growth rate and stature in adulthood but also adult obesity, diabetes, hypertension, and schizophrenia were linked to reduced nutrient availability in utero.4 Importantly, the time of exposure during gestation plays a central role in determining disease type and susceptibility (Table 1). As an example, 2-h glucose concentrations (a predictor of insulin resistance) are elevated in adulthoods exposed to famine during the second and third trimesters of development but not the first trimester.5 In con-
Fetal programming can be summarized as the process whereby the fetus adapts to its uterine environment. This adaptation occurs in response to nutrition and other environmental stimuli. Importantly, an identical stimulus can have a vastly different effect depending on the stage of fetal development (i.e., first versus third trimester) during which it occurs. The adaptive process is rooted in evolution and likely intended to provide an advantage to the fetus after birth. For example, a poorly nourished fetus would predict that nutrient availability will be scarce later in life and develop an extremely efficient metabolism. If, in fact, the nutrient availability is high later in life, this metabolic efficiency would result in the deposition of fat. Indeed, some horses are described as “easy-keepers”—a “condition” that may result from the metabolic programming of the fetus in utero.

When environmental cues during prenatal life incorrectly predict the postnatal environment, the offspring is inappropriately programmed to the environment, leading to an increased prevalence of disease later in life. This disparity between predicted (programmed) and actual environment creates a mismatch that has been observed in the wild, in society, and under experimental conditions. For example, vascular cholesterol buildup in pigs receiving a diet high in saturated and trans fatty acids is prevented when their mothers were fed the same diet during gestation. In sheep, a mismatched prenatal and postnatal diet leads to cardiovascular dysfunction and altered renal function as adults.

### 3. Maternal Undernutrition

In livestock production, drought during gestation is the most common cause of a mismatched prenatal and postnatal environment. Thus, the preponderance of available literature regarding maternal nutritional status and its effects on fetal programming is derived from studies of maternal undernutrition. From a livestock perspective, insufficient nutrient supply is common across all species because of poor forage quality and/or availability, excessive temperatures resulting in heat stress that deprives the fetus of nutrients, poor management practices, and even twinning in typically monochorous species.

In both clinical and agricultural settings, birth weight is the predominant indicator of prior nutrient availability in utero. However, to complicate matters, in the horse, experimentally induced maternal undernutrition, as well as undernutrition induced by maternal illness, showed no influence on birth weight. There are questions regarding the level of undernutrition and weight loss in these studies.
More nutritionally defined studies are needed in the horse. Nonetheless, subsequent observations that low birth weight is correlated with adult onset diseases such as cardiovascular disease and metabolic syndrome highlight the importance of this simple measure on predicting and potentially managing potential health risks. For example, children born with low birth weight had elevated levels of glucose and insulin in response to glucose challenge at 4 yr of age.10 Thus, although foals born to mares with nutritional deficiencies or excesses may seem normal at birth, studies are needed to monitor them for problems, especially metabolic problems, later in life. Experimental evidence in a variety of species and models has provided a wealth of knowledge regarding the mechanisms by which reduced nutrient availability in utero gives rise to adult disease. In rats, maternal mechanisms by which reduced nutrient availability results in impaired growth and metabolic function in the fetus or its organs, have been investigated in a number of livestock species18 and go well beyond animal health to factors that could potentially affect performance. In both pigs and sheep, IUGR results in decreased skeletal muscle-fiber number, increased deposition of adipose tissue, and increased connective-tissue content.19–22 Collectively, these alterations in normal development result in reduced growth performance, including both whole-body and skeletal muscle-growth rates and reduced nutrient use. Given the combined effects of impaired vascular function and suboptimal skeletal muscle and whole-body growth, it has been hypothesized that the athletic performance of horses may be highly susceptible to fetal programming.23

4. Maternal Overnutrition

Among livestock species, obesity is most often encountered in horses as views shift from one of a primarily livestock species to one of recreation and companionship. The civilization of the horse in the Western culture has followed a similar trend to that of humans and has increased the incidence of obesity and its associated metabolic disorders in both species.24,25 There is little experimental evidence to predict the long-term consequences of maternal obesity in the horse. However, maternal obesity has been linked to obesity and metabolic perturbations in the offspring of a number of species. In non-human primates, a maternal high-fat diet has been shown to alter the fetal hepatic metabolome and promote the development of non-alcoholic fatty liver.26–28

In rats, feeding a high-fat diet during pregnancy or during suckling induces cardiovascular dysfunction characterized by elevated systolic blood pressure and impaired endothelium-dependent relaxation.29 Importantly, response to maternal dietary treatment differed between male and female offspring, with female offspring being more susceptible to elevated systolic and diastolic blood pressure in response to high-fat diet during gestation and/or lactation. Feeding the high-fat maternal diet altered renal function but not weight of the kidney or glomerular number.30 Notably, in application to the horse, in addition to altered cardiovascular function, a maternal high-fat diet during pregnancy also resulted in impaired glucose homeostasis in offspring, characterized by elevated plasma insulin levels at 1 yr of age.31 Similar observations have been made in offspring of pregnant mice fed a high-fat diet, including increased adiposity, hypertension, and insulin resistance.32,33

In sheep, maternal overnutrition has an array of consequences on fetal organ development. Maternal overnutrition results in an increase in fetal weight at mid-gestation, with a number of fetal organ and tissue weights increased proportionally with the increase in overall weight.34 However, at term, birth weights of lambs from overnourished dams are not different compared with normally fed ewes.35,36 These findings suggest that although maternal obesity does not affect birth weight, the pattern of development is altered by maternal overnutrition. To this end and again, directly relevant to the horse, subsequent studies have indicated that maternal obesity down-regulates genes involved in the development of skeletal muscle and liver metabolic function in the fetus.37,38 Importantly,
these changes in gene and protein expression in response to maternal overnutrition result in increased fetal and/or neonatal adiposity. Therefore, although birth weight in the overnourished offspring does not change, the body composition is significantly altered to more fat and less muscle, likely having considerable impacts on growth and performance. We have found that maternal overnutrition results in impaired glucose uptake and diminished growth rate to postnatal day 210 compared with controls growing in an identical nutritional environment from birth.

Although little information is available in the horse, the data available support similar effects of maternal overnutrition in this species. Feeding mares a high-starch diet during late gestation produced a trend to reduced insulin sensitivity of foals from postnatal day 5 to postnatal day 160. Maternal overnutrition during late gestation also reduced colostrum immunoglobulin G (IgG) content, whereas levels of protein and fat in colostrum were unchanged. The long-term consequences of these observations require further study. Additionally, given that the preponderance of evidence regarding obesity in non-livestock species involves the feeding of high levels of fat, which are fed at low to moderate levels in the horse, studies are needed to determine the effects of high-caloric diets consisting of high levels of starch on the consequences of maternal obesity to the developing equine offspring.

5. Uterine Capacity

The uterine versus fetal (i.e., genetic) contribution to fetal growth has been of interest in a number of species, including the horse. Indeed, ovum donation and embryo-transfer studies in humans indicate that birth weight is highly correlated with weight of the recipient but not the weight of the ovum donor, indicating that the uterine environment in which the embryo develops is more important than the genetic contribution to birth weight.

Similarly, the mare is famous for her ability to control the size of the foal that she carries, first shown by crossing Shetland ponies with shire stallions and vice versa.

Over the past decade, a series of studies conducted by Dr. Twink Allen’s group in Cambridge has used embryo transfer to determine some effects of the uterine environment on fetal and postnatal growth and development in the horse. This group performed within-breed embryo transfer in either Thoroughbred or pony mares as well as the reciprocal crosses—transfer of a Thoroughbred embryo to a pony mare and its reciprocal. Data from these studies clearly indicate that the maternal uterine environment plays a role in regulating not only fetal but also postnatal development. Transfer of a pony embryo into a Thoroughbred mare resulted in a greater than 50% increase in birth weight compared with a pony foal born to a pony mare. Reciprocally, a Thoroughbred foal born to a pony mare was 37% lighter than a Thoroughbred foal born to a Thoroughbred mare. The genetic contribution was evident also in that the birth weights of foals from the between-breed transfers were intermediate to those of either within-breed transfer offspring. Thoroughbred foals born to pony mares grew at a faster rate than Thoroughbred foals born to Thoroughbred mares, whereas the opposite was true in foals born to pony mares. At 36 mo, Thoroughbred foals carried in ponies had significantly smaller cannon bone circumference, elbow-to-knee length, and kneefoetlock length than did control Thoroughbred foals, whereas in ponies carried in Thoroughbreds, these values, as well as body weight at 36 mo, were all significantly greater than those in control ponies.

Evaluation of cardiovascular and metabolic function in early postnatal life indicates that providing a luxurious uterine environment (pony embryo in a Thoroughbred mare) results in an increased basal arterial blood pressure and baroreflex threshold, decreased baroreflex sensitivity, and altered response to stress as well as elevated basal insulin and insulin secretion in response to glucose infusion. In contrast, Thoroughbred foals gestated in pony mares exhibited normal basal arterial pressure and baroreflex threshold but exhibited enhanced baroreflex sensitivity and increased production of catecholamines in response to acute stress. These data support the conclusion that intrauterine environment may alter cardiovascular function and postnatal insulin secretion as well. Further studies are needed to determine if these physiologic changes continue into adulthood.

6. Mechanisms of Fetal Programming

How is a permanent alteration in the function of a metabolic system induced during fetal life? The genetic code established by the DNA sequence essentially does not change after fertilization. It has long been questioned how the known miniscule rate of change to the genetic code could account for the array of phenotypic variation among genetically similar individuals. Indeed, this question led to a search for mechanisms of differential regulation of gene function within animals of a given genetic makeup. This investigation resulted in the now widely accepted observation that changes in gene expression can be modified without any change in the DNA sequence. These changes in expression (i.e., whether a gene is being used (transcribed) and how often and under what circumstances) can be heritable—either mitotically (i.e., to the daughter cells after mitotic cell division) or more rarely, meiotically (i.e., to the next generation through the eggs and sperm). The study of this phenomenon has been termed “epigenetics,” derived from the Greek prefix epi that means over or above.

At present, three mechanisms have been identified that serve to regulate gene expression: DNA methylation, modifications of the histones (the proteins around which the DNA is wrapped), and...
RNA-based mechanisms such as non-coding RNA or inhibitory RNAs. These epigenetic modifications can alter the magnitude of the expression of that particular gene. The addition of a methyl group to the cytosines in a particular DNA sequence results in suppression of gene expression (the transcription machinery fails to bind to it) and thus, causes suppressed function of that gene. Conversely, removal of methyl groups will increase the activity of a gene. These methylation events occur at discrete locations within genes, and some genes are more susceptible than others to this process. For example, the glucocorticoid receptor gene is a methylation-sensitive gene. Is there a specific example of the impact of this?

Histone modifications cause these proteins to either tighten or loosen their hold on the DNA wrapped around them, thus making genes more or less available for transcription.

Non-coding RNAs can bind to DNA and RNA having the complementary nucleotide sequence. They have been recently found to regulate both transcription of genes, by binding to areas on the DNA, and the function of miRNAs already transcribed from the genes, either inhibiting protein production from the mRNA or actually causing destruction of the mRNA. This is an active area of research.

Environmental cues, such as nutrient availability, stress, or environmental pollutants and toxins, seem to be primary triggers to induce changes in the epigenome (the epigenetic pattern regulating DNA function). The extent to which cells are able to respond to these cues lies in large part with the plasticity of the cells in question. Thus, the developing embryo and fetus are prime targets for epigenetic modifications. Studies are now directly investigating the epigenetic effects of intrauterine environment; for example, in primates, a 30% reduction in maternal nutrient intake caused alteration of methylation status in the kidney both halfway through and at the end of gestation. These data suggest that even a relatively mild nutrient restriction can induce epigenetic alterations and may alter organ function.

7. Amino Acid Nutrition and Epigenetics

Of the domestic livestock species, the horse has the most varied nutritional management. An encompassing standard of nutritional requirements or recommendations, based on scientific research, is lacking. Specifically, the importance of certain nutrients such as amino acids and other micronutrients has not been established. The importance of amino acids in supporting fetal growth and development is an emerging field in many livestock species and will undoubtedly shape the future of nutritional management in the horse. In pigs, arginine supplementation to pregnant sows increases litter size by two piglets. In sheep, maternal arginine administration during late gestation increased fetal peri-renal brown adipose-tissue development, which may enhance the neonates’ ability to combat cold exposure at birth. Restriction of essential B vitamins, folate, and methionine during the periconceptual period in sheep resulted in altered DNA methylation, insulin resistance, and elevated blood pressure, observed most notably in adult male offspring. Given the myriad of functions that amino acids play in physiology, it is not surprising that a group of amino acids plays a critical role in maintenance and regulation of epigenetic status. Specifically, methylation of DNA requires donation of a methyl group derived from S-adenosylmethionine. S-adenosylmethionine is synthesized from the amino acid methionine. In addition to its role as a methyl donor, methionine is also required for the synthesis of other amino acids and non-protein products. Synthesis and metabolism of the amino acids are interdependent; thus, it is not surprising that overall amino acid and micronutrient availability may alter the epigenetic code through changes in DNA methylation as well as by histone modifications.

Although extremely complex, these data highlight the delicate nutritional balance regulating development. The bounty of supplements that are currently available for equine use is overwhelming. It is imperative that we use caution when giving nutritional supplements to pregnant mares, because using an inappropriate type, dose, or combination may have permanent consequences on the developing fetus.

8. Behavioral Programming

In addition to programming various aspects of health and disease, the prenatal and early postnatal environment can also give rise to behavioral programming. Among livestock species, the benefits or consequences of behavioral programming are most important in the horse, given the variable requirements for companion qualities, willingness, trainability, and desire to compete.

The majority of scientific evidence related to behavioral programming comes from research in rodent and primate models. In rats, maternal licking and grooming (LG) of pups at birth is a measure of mothering ability. It seems to be heritable (i.e., pups of a high LG mother will be high LG when they give birth). However, cross-fostering of offspring to mothers exhibiting different levels of LG shows that this trait is regulated by the pups’ experience, not by its genetic makeup. Thus, LG is regulated at the epigenetic rather than genomic level. In addition, pups of high LG mothers also show reduced fear and lower stress responses as adults. Maternal stress can also alter the pattern of LG, which will then be conveyed to the offspring.

How can an experience in the first 7 days after birth affect stress responses in the adult? Further studies showed that high LG caused reduced methylation of the gene encoding the glucocorticoid receptor in the hippocampus. Thus, pups of high LG mothers have higher numbers of glucocorticoid receptors in this area, resulting in enhanced glucocorticoid...
ticoid feedback sensitivity; thus, there is a decreased release of adrenocorticotropic hormone (ACTH) and therefore, a damped stress response. This is not the only change associated with high LG—studies have shown that there are epigenetic changes in over 100 genes associated with this experience.

These findings on LG behavior in the rat may have direct and important relevance to the horse. The capacity for horses to deal with stress, respond correctly to humans and training situations, and tolerate new environments is extremely important to their function. It is well-known that orphan foals, brought up without normal mothering from a mare, can be difficult to train as adults and even dangerous to humans. It is likely that differences in foaling environment, birthing stress, level of human contact, and perinatal morbidity all play a role in establishing the permanent behavioral response of the foal in adulthood.

This lends new insight into the practice of “imprinting” of foals, as described by Dr. Robert Miller and others. It has been shown that tactile conditioning of foals at birth and at 24 h reduced resistance to touching of the legs and picking up the hind feet at 3 mo of age compared with unconditioned controls.59 However, as shown with orphan foals, we probably do not yet know the neonatal environment that best promotes what humans would consider the most successful equine adult behavior. A recent study by Henry et al.60 found that early exposure to a motionless human improved the foals’ reaction to humans, whereas forced stroking and handling did not improve later human/foal relations. Interference with development of the fetal/maternal bond by assisting with the first suckling caused foals to avoid human approach and physical contact at 2 and 4 wk of age, respectively.60

The role of maternal experience (stress during pregnancy) in fetal programming of adult health and disease has also been investigated. Interestingly, young adults of normal birth weight but whose mothers had experienced psychosocial stress during gestation showed an increase in the incidence of primary insulin resistance and a lipid profile indicative of metabolic syndrome.61 In addition, these individuals exhibited altered immune and endocrine function as well as compromised cognitive function.62–64 These findings again have direct application to the horse. For example, does trailersing a pregnant mare for hours to the breeding farm or foaling shed have permanent effects on the health of the foal in utero? Are there effects of maternal stress during pregnancy that will not be seen until this foal is an adult? The possibility exists, and much further study in this area is needed.

An interesting aspect of LG behavior in the rat is that female offspring of mothers with low levels of LG exhibited increased rates of sexual receptivity compared with female offspring from high LG mothers.57 Furthermore, 80% of female offspring from low LG mothers became pregnant under a controlled receptivity test compared with 50% of offspring from high LG mothers. These data highlight an evolutionary strategy for propagation of the species, one in which quantity of offspring produced is emphasized with a reduced postnatal investment (versus one in which maternal energy is invested in rearing for and ensuring maximal survival of fewer offspring). It is tempting to speculate how these findings could have application both to humans and horses. Is increased sexual activity in female offspring the direct effect of poor mothering? Perhaps increased mothering could serve as a preventative treatment for later heat-related behavior that interferes with a filly’s performance.

All of these maternal-related factors apply to an increasingly common procedure in the horse (i.e., embryo transfer). By transferring an embryo into a recipient mare, that embryo is going to have epigenetic alterations in response to its environment, both in utero and neonatally, that are different from those it would have experienced if left to gestate in the donor mare. How do these changes affect both behavior and physiology? Very little work has been done in this area.

Given the current evidence available in other species that experience in utero and immediately after birth can permanently alter not only behavior but metabolism and the clear ability for experience to alter the human/foal relationship, we must begin to consider that these interactions may have benefits and/or consequences not only with the present individual but also its future offspring.

References and Footnotes


*Satterfield MC and Wu G. Personal observations, 2010.