Developmental programming in equine species: relevance for the horse industry

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Implications

- Broodmare nutrition, in particular the use of concentrated feed, could affect the foal’s metabolic and joint health, inducing lameness and reducing sport performance.
- Embryo biotechnologies are widely used in the horse industry. The choice and nutrition of the surrogate dam partly determines the adult size and health of the offspring.
- Better broodmare nutrition may be an easy and cheap means of preventing joint diseases.
- Equine obesity and metabolic diseases are raising concerns for veterinarians. There is a need for better nutrition of horses, in particular in mares during gestation.

Key words: DOHaD, foal, mare, osteochondrosis, pregnancy

Introduction

Economic importance of the horse industry

According to statistics collected by the Food and Agriculture Organization of the United Nations (www.fao.org/faostat/en/), the worldwide population of horses was estimated at 58 million head in 2014, of which 55.2% are located in the Americas (with a third in North America), 24.3% in Asia, 10.3% in Africa, and 9.4% in Europe. This population is heterogeneous depending on continents, but the percentage of horses dedicated to leisure and sport activities is steadily increasing in westernized countries. In Europe, the equine sector provides 400,000 full-time jobs, generating revenue above €100 billion annually, and the number of horse riders increases by 5% per year (http://www.europeanhorsenetwork.eu/the-horse-industry/).

Athletic performance and use of horses

Horses are mainly used for sport or work. Athletic performance in horses is the result of multiple variables, including genetics, health, training, temperament, nutrition, and rider’s ability. Osteoarticular health is of uppermost importance. One major burden for the breeding industry is osteochondrosis, a perturbation in cartilage ossification that affects up to 44% of young horses and reduces their ability to compete (Verwilghen et al., 2013). Osteochondrosis is known to be due to both genetic and environmental factors.

Depending on the equestrian sport, horse careers differ. For example, Thoroughbreds compete in flat-racing when they are still growing, as many top-ranking races are devoted to 3-yr olds. In other sports, such as show jumping, the sport career may be long and intensive, up to 15 to 20 yr of age. Reproductive use takes place either after the sports careers or in combination with performance through semen freezing in males and embryo transfer in females. Alternatively, when horses are kept for leisure, they are often considered as companion animals, given relatively little exercise and kept until old age.

Developmental origins of health and disease and epigenetics

In mammals, developmental conditions (e.g., maternal nutrition, stress, or exposure to pollution) at the time of conception or during pregnancy and the neonatal period are known to affect long-term postnatal health, and are referred to as “Developmental Origins of Health and Disease” (DOHaD).

Non-communicable diseases such as diabetes (perturbation of the glucose metabolism), obesity, and cardiovascular diseases, but also neurological and osteoarticular disorders, have been shown to have developmental roots.

The biological basis for DOHaD is epigenetic mechanisms that are induced by modifications of the cellular environment. The epigenetic machinery modifies the expression of genes without altering the DNA structure of the genes, either by hampering or enabling the access of transcription factors to the DNA, or by acting at the post-transcriptional level (i.e., after the genes have been translated into molecules conveying the information to the cellular machinery; Jammes et al., 2011). Although they are reversible, epigenetic marks are maintained with cell division. They are considered as the “memory of the cell” and can induce physiological effects a very long time after the event that caused them occurred took place (Figure 1). The prenatal and early postnatal periods are considered as critical periods because the epigenetic plasticity of developing organism makes it very sensitive to its environment.

Scope of the article

Thus, in the equine species, maternal environment, such as broodmare metabolism and nutrition, or the use of reproductive biotechnologies, may affect feto-placental development, foal growth, and subsequent adult health, thus impacting offspring performance and longevity.

This article aims to summarize existing knowledge on long-term effects of breeding conditions and reproductive biotechnologies in the equine species, with special reference to their unique use as performers. Future directions both in research and for improvement of field management are discussed.

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Maternal Nutrition

The first environmental factor that could affect fetal development and long-term offspring development is the nutrition of the dam.

Nutritional needs during gestation: Seasonal adaptations

The horse being a seasonal mammal, pregnancy usually starts in mid-spring and lasts 11 mo. In feral conditions, horses depend on vegetation availability for feeding throughout the year, with subsequent seasonal variations in body condition (higher in summer and lower in winter; Kuntz et al., 2006; Dawson and Hone, 2012). Thus, pregnant mares in early gestation (spring and early summer) have access to abundant resources and can store energy. In mid-pregnancy (late autumn and winter), food availability is reduced, and mares use their reserves to support fetal growth (Figure 2), with the foal gaining more than 50% of its birthweight in the last 3 mo of pregnancy. Nevertheless, most nutritional recommendations advise to feed domestic broodmares at maintenance levels until 5 mo of pregnancy and to subsequently increase feed energy levels to account for increased fetal demands (National Research Council, 2007; Martin-Rosset, 2012).

Breeders practice

Data regarding broodmare nutrition in the field are scarce. In Europe, late-gestation mares are generally fed 10 to 40% above nutritional recommendations for their gestational age (Bergero et al., 2006; Winkelsett et al., 2006), and even in pasture, mares often are most of the time supplemented with cereal concentrates (Jouven et al., 2013; Van der Heyden et al., 2013).

Broodmare nutrition can affect offspring health: Scientific evidence

Although maternal nutritional restriction or excess does not seem to affect foal weight at birth, except in cases of extreme undernutrition, glucose metabolism and osteoarticular development of the foal may be affected by inadequate maternal nutrition (Peugnet et al., 2016).

Epidemiological observations in the Belgian Ardennes showed that foals born to dams fed concentrated feed during pregnancy were eight times more likely to develop lesions of osteochondrosis compared with foals born to dams fed forage only (Figure 3; Van der Heyden et al., 2013). These data were confirmed in a controlled study using Saddlebred mares fed forage only or forage and concentrates although the difference in the incidence of osteochondrosis was not as large as in the epidemiological study, possibly because of the limited number of experimental animals (Peugnet et al., 2015). Foals born to dams fed forage only, however, had thinner cannon bones, were less efficient in regulating their blood glucose,
Figure 2. Physiological evolution of the mare’s metabolism during gestation.

- **Early gestation**: Facilitated anabolism
  - 1. Increase in the quantity of glucose absorbed during meals
  - 2. Glucose storage in the form of glycogen and fat in liver, muscles and adipose tissue

- **Late gestation**: Energy catabolism to sustain fetal growth
  - 1. Increase in the quantity of glucose absorbed during meals
  - 2. Hepatic production of glucose, degradation of glycogen in muscles
  - 3. Degradation of muscular proteins and adipose tissue fatty acids, hepatic production of ketones to cover the needs

Figure 3. Differences in the incidence of osteochondrosis—a debilitating articular disease affecting young horses—in foals according to maternal nutrition (after Van der Heyden et al., 2013).

- **Concentrates during gestation**: Osteochondrosis
  - Prevalence: 33 to 38%

- **Forage only during gestation**:
  - Prevalence: 4%

△ by 8X of incidence of osteochondrosis
and had delayed testicular maturation compared with those born to dams fed forage and concentrates (Robles et al., 2017).

**Biological basis for the concomitant effects on glucose metabolism, bone growth, and testicular development**

The data described above show that altering nutrition in pregnant mares can induce effects in the foal on glucose homeostasis and bone and testicular development, most probably as a result of the cross-talk between energy, bone and testicular, or ovarian metabolism (Confavreux et al., 2009; Figure 4). Indeed, organs such as pancreas, bone, fat, and sexual organs (testicles and ovaries) communicate and regulate each other’s function through the secretion of hormones (insulin, osteocalcin, adipokines, and sex steroids being among the key factors). Thus, any dysfunction in one organ may affect the whole system.

Although the exact inter-relation between these organs has not yet been described in fetal life, the occasional observations of osteochondrosis lesions in newborn foals already confirm the prenatal roots for this multifactorial disease (Olstad et al., 2007). Moreover, since testicular and ovarian development takes place in utero in horses, like in most domestic species, effects on testicles may also be due to prenatal developmental conditions. It is, however, important to underline that the quality and quantity of maternal lactation and the exercise of the foal have not been analyzed in the currently available models, and that they may have an important role in modulating the post-natal effects.

**Consequences for the industry**

The economic burden of osteochondrosis on the industry is related to the cost and length of treatment (surgery is needed in the most advanced stages of the disease) and the sportive consequences such as delayed training and poorer performance (Verwilghen et al., 2013) but also the refusal of certain studbooks to accept horses affected by this condition. The systematic rejection of stallions affected by the disease as registered stallions did not enable a significant reduction in the incidence of the disease. This is possibly due to the fact that some horses are operated on and the clinical signs of osteochondrosis removed before they are registered as breeders but also as a result of the multifactorial origin of the disease. Improving maternal nutrition may be one of the so far unexplored ways to prevent this condition. It is also relatively inexpensive compared with costly surgical treatment.

Moreover, experimental observations show that metabolism, bone strength as assessed by cannon bone width, as well as reproductive function could be affected by maternal nutrition as demonstrated in other species. Proper glucose metabolism is essential for performance as glu-
cose is the major provider of energy during exercise. Several studies indicate that metabolic diseases in horses reduce the individual’s performance and increase veterinary costs to the owner (Kearns et al., 2002).

The solidity of cannon bones is also important for sports such as steeple-chase and show jumping where the combined weight of the horse and its rider at reception are supported by the front legs. Finally, high-performing horses may be used for reproduction until 20 yr of age, and the maintenance of their fertility is an important concern.

**Periconceptional Development and Embryo Biotechnologies**

**Scientific context**

The pre- and/or periconceptional periods (before and just after fertilization until the embryo blastocyst stage) are critical for DOHaD (Steegers-Theunissen et al., 2013; Sinclair and Watkins, 2014). Deep epigenetic modifications occur during the development of the gametes (spermatozoa and eggs), fertilization, and the early stages of embryonic development. These modifications can be altered by the environment in vivo, in particularly through maternal and/or paternal nutrition, but also in vitro, in the context of Assisted Reproductive Technologies (ART) (Chavatte-Palmer et al., 2016).

**Use of reproductive technologies in horses**

In horses, the use of ART depends on the breed used. The Jockey Club, in charge of the Thoroughbred studbook, demands that all foals are born after natural breeding of their dams. Trotter breeds such as the French “Trotteur Français” accept that artificial insemination is used, but both the mare and the stallion need to be present on the farm. This enables a wider use of stallions as the collected sperm can be used for several mares. Most other breeds will accept foals that are born after insemination of the dam with shipped semen (enabling the dissemination of genetics) or after embryo transfer (the embryo of the genetic mother is transferred to a surrogate dam). So far, only a limited number of studbooks will register cloned foals.

To date, the most popular embryo technology in the equine species is embryo transfer, which involves the collection of one embryo from a donor mare that is transferred immediately or after shipping in conservation medium (24 to 48 h) into a recipient mare. The International Embryo Transfer Society reported that more than 20,000 in vivo produced equine embryos were transferred worldwide in 2015 (Perry, 2016). Although procedures such as intracytoplasmic sperm injection (ICSI) and ICSI followed by in vitro embryo culture (ICSI-C) as well as cloning are now developed in the industry (Galli et al., 2014; Hinrichs et al., 2014), the number of foals born from these procedures remains very limited (around 1,000 foals/year worldwide).

**Effects of the size of surrogate dams**

The ability for an individual to reach its genetic potential in terms of adult size partially depends on the size of its dam, as elegantly demonstrated by Walton and Hammond (1938) in the first half of the 20th century using cross-breeding between large Shire horses and small Shetland ponies. Crossbred offspring whose dam was a Shetland pony were smaller at birth and remained smaller as adults than those whose dam was a Shire mare. Almost 50 yr later, Polish pony embryos transferred into draft mares...
were shown to produce foals that were larger at birth and remained larger as adults compared with those that had been transferred into mares of their own breed (Tischner, 2000). These data have been confirmed more recently by two independent studies using embryo transfer between breeds of different sizes as a model (Figure 5). The transfer of pony embryos into mares of larger breeds consistently increased fetal growth, and excess size was maintained until adulthood. In contrast, foals from a larger breed born to pony mares were small at birth and only partially caught up to controls of the same breed (Allen et al., 2004; Peugnet et al., 2014). Moreover, excess or reduced fetal growth was associated with abnormalities in the regulation of blood pressure at birth as well as metabolic perturbations that could persist up to 2 yr of age (Peugnet et al., 2016). Of particular interest for the athletic performance of the young horse, the risk of developing lesions of osteochondrosis was increased at 6 mo of age. Osteochondrosis has been associated with greater plasma glucose and insulin concentrations after the meals (indicating a less efficient control of glucose metabolism) in young horses, and thus these observations may be related to the metabolic dysfunction.

**Effect of embryo biotechnologies**

In horses, so far, no adverse effects of embryo transfer, ICSI, ICSI-C, and cloning on postnatal growth and adult health have been reported in horses (McKinnon and Squires 2009; Johnson et al., 2010; Galli et al., 2014; Hinrichs et al., 2014).

**Consequences for the industry**

In commercial embryo transfer units, surrogate dams are usually chosen for their breeding soundness, maternal abilities, and low cost. Alternatively, the client may also impose the choice of the recipient mare to reduce costs and for personal convenience. Although extreme size differences between the embryo and recipient genetics are rarely encountered, small differences (such as the transfer of embryos of large show jumping breeds into smaller size Quarter Horse mares) are common. Consequences of the effects on metabolism and osteochondrosis have been discussed with maternal nutrition.

Differences in adult size induced by embryo transfer may jeopardize the registration in a studbook as size limits are imposed. On the other hand, breeders may take advantage of these mechanisms. For example, registration in a pony breed and access to pony competitions depend on the animal size: choosing a smaller recipient mare may help to ensure that the offspring of a larger individual remains suitable for registration as a pony. To date, consequences of these observations on further performance remain to be determined although a few studies indicate that smaller size is associated with poorer prices at Throughbred yearling sales (Pagan et al., 2005) and poorer racing performance in Throughbreds (Smith et al., 2006) and Norwegian trotters (Dolvik and Klemetsdal, 2010).

In terms of embryo biotechnologies such as ICSI, ICSI-C, and cloning, work in animal models and in humans indicate that their use may affect fat deposition, glucose metabolism, and cardiovascular function in offspring (Hart and Norman, 2013). Moreover, there is ample evidence in animal species that the nutritional status of the embryo donor and recipient can also affect postnatal health (Sinclair and Watkins, 2014). Since these technologies are still new in horses, data evaluating their impact on performance are currently lacking.

**Perspectives**

In humans, DOHaD have been demonstrated as one of the causes for the current obesity epidemics. In horses, overweight and obesity are becoming a health issue with a prevalence ranging from 2% in animals in training to 70% depending on the use and breed of the animals. The equine metabolic syndrome that compares with the human condition is also a recent concern among veterinarians (Frank et al., 2010). More work is needed to explore the biological bases of the DOHaD in horses. Tools for the study of epigenetic modifications need to be developed, taking advantage of rapid methodological progress in other species. Finally, the data presented here plead for a better definition of broodmare nutritional requirements and for improved management of pregnant horses to prevent equine diseases and improve welfare. In view of the raising concern of westernized society for animal welfare and of the increased use of the horse as a companion animal, better preventive strategies for diseases prevention and for improving welfare are needed and may be offered through the improvement of pre- and postnatal developmental conditions.

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