Nutrition of Broodmares

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KEY WORDS

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KEY POINTS

- In normal conditions, forage availability can match mares' energy and protein needs but low forage quality or breeding out of season requires nutritional supplementation. Micronutrient availability, however, should be verified and often requires supplementation.

- Attention needs to be placed not only on the quantity of energy and nutrients but also on their quality and characteristics.

- Mare nutrition and adiposity can influence the foal's long-term health and metabolism (Developmental Origins of Health and Disease; DoHaD) and therefore excess nutrition can be as deleterious as feed restriction.

- There is a need for more research on broodmare nutrition, taking into consideration genetics, breed, breeding conditions and environment.

SYNOPSIS

Forage availability should cover most needs for mares bred during spring/summer. Nevertheless, out-of-season breeding, lack of access to pasture or of good quality forage calls for nutritional supplementation. Current evaluations of broodmare needs are based on fetoplacental tissue requirements but do not consider endocrine changes nor the fact that maternal diet quality affects long-term foal health. This paper first reviews pregnant mares' current nutritional recommendations. Secondly, fetoplacental developmental stages during gestation are outlined, defining critical periods in the context of the Developmental Origins of Health and Disease (DOHaD). Lastly, examples of how maternal nutrition affects long term foal health are presented.

INTRODUCTION

Nutrition and reproduction are central facets of life, highlighting the critical importance of optimal nutrition for the broodmare. Our goal with this review is to provide the reader with a solid foundation of knowledge regarding:

1) core broodmare and fetal physiology, as well as maternal nutritional requirements,

2) the influence broodmare nutrition can have on the future health and performance of the foal.

This review should enable readers to move beyond the basic dietary energy and nutrient requirements and consider more precise formulation of diets for broodmares being kept in a wide range of different environments.

The first step in any nutritional evaluation should be to evaluate the mare and the performance sought. Once this has been well characterized, the diet and management best suited to that scenario can be formulated. Gestation and lactation result in substantial increases in nutritional requirements. Estimates of energy and nutrient requirements developed by equine nutritionists represent an excellent starting point for formulating a broodmare's diet[1–3].

Nutrition, along with day length and ambient temperature, are important environment variables. Over tens of millions of years of evolution, horses developed a seasonally polyestrous reproductive physiology that resulted in most foals being born in late spring and early summer, thereby synchronizing the nutrient requirements of late gestation and early lactation with environmental energy and nutrient availability.

While those caring for broodmares can easily evaluate the mare and her environment (day length, ambient temperature, and forage/diet characteristics), it is more challenging to know the condition of the fetus or modify its environment. A primary histotrophic nutrition (based on uterine secretions) transitions to hemo (based on exchange between maternal and fetal bloods) and histotrophic nutrition following implantation[4]. The fetus progresses through various developmental stages all of which may be influenced by the dam's nutrition. A growing body of research highlights the importance of the link between maternal nutrition and developmental programming of the fetus.

Nutrition is customarily evaluated as a balance between the requirements of the horse and the dietary supply. The objective is to find a balance that optimizes the long-term health and performance for the broodmare and her foal. Finding this balance requires an understanding of over- and undernutrition as well as the changes in requirements according to physiologic status. Furthermore, we now appreciate that optimal nutrition goes beyond simple quantities and requires consideration of the quality and form of the dietary energy and nutrients.

With the increasing incidence of obesity and metabolic syndrome in horses, in addition to effects on mare fertility[5], unforeseen effects may result, especially in terms of offspring health and metabolism. Conversely under moderate maternal undernutrition, foal birth weight is not affected but long-term metabolic consequences may still be observed in offspring. There is a relatively clear connection between glucose/insulin homeostasis and metabolic consequences. However, other dietary components should also be considered, despite the fact that current knowledge is limited.

KEY POINTS

- **Recommendations for dietary energy, protein, vitamins, minerals and water are available, but these should be considered a starting point, from which the unique characteristics of each breeding operation should be further considered.**
- **Practitioners should consider the environment that a broodmares' physiology has been well adapted to as they make choices regarding diet and management during different stages of gestation.**
- **Knowledge of the negative implications of over and under-nutrition, the metabolic and developmental impact of fiber, nonstructural carbohydrates and fats as dietary energy**

sources, and the potential benefits of precision feeding using supplements or ration balancers is invaluable when formulating diets for broodmares.

CURRENT NUTRITION RECOMMENDATIONS

GENERAL ESTIMATION OF BROODMARE NEEDS

Intake during gestation needs to meet mare plus fetal growth requirements. Studies on fetal growth, however, are limited. Fetal growth curve data are based on aborted or stillborn animals[6–8], thus potentially underestimating fetal growth at the end of gestation[1].

Historically, mare gestational nutritional requirements have been calculated based on the following assumptions:

- Accretion of uterine and placental tissues takes place mid-gestation[7,8]. It is assumed that fetal adnexal tissue (placenta, amnion) and mammary development are linear to that of the fetus, as observed in the cow[9], but this has not been demonstrated in the horse[10].
- Fetal growth is best represented by an exponential growth curve with rapid fetal development occurring from day 240 of gestation to parturition[10].
- The foal's weight at birth is assumed to be approximately 10% of the dam’s nonpregnant mature body weight (BW).
- Uterine and placental tissues are metabolically more active (66.6kcal/kg) than the rest of the body (33.3kcal/kg BW) and therefore have higher energy needs[10].
- The efficiency of using digestible energy (DE) for depositing fetal and placental tissue during gestation is assumed to be 60%[11].

Most of the factors above are considered in the estimate of energy and nutrient requirements for pregnant broodmares that have been developed around the world. Differences between the various estimates are often due to variation in interpretations of fetal, placental, and uterine growth data and estimates of changes in mare metabolism during gestation[12] (Personal communication, Manfred Coenen).

ENERGY AND PROTEIN REQUIREMENTS AND RECOMMENDATIONS

Lactation and rapid fetal development in late gestation represent periods of high requirements for broodmares[1,10]. Non-lactating mares in early and even mid-gestation have energy and protein requirements either at or close to maintenance levels (**Figure 1**). Estimated energy requirements in late gestation rise to between 1.3- and 1.5-times maintenance levels, while lactation can result in a doubling of energy requirements.

Horses obtain energy effectively from their environment, primarily from forages. Total dry matter intake (DMI) will likely range from 1.5 to 3.0% BW, and in most cases at the higher end of this range in pregnant or lactating broodmares. Forages have most of their potential energy stored in the chemical bonds of structural and nonstructural carbohydrates and horses have evolved for optimal utilization of this particular environmental dietary energy source. Therefore, caregivers should focus first on providing dietary energy from forages. Based on a host of published information, a rough approximation of the DE content of most forages is between 1.5 and 2.5 Mcal/kg, with more mature forages usually providing less DE[1–3]. For most mares, forages should make up at least 50% of their daily DMI, and in many cases may be close to 100%, if no energy rich concentrate/complementary feed or vitamin and mineral supplement is needed. Once dietary forage has been optimized, attention can shift to concentrates and/or vitamin and mineral supplements.

Mares in late gestation provided concentrates, in addition to grass forage, maintain body condition and weight better than those on forage alone[13]. Grains and concentrates should really be viewed as a complement or supplement to the energy and nutrients provided in the base forage diet (see Chapters 1, 3 and 4). Grains contain high concentrations of starch, a nonstructural carbohydrate that can be a valuable dietary energy source for broodmares. An approximation of the DE content of grains is 2.5 to 3.8 Mcal/kg of dry matter (DM)[1–3], with some high fat grains providing even more. The DE in most commercial concentrates containing mixtures of nonstructural carbohydrates, fiber, and vegetable oil/fat will range from 3.0 to 3.8 Mcal/kg DM. In the vast majority of scenarios, grains and concentrates should not constitute more than 50% of the daily DMI. A range of 5 to 30% is probably appropriate in most cases, depending on the BCS of the mare, her health, and the quality and availability of the forage. More details on starch intake are developed in Part 3 (Developmental Origins of Health and Diseases; DoHaD).

Horses are somewhat unique as a grazing species due to their gastrointestinal anatomy and their ability to digest and utilize dietary fats[14]. The location of the small intestine prior to the cecum and colon, allows the horse digestive and absorptive capabilities for fats before those fats reach the primary microbial populations in the cecum and colon. Adding dietary fat therefore can be an effective strategy to increase dietary calories for broodmares[15] while limiting the potential negative effects of excess starch on offspring health (see Part 3). Research indicates that horses are likely capable of digesting and absorbing dietary fat at concentrations of up to 200 g/kg DM density[14]. The increased energy density of added vegetable fat/oil provides several possible benefits, including flexibility to maximize fiber without sacrificing energy intake, especially when energy requirements are high and DMI may be limited, and potential improvements in fat soluble vitamin absorption[14,16]. While horses may be capable of digesting and absorbing upwards of 15% to 20% dietary fat, forage contains only 2% to 4% fat/lipid. In the authors' opinion, the potential benefits of adding fat are more likely to be seen in the range of 5% to 10% dietary fat on a DM basis, calculated by evaluating both forage and concentrate fat intake. The quantity and ratio of dietary omega-6 and omega-3 fatty acids may influence inflammation, alter cell membrane fluidity, and gene expression but much more work is needed in this area[17]. Most forages are rich in omega-3 fatty acids (e.g. alpha linolenic acid), so diets that contain at least 50% forage are more likely to have a relatively low omega-6-to-omega-3 ratio[17]. If additional omega-3 fatty acids are desired, flaxseed or flax oil would likely be the most practical to incorporate into the ration, but other sources such as fish and algae oils can also be used depending on the budget and the palatability of the oil for the individual animal.

The pattern of change in crude protein (CP) requirements during gestation is similar to that of DE (**Figure 1**). The CP requirements for non-lactating early gestation mares are near or at maintenance levels and rise exponentially in the last third of gestation and rise again during lactation. Estimated CP requirements vary around the world, likely due to different assumptions of protein utilization for fetal, placental, and uterine development, as well as protein as an energy source in late gestation.

Of greater importance is protein quality, specifically digestibility and amino acid composition. Under most circumstances, feeding the broodmare a higher quality protein will improve the mare's and developing fetus's ability to utilize amino acids for tissue development. Available protein can be estimated by subtracting the acid detergent insoluble nitrogen and the non-protein nitrogen from the CP to provide a better estimate of the protein available for absorption in the horse's small intestine[1]. This information can be provided on demand by most laboratories. The quality of the dietary protein is also improved by providing a composition of essential amino acids that most closely meets the requirements of tissue development[18]. More research is needed to uncover knowledge of broodmare's amino acid requirements, but it is assumed that lysine is the first limiting amino acid and its concentration is approximately 4.3% of the CP requirement[1]. Quality protein sources include soybean meal, alfalfa, and certain milk byproducts, due to their amino acid composition.

VITAMINS, MINERALS AND WATER REQUIREMENTS AND RECOMMENDATIONS

Essential vitamin and mineral requirements needed to support optimal embryo and fetal development are not clear. Vitamins A and E are normally high in fresh forages (**Table 1**)[19,20]. Vitamin D requirements should be met by the horse having sunlight exposure, thus mares maintained predominantly indoors may require additional vitamin D from the diet (see also Chapter 4). Horses maintained in an environment where they have sufficient access to immature fresh forages during late gestation and early lactation will likely be meeting their requirements. In contrast, dried hay (and especially hay that has been stored for a long time) will not be sufficient to maintain adequate Vitamin A and E levels in pregnant mares when fed for several months[21]. Serum vitamins A and E concentrations are higher in summer when pregnant mares are in pasture, compared to in winter, when they are typically stabled and fed preserved forage[19,22]. Various B-vitamins are found in forage and are also produced by microbes within the equine digestive tract. However, there is little known regarding B-vitamin requirements during gestation or their relative concentrations in forages.

Table 1. Ability of forage to meet broodmare fat soluble vitamin requirements

Vitamin	NRC Requirement (500 kg of body weight)						Is forage adequate?[2]
	0-120 d gestation	120-250 d gestation	250-340 d gestation	0-30 d lactation	Fresh forage content (unit/day)[1]	Hay content (unit/day)[1]	
Vitamin A (kIU)	30	30	30	30	*55-2,418[3]*	*3.6-593[3]*	Likely inadequate in preserved forage/hay
Vitamin D (IU)	3300	3300	3300	3300	*341-19,800*	*990-61,160*	Yes, if sun-exposure is not greatly limited. Higher values found in sun-dried forage/hay compared to fresh pasture.
Vitamin E (IU)	800	800	800	1000	*147.5-6,556[4]*	*164-3,458[4]*	Likely inadequate in preserved forage/hay

[1]Forage and hay calculations based on 500 kg BW horse and 2% BW (DM basis) consumption of forage only
[2]For more information, see Chapter 4 (Forage)
[3]Calculated using the conversion 1 mg β-carotene = 333 IU Vitamin A during pregnancy[20]
[4]Calculated using the conversion 1 mg ⍰-tocopherol = 1.49 IU Vitamin E[1]

Mineral supply from pasture and hay is influenced by soil factors, plant species, state of vegetative growth, and fertilization/irrigation[23,24]. Therefore, specific recommendations regarding the need for mineral supplementation are difficult to state as they are dependent on grass type, geographic location and season of the year. Many fresh forages will meet the macromineral needs (see also Chapters 1 & 4) of the mare during gestation for calcium (Ca), phosphorus (P) and potassium (K) but may be low in sodium (Na) and some trace minerals, including copper (Cu), zinc (Zn) and selenium (Se)[23,25–28] (**Table 2**).

Table 2: Ability of forage to meet broodmare mineral requirements

Mineral	NRC Requirement (500 kg)						Is forage adequate?[2]
	0-120 d gestation	120-250 d gestation	250-340 d gestation	0-30 d lactation	Cool season grass	Warm season grass content	

					content (unit/day)[1]	(unit/day)[1]	
Calcium (g)	20.0	28.0	36.0	59.1	*20-86*	*24-89*	Yes, though supplementation may be needed during early lactation.
Phosphorus (g)	14.0	20.0	26.3	38.3	*12-31*	*15-98*	Yes, though supplementation may be needed during late gestation or early lactation if consuming mature (seed heads present) cool season grass.
Potassium (g)	25.0	25.0	25.9	47.8	*37-269*	*63-298*	Yes
Sodium (g)	10.0	10.0	10.0	12.8	*0-36.8*[3]	*Not reported*	Supplementation is often needed.
Chloride (g)	40.0	40.0	40.0	45.5	*19.6-144*[3]	*Not reported*	May require supplementation..
Copper (mg)	100.0	100.0	125.0	125.0	*39-87*	*43-143*	Most cool season grasses are too low in Cu to meet the requirements throughout gestation. Some warm season grasses may provide adequate levels.
Zinc (mg)	400.0	400.0	400.0	500.0	*149-273*	*196-634*	Supplementation needed.
Selenium (mg)	1.0	1.0	1.0	1.25	*0.5-0.7*	*0.3-4.0*	Depends on geographical region.

[1]Grass value calculations based on 500 kg BW horse and 2% BW (DM basis) consumption of forage only
[2]For more information, see Chapter 4 (Forage)
[3]Values for general grass pasture obtained from https://equi-analytical.com/common-feed-profiles/

Water requirements during gestation seem to be similar to that of maintenance. Observed intakes range from 5.1 L/100kg BW[29] to 6.9 L/100kg BW[30] in pregnant mares and from 11.9 L/100kg BW to 13.9 L/100kg BW in lactating mares[1] and are influenced by several factors, including DMI and environmental temperature. Availability of water is especially important for horses consuming dried preserved forage, such as during cold seasons and when stalled[31,32]. Water restriction during pregnancy results in decreased feed intake and loss of BW[30]. Thus, water restriction should be avoided.

KEY POINTS

- **Published energy and nutrient recommendations are best viewed as starting values which can be tailored to meet individual scenarios.**

- **Mares' requirements are continuously changing based on stage of gestation and lactation.**

NUTRITION OF THE BROODMARE: ADDITIONAL FACTORS TO CONSIDER

GESTATION: A UNIQUE PHYSIOLOGICAL STATUS AFFECTING METABOLISM

In early gestation, mares have more efficient glucose absorption ~~sugar ingested~~, which results in a higher postprandial blood glucose[33]. They also have an enhanced endocrine pancreatic response, resulting in increased postprandial insulin secretion and basal hyperinsulinemia compared to non-pregnant mares[33]. Therefore, mares dedicate the first part of gestation to glucose storage as fat or glycogen in peripheral tissues (adipose tissue, muscles and liver), in order to stock up for when fetal needs will increase. This is called "facilitated anabolism"[34]. At the end of gestation, mares become more insulin resistant, have decreased peripheral tissue glucose tolerance and decreased pancreatic β-cell sensitivity, limiting the glucose storage in maternal tissues[33,35–37]. These changes are associated with a pronounced increase in glucose absorption after meals[33,37,38] (**Figure 2**). These physiological adaptations coincide with a strong increase in feto-placental glucose requirements at the end of gestation: almost 75% of the circulating maternal glucose is used by the uterus and fetal tissues[39].

Age and parity may alter these metabolic changes in mares. For instance, primiparous mares have been shown to have higher insulin responses to feeding compared to multiparous mares in late gestation, which could mean impaired metabolic adaptation to gestation in primiparous dams[36].

In late gestation, mares have an increase in lipid mobilization, as observed through the following:

- Increased serum β-hydroxy-butyric acid[40] concentrations, a ketone which serves as an alternate ATP/energy substrate when glucose availability is low[41].

- Some authors observed an increase in plasma triglyceride concentration reaching a plateau from the 7th month of gestation onwards[42,43], however, not all have observed such change[40,44].

- Plasma cholesterol concentrations are stable during gestation[45] and lower than in non-pregnant mares[40], which may result from use of cholesterol for steroid synthesis[46,47].

Nitrogen metabolism also adapts to the pregnant state. During gestation, plasma total proteins concentration varies[40,42–44,48], though lower than in non-pregnant mares[40,43,44]. Plasma urea concentrations are higher in late gestion mares than in non-pregnant mares, reflecting the increased need for amino acids to support anabolic processes during gestation[40,44].

Metabolic changes are summarized in **Figure 3**.

KEY POINTS

- **During the end of gestation, maternal metabolism allows for maximum glucose redirection to the fetus to meet its needs for full growth.**
- **The high use of glucose by the fetus leads the mare to use mainly her lipid reserves to meet both her own and fetal needs. This highlights the importance of the first months of gestation for the build-up of lipid reserves. A BCS of 5 (1-9 scale), however, should be targeted to avoid the detrimental effects of overweight and obesity on both maternal and fetal health.**
- **If there has been insufficient lipid storage earlier in pregnancy the mare will need to also draw on her protein reserves to meet her own as well as the energy needs of the fetus.**

CONSIDERING THE SEASON IN MARE'S NUTRITION

Providing optimal nutrition for broodmares requires consideration of multiple factors, each likely to be changing daily over the ~340 days of gestation. Feral and semi-feral horses manage excellent reproductive performance without human intervention[49–51], even though body condition loss is often observed in late gestation[49,50,52]. Their success may be attributed to reproductive and feeding strategies that evolved over 30 to 40 million years in temperate grassland environments enabling adaptation to an environment that changed in a predictable manner with each season. Feral and semi-feral horse herds today still foal and breed during some of the longest days of the year[49,51]. The connection of reproductive patterns with day length and nutrition are well recognized[53], with an evolutionary benefit to synchronizing reproduction, growth, and lactation needs with the environmentally available energy sources.

The diet is the primary source of energy, with adipose (and muscle) tissue serving as a supplementary source when the diet is limited. For domesticated broodmares, the responsibility for nutritional and reproductive management is that of the caregivers. In addition to gestational needs, changes in energy requirements due to thermoregulation, feed acquisition and disease in broodmares have been poorly studied. Optimal broodmare nutrition can be achieved by understanding and accounting for each of these factors.

In **Figure 1**, the DE and CP requirements of broodmares during gestation are overlaid on day length, one of the primary environmental variables that leads to changes in ambient temperature and forage growth and availability. The day length curves represent day length experienced by mares that were due in mid to late-winter (February), early to mid-spring (April), or early summer (June) in the northern hemisphere. Those feeding broodmares can examine this figure and not only consider what the broodmare's requirements are, but also the environmental energy and nutrient sources and sinks by which she is being influenced. Here are a few examples.

- <u>A broodmare at 320 days of gestation and due in February</u> has both high DE and CP requirements, yet day length is short, and ambient temperatures and fresh forage availability are low. Her caregiver should provide high quality preserved forage and consider a complementary concentrate feed to provide the required DE and CP (including essential amino acids). <u>Another broodmare at the same stage in gestation, but instead due in June</u> has identical requirements, but is in an environment where day length is long, and ambient temperatures and fresh forage availability are high. In this case, the caregiver can provide significantly less complementary feeds, based on what the mare's environment provides.
- <u>A lactating broodmare at 40 days of gestation and due in February</u> has high DE and CP requirements, primarily related to lactation. Day length is increasing, but ambient temperature and fresh forage availability are just beginning to rise. To meet the nutritional demands of early lactation she will require high quality preserved forage and possibly a complementary concentrate. <u>Another broodmare at the same stage in gestation, but instead due in June,</u> has identical requirements, but is in an environment where day length has already peaked, leading to higher ambient temperatures and likely plentiful fresh forage availability.
- Finally, **Figure 1** highlights a nutritional opportunity during early and mid-gestation to increase a broodmare's body condition score (BCS) when energy and nutrient requirements are low (in a natural environment, *i.e.* if the mare is not overweight/obese). Obviously, this period is shorter in the lactating mare, extending from late lactation to the last third of gestation. An example would be a mare who foaled in mid-spring, was bred a month later, and her foal had been weaned at the end of the summer. She should be able to take advantage of good fall forage and relatively low energy and nutrient requirements of mid-gestation to increase her BCS. Another example would be a mare with no foal at her side, bred in March, taking advantage of spring forage to improve BCS. It will be much more challenging to increase BCS during early lactation, even with good spring forage, or in late

gestation, because energy will be partitioned away from the mare and to the rapidly developing fetus.

Body condition score (BCS) is an indicator of energy balance. BCS is a standardized subjective evaluation of subcutaneous fat stores. The evidence seems to indicate a moderate BCS of 5 (1-9 scale[54]) and 3 (1-5 scale[55,56]) in the broodmare as a target through gestation. Fat stores provide energy when the less predictable short-term environmental patterns of dietary energy result in a deficit. Maintaining sufficient energy savings can help weather some of the unpredictability in other energy sources and sinks. The majority of evidence indicates that domesticated broodmares are best managed by maintaining a moderate BCS, but there remains some lack of clarity regarding how changing planes of nutrition, and even changing BCS, may have positive or negative impacts on reproductive performance and progeny success[5]. Future work should focus on investigating how dietary energy and stored energy are communicated to the gonadotropic and somatotropic axes to influence reproduction and growth[57]. The knowledge uncovered here might allow for more precise and nuanced modifications of the diet through gestation to optimize health and performance of the offspring, but also the continued reproductive performance of the broodmare.

Key points

- **Feral mares are bred and foal in the longest days, which coincides with increased nutrient availability from grazing. In mid-gestation (120-250 days of gestation), the fiber content of the forage increases and nutrient availability, as well as day length decrease. Late gestation (250-340 days of gestation) begins with low nutrient availability and short-day lengths, but rapidly increasing nutrient availability coincides with exponential fetal growth.**
- **Body condition of the mare represents her energy savings, and hence her ability to provide for a rapidly developing fetus in late gestation and reproduce in the following breeding season. In most situations it is prudent to maintain a moderate BCS (5/9) throughout gestation, with the**

understanding that during lactation and late gestation, energy partitioning will first direct resources to milk and fetal development.

FETAL NUTRITION DURING PREGNANCY

DEVELOPMENT AND ROLE OF THE PLACENTA

During the first 40 days of the embryo's life, histotrophic secretions (endometrial glands secretions) are the main source of nutrients for the embryo[4]. Briefly, the embryo enters the uterus around 6-7 days post-ovulation[58]. At this time and for the next 20-25 days, the embryo is surrounded by a capsule composed of glycoproteins which regulates the assimilation of uterine secretions[4,59]. Between 20 and 30 days, the embryo capsule disintegrates, so that the trophectoderm (precursor of placenta) is directly in contact with the endometrium[60]. Trophoblastic cells (placental epithelial cells involved in feto-maternal exchanges) develop and protrude into the endometrial glands which facilitates histotrophic nutrition prior to implantation[61,62]. Thus the embryo is solely dependent on the uterine environment for its development during this period.

The following nutrition and metabolic factors affect the uterine environment in the mare:

- Maternal obesity and excessively increased insulin resistance (knowing that insulin resistance is increased physiologically in response to gestation) in mares has been shown to increase the expression of genes and/or proteins involved in inflammation, lipid homeostasis, growth factors and cell stress in uterine secretion, endometrium and embryos[63,64]. Moreover, alterations in concentration of lipids involved in cell membrane integrity and signal transduction was observed in embryos of obese mares[63].
- Conversely, supplementing the diet of overweight mares with omega-3 fatty acids rich fat sources has been shown to increase the expression of genes involved in embryo and trophoblast development[65] and to decrease expression of proteins involved in

inflammation[64]. Nevertheless, this has not been shown to overcome adverse effects of maternal obesity.

Although there is so far little knowledge on specific nutritional needs in early gestation in the mare, the quality of maternal nutrition should not be neglected at this stage.

The placenta is a complex organ involved in gestation maintenance, feto-maternal exchange, metabolism, hormones synthesis and immunity. In the mare, two different placentas develop during gestation (**Figure 4**):

-**A transient trophoblast** (chorionic girdle) from 30 to 120-140 days of gestation[4].

-**A definitive non-invasive placenta** that forms close interdigitations (microcotyledons) with the endometrium from 40 days. Two trophoblasts are involved in feto-maternal exchanges. The hemotrophic trophoblast lines the microcotyledons in close contact with the endometrium[66] and ensures exchanges between maternal and fetal bloods. The histotrophic trophoblast is located at the basis of microcotyledons and collects uterine gland secretions. Therefore, both hemotrophic and histotrophic nutrition play essential roles for fetal development.

Of interest is that the placental microvilli lengthen and branch out throughout gestation[67] and can adapt to a certain extent to adverse maternal nutritional conditions. For instance, in moderately undernourished mares, normal fetal growth was observed[68] thanks to placental adaptations:

- increased volume of microcotyledonary vessels and
- increased expression of genes involved in amino and fatty acids catabolism as well as vitamin transport[69].

However, placental structural adaptations cannot overcome severe undernutrition[70].

DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASES (DOHaD) AND CRITICAL PERIODS OF EMBRYO AND FETAL DEVELOPMENT

The concept of Developmental Origins of Health and Diseases (DOHaD) stipulates that fetal adaptations to an adverse *in-utero* environment induce permanent changes in the fetus that are revealed as the individual ages or in the presence of an adverse postnatal environment. First demonstrated in humans[71,71,72], this phenomenon has also been shown in animal models and domestic animals[73,74], including horses[75–78].

Adverse effects on fetal and post-natal development have been shown to differ, depending on the gestational stage at which they were applied[72]. This implies the existence of *critical periods of development* that are directly correlated to the timeline of fetal organ development and maturation. Mechanisms underlying these effects involve modification of gene expression without changing of DNA structure (epigenetic mechanisms[79]) which are sensitive to the environment and can persist until adulthood.

Critical periods of development can be defined depending on the organ concerned (**Figure 5**). In the horse, by day 35, the embryo has completed most of its organogenesis[4,80] and is referred to as a fetus[4]. This time of gestation also coincides with the onset of placentation. These differences between organs have an important impact on fetal development in response to maternal feeding as the embryo/fetal organ development and maturation is dependent on the maternal environment (metabolism and nutrition). For more detailed information and references, see Figure. 6.

Although DOHaD and the importance of critical periods have been well described in animal models and some domestic species, so far, only limited data are available in horses. Nevertheless, maternal nutrition has been demonstrated to affect foal metabolism, onset of osteochondrosis and the maturation of reproductive organs (**Figure 7**)[78].

KEY POINTS

- **The horse embryo depends solely on nutrients from uterine glands until 30-40 days post-ovulation. Uterine environment may vary depending on maternal nutrition and metabolism.**
- **Obese mares may have a more inflammatory endometrial environment, which could impact embryo health and development in the first month of gestation.**
- **Organogenesis is largely completed at 40 days post-ovulation, but organs continue to mature afterwards, and critical periods of development vary between organs.**
- **Maternal environment (and nutrition) from early gestation has long term effects on offspring development.**

DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

Nutrition of the broodmare during gestation and lactation is not only important for her own health and fertility but also for the development and health of her foal. The limited information available in horses is detailed below.

<u>MATERNAL ENERGY RESTRICTION</u>

Moderate (70-80% of energy requirements) undernutrition does not appear to affect *in-utero*, nor pre-weaning postnatal growth of the foal[68,81,82]. Placental[69] (increased vascularization and nutrient transport) and maternal[68] (decreased insulin secretion following a glucose challenge, lipid mobilization) adaptive mechanisms seem to be sufficient to sustain fetal growth. However, moderate maternal undernutrition is associated with delayed testicular maturation at 12 months of age (beginning of puberty), reduced insulin sensitivity at 19 months of age, and reduced cannon width from 19 months of age[83]. Furthermore, severe undernutrition leads to *in-utero* growth retardation (IUGR)[70].

ENERGY OVERFEEDING AND OBESITY

Because horses are herbivorous, their body condition varies according to season and the nutritional availability of pasture[52]. A healthy horse in outdoor conditions will generally have a higher BCS in summer than in winter[84]. A horse is considered overweight when its BCS exceeds 6[54] (or 3.5 if using the 1-5 scale[55,56]) and obese when its BCS exceeds 7[85] (or 4 using the 1-5 scale). Obesity can also be chronic, as some horses maintain a high BCS throughout the year, with no seasonal variation[84]; see Chapter 7.

A mare can be overweight due to short-term overnutrition during pregnancy ("excess gestational fat deposition"). Alternatively, obesity can result from long-term overnutrition and/or metabolic disease. These two scenarios may have different effects on foal health and development:

- Obesity in late gestation: Overnutrition during pregnancy, leading to obesity in late gestation, does not affect foals' birthweight[81,86]. Nevertheless, reduced weight and thoracic perimeter at 2 months of age have been described when excess maternal nutrition is continuous from 2 months gestation[87]. This may be due to decreased milk production in overnourished mares during the first 2 months of lactation[87]. When overnutrition begins later (8th month of gestation), foals' growth between birth and 3 months of age was not affected[82] (later effects have not been studied).
- Long-term obesity: Maternal obesity from the time of insemination, together with decreased insulin sensitivity and increased plasma concentrations of inflammation biomarkers in late

gestation, did not affect the birth weight, nor the growth of foals when monitored until at least 18 months of age[88,89].

- Nevertheless, maternal obesity was associated with increased systemic inflammation, decreased insulin sensitivity and increased incidence of osteochondrosis in foals[88]. Maternal adiposity at the base of the tail, as measured by ultrasound, has also been positively correlated to the same measurement in 4 months old foals[90].

THE SOURCE OF ENERGY MATTERS

USE AND EFFECTS OF STARCH

Epidemiological observations demonstrated that risks for a foal to develop osteochondrosis were 11-fold higher when the broodmare's diet included 'concentrated feeds' during gestation, compared to forage only[91]. This study, however, did not consider the quantity nor the source of the concentrates given to the mares, albeit experimental studies performed using starch-rich barley as a concentrate validated this observation[68]. Nevertheless, in field conditions, forage is sometimes not available in sufficient quantity and quality to cover the mare's needs and the provision of an energy-rich concentrated feed remains a practical necessity. To decrease the potential detrimental effects of starch on foal development, starch quantity per meal and per day has to be closely monitored.

The results from several experimental studies, where the source of energy used was known can help build these recommendations:

- Study 1[68]: Mares received 1.7g of starch/kg BW per meal as barley in addition to hay and haylage, or hay and haylage only during the last 4.5 months of gestation. Mares fed with barley produced more foals affected with osteochondrosis lesions at six months of age (45%) compared to mares fed with hay and haylage only (17%). This difference was not observed at 12 and 18 months of age, with some lesions spontaneously resolved and some new lesions in different individuals[83].
- Study 2 (*MR, PCP personal communication*): Mares were fed with either a maximum of 0.75g of starch/kg BW per meal (range 0.40-0.75, n=5), or a minimum of 1.1g of starch/kg BW per meal

(range 1.1-1.6, n=5) during the last 2 months of gestation. Both groups received the same amount and type of forage during gestation. As a result, 12-month-old foals born to mares fed the high-starch meals experienced a higher incidence (80%) of osteochondrosis lesions, as compared to foals of mares fed low-starch meals (20%) (**Figure 8**). Unfortunately, foals were not monitored further.

These results indicate that excess starch provided to mares per meal has an impact on the osteoarticular development of foals. However, the role of starch source has not been fully investigated. **Regardless, for now, the authors recommend that starch+sugar intake in pregnant mares should not exceed 1g/kg of BW per meal**. Limits per day are currently unknown.

Feeding the broodmare with starch-rich concentrates may also affect colostrum quality as it has been shown to decrease the IgG colostrum concentration at birth, in comparison to mares fed with forage only[12,92]. A French epidemiological study showed that mares producing foals with osteochondrosis lesions had colostrum that was poorer in IgG compared to mares that produced foals that remained healthy[93].

USE AND EFFECTS OF FATS

Because the diets richer in starch were also more energy dense in the previously mentioned studies, discrimination between the effect of starch and that of energy content *per se* on the foals' osteoarticular development is impossible. Feeding pregnant mares with a diet rich in starch (corn, >1g starch/kg BW per meal) was shown to alter the glucose metabolism of foals during pre-weaning growth compared to a diet rich in lipids (corn oil, <0.15g starch /kg BW per meal, 14% DM fat). These results indicate that vegetable oil/**fat may be a good way to increase the energetic density of the diet** of pregnant mares, without increasing the starch content, thus limiting detrimental effects on foal's development[94].

Fatty acid composition may be important as some fatty acids have immunomodulatory properties, and could therefore affect pathways involved in fertility, but also in inflammation. Essential fatty acids are also involved in fetal neuronal development. Thus, dietary fatty acids could affect maternal/uterine environment quality and subsequently embryo and fetal development. This is particularly important for mares fed dry forage and concentrates, as omega-3 to omega-6 ratios are low in dry forage and especially in cereals, in contrast to grass and fresh forage[17,95,96]. As horses evolved grazing on fresh grass, the dietary omega-3 contents and omega-3/omega-6 ratio are most probably important, especially around conception. However, there is little confirmed information regarding the exact quantities and ratios that might be optimal.

For now, supplementing mares with fat sources rich in omega-3 fatty acids has only been shown to increase the total omega-3 and DHA transfer from the dam to the fetus at birth[97,98] and to increase lymphocyte proliferation in 7 days old foals[99]. No other effects on colostrum quality nor foal growth have been demonstrated[97–99]. The effects of maternal supplementation with omega-3 fatty acid during gestation on maternal and foal metabolism are still unknown. Both fish/algae and flaxseed oils have been studied independently but more studies are needed to compare the efficiency of both sources. For now, we recommend flaxseed oil as it is more practical.

USE AND EFFECTS OF PROTEINS

Protein needs are increased during gestation in mares. The quality of proteins, and especially the content of essential amino acids, such as lysine and threonine, is crucial for foal development[1]. It must be noted that although the role of protein excess in foals in the development of osteochondrosis lesions has been ruled out[100], to the best of our knowledge, no studies have been performed on the effect of overall protein quality fed to broodmares on the long-term health of the foals.

As an example, L-arginine is an essential amino acid during pregnancy and growth in the horse. The particular abundance of arginine in mare's milk seems to indicate that L-arginine may be needed in much larger proportions in foals than in the offspring of other species[101]. Supplementation with L-arginine (100g/day) during the last 4 months of gestation to primiparous mares improved their glucose metabolism, increased placental expression of genes involved in glucose and fatty acid transport but did not affect placental and birth weights nor growth of foals monitored until 2 months of age[48]. In a study where mares' parity was not described, supplementation with 100g of L-arginine/day from 21 days before foaling increased uterine arterial blood flow and shortened the gestational length by 12 days, without affecting the placental and foal weight at birth[102]. Effects on gestational length were, however, not observed in another study supplementing 50g of L-arginine/day in pregnant mares from 90 days before foaling[103].

Further studies are needed to determine the effect of protein deficiency or excess, as well as the effect of protein quality during gestation, on foals' long-term health. Interestingly, alteration of protein intake in other species has been shown to affect the behavior, health and lifespan of the offspring[104]. Because some amino acids can affect absorption and cell use of other amino acids, dietary amino acid contents should be checked in accordance to the known optimal ratios, which may change between the different physiological states[1].

KEY POINTS

- **Over and undernutrition of the mare are detrimental to foal's health. The energy content of the mare's feed should be considered according to mare's BCS and DMI.**
- **Forage should be the basis of a broodmare's diet.**
- **Broodmares should not receive more than 1g of starch+sugar/kg BW per meal in order to limit detrimental effects of non-structural carbohydrates on foal's metabolic and osteoarticular health.**
- **Vegetable oil/fat may be a good way to increase the energy content of the diet.**
- **The quantity (and ratio to omega- 6 fatty acids) of omega-3 fatty acids may be important.**

FEED SUPPLEMENTS: USEFUL FOR IMPROVING THE HEALTH OF THE FUTURE FOAL?

As presented in section 1.3, geographic location, season, soil factors, plant species, state of vegetative growth and fertilization/irrigation can affect the amino acid and fatty acid profiles, as well as the vitamin and mineral content of the forage. Moreover, utilizing cereal grains to provide additional energy can alter the balance between minerals. Depending on these factors, supplementation is not always needed and should be carefully thought out.

Some vitamins and minerals easily cross the placenta to be delivered to the fetus during gestation. On the other hand, some are weakly transported through the placenta and their storage in colostrum is therefore essential for the newborn foal. For instance, vitamins A and E are poorly transferred through the placenta but are concentrated in colostrum[105], while iodine is actively transferred through placenta and is also rich in colostrum and milk[106]. Some other nutrients, like copper, will be stored in the fetal liver during gestation and used during fetal growth to compensate for low milk concentrations[107].

VITAMINS

Studies on the effects of vitamin excess and deficiency on the health of foals are lacking. In other species, it has been shown that maternal imbalance in D and B-group vitamins could affect not only *in-utero* growth, but also long-term growth, metabolism diseases and behavior of the offspring[108]. As presented in section 1.3, vitamin E, D and β-carotene concentrations are high in fresh grass in spring and summer, implying that supplementation may not be needed if mares are kept in pasture and bred between spring and summer[21]. Conversely, mares bred out of season or fed dried forage would benefit from vitamin supplementation as observed in the following studies.

Natural vitamin E (RRR-α-tocopherol) oral supplementation, fed above the NRC requirements (200-300%)[1,] has been shown to increase the concentration of vitamin E in colostrum, milk and plasma of neonatal foals as well as the immunoglobulin concentration in colostrum and plasma of 3 days old foals, compared to mares fed a diet deficient in vitamin E (15-30% of NRC requirements)[109]. Moreover, oral supplementation with β-carotene (1000mg/day) to mares fed with hay and concentrates from 2 weeks before foaling, increased the concentration of β-carotene in colostrum and plasma of foals at 1 day of age[110]. To our knowledge, long-term effects of these supplements on foal health and development have not been studied yet. The observed increased insemination success with β-carotene supplementation, however, may imply an effect on the uterine environment, and then, on embryo and fetal development[110].

MINERALS AND MICROMINERALS

CALCIUM AND PHOSPHORUS

The effects of an inverse calcium:phosphorus ratio during pregnancy have not been studied but may negatively impact the foal's bone and articular development. Mares consuming diets providing 80% of the NRC requirement for calcium (Ca:P = 1.1, lower limit) during late gestation gave birth to foals with thinner and weaker cannon bones which persisted through the period of observation (10 months of age)[111].

COPPER

Copper is a micromineral essential for the development of cartilage and bone. Although pregnant mare copper supplementation above NRC requirements (200-300%) have been shown to decrease the prevalence of articular cartilage lesions in growing foals[112,113], there is, so far, no substantial evidence that pregnant mares should be supplemented over the recommendations if the diet is correctly balanced (especially the Cu:Zn ratio)[1,114]. Maternal copper supplementation does not affect milk copper concentration nor foal plasma concentration but increases foal liver copper storage[107]. These results imply that fetal liver copper storage is essential for foal's osteoarticular health, especially because supplementing the foal after birth will not counter the detrimental effects of *in-utero* deficiencies[113].

SELENIUM

Selenium deficiencies during gestation have been associated with white muscle disease in foals, a myodegenerative pathology, affecting skeletal and cardiac muscles and leading to the death of the foal in most cases[115]. The form of selenium distributed to pregnant mares is potentially important, as inorganic and organic selenium are not absorbed and incorporated into body tissues equally[116,117]. In fact, supplementing the mares with selenium yeast in the 2 last months of gestation increased the expression of genes involved in proliferation and cellular immune response in lymphocytes of growing foals, compared to mares supplemented with sodium selenite[118], which may imply improved foal immunity. Moreover, supplementation with selenium yeast (0.65ppm *vs*. 0.35ppm Se in total diet [650% *vs*. 350% of NRC requirements]) during the last 4 months of gestation has been shown to increase the selenium concentration in the plasma and muscle of foals, but without affecting the glutathione peroxidase activity in foal's plasma[121]. Special caution must be paid to selenium excess as optimal range is narrow, i.e., the level of toxicity close to the recommended amounts (0.2mg/100kg BW is recommended in pregnancy, safe upper limit is considered to be 1mg/100kg BW[1]). Organic forms of selenium may have a stronger beneficial effect on foal development compared to inorganic selenium, but more studies are needed to confirm these effects.

IODINE

Thyroid function is involved in metabolism, bone development and growth. Foals have a very high iodine serum concentration at birth that slowly decreases during growth[106], which correlates to triiodothyronine (T3) and thyroxine (T4) plasma concentrations[88]. Transplacental iodine transport may therefore be efficient. Strong excess or deficiency in iodine of the maternal diet have been linked to congenital hypothyroidism in foals, which can be characterized by thyroid gland hyperplasia and musculoskeletal abnormalities in foals as well as increased gestational length[119]. More work is needed to study the effects of iodine intake on long-term foal development. Seaweed supplementation can cause iodine excess, therefore, iodine levels in seaweed supplements have to be carefully considered before feeding seaweed to pregnant mares.

OTHERS

Other minerals and microminerals are also involved in metabolism regulation (chromium), inflammation and oxidation (iron), bone and teeth development (fluorine) and their imbalances may also impact the long-term health of the offspring. Mineral supplementation should be developed in accordance with the balance between minerals and microminerals, as it can affect their absorption and cell use[1]. More work is needed to develop specific recommendations for pregnant mares.

USE OF PROBIOTICS

The intestinal microbiota in early life can impact metabolic health, growth and behavior of the individual[120]. The equine microbiota influences the risk of resistance to endo-parasites[121], colic[122] and metabolic syndrome[123]. However, few studies have focused on the effect of the mare's gut microbiota on foal health. Few prebiotics have been tested in pregnant mares so far, despite a large number of yeast and bacteria strains ~~(genetic variants)~~ available on the market. Effects observed from one strain of probiotics cannot be extrapolated to other strains. Safety of strains and effective dosing are unknown, which calls for a cautious use of these nutraceuticals in mares (see also Chapter 2 and 10).

Pregnant mare probiotic supplementation, however, has shown some beneficial effects on foal health and development. Pregnant mares were supplemented with live yeast (*S. cerevisiae* CNCM-I1079, 7.10^{10}CFU/day) from 8 days before to 4 days after foaling. Their foals had a decreased quantity of *E. coli* and enterobacteria in the feces at 10 days of age, an increased proportion of normal-looking feces and a tendency to an increase in the average daily gain from birth until 20 days of age[124]. In another study, the supplementation of pregnant mares with fermented feed products from 45 days before foaling until 60 days after did not affect the fecal pH of mares, nor the fecal concentration of culturable bacteria, but increased the maternal fecal proportion of acetate. Moreover, foals born to supplemented mares had an earlier establishment of gut microbiota and gut function, possibly leading to an increased weight between 19 and 60 days of age[125].

In conclusion, studies on the long-term effect of probiotics during pregnancy and/or growth are needed to help develop recommendations on the use of these supplements in breeding horses. It is also worth noting that safety in pregnant animals has not been tested for most supplements.

The effects of other nutritional supplements on other aspects, such as muscular and cardiovascular development as well as bone strength and resistance remain to be studied in the horse.

KEY POINTS

- **Supplementation must be carefully thought out as some supplements have not been tested for safety.**
- **Vitamins and minerals in excess can be as detrimental as deficiencies for the health of both the mare and the foal.**
- **Nutritional balance is important when supplementing amino acids, vitamins and minerals.**
- **More studies are needed to confirm beneficial effects of supplements in pregnant mares on long-term health of the foals.**

CONCLUSION

Although the basic recommendations for broodmare nutrition are known, as described in the first part of this chapter, there are many variations in the needs of mares according to season and possibly breed, age or even parity. Combined with the discordant studies, more research is therefore needed. The mare's diet can positively or negatively affect her foal's health. Limiting intake of especially starch and sugar rich concentrates and maximizing the intake of forage may help prevent non-transmittable diseases such as osteochondrosis and possibly, in longer term, equine metabolic syndrome. Long term studies are urgently needed to answer these questions.

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FIGURE LEGENDS

Figure 1. Pregnant lactating and non-lactating mares' energy and protein requirements relative to maintenance, according to time of gestation and compared with day length. The symbols represent the digestible energy or crude protein requirements relative to maintenance of broodmares with or without a foal at their side during gestation as determined using the French, German, and North American feeding standards for horses[1–3]. The lines represent the calculated day length at any point during gestation for foals due in February, April, or June for a northern latitude of 35 degrees, as an indicator of ambient temperature and forage availability. The colored regions represent important stages of gestation, represented by pre-implantation, endometrial cups and organogenesis (0-120 d),

moderate fetal and organ growth (120-240 d), and rapid fetal development (240-340 d). Those interpreting this figure should consider the synchrony between day length, a proxy for the broodmare's environment, and energy and nutrient requirements during gestation. (Data from references 1-3.)

Figure 2. Evolution of glucose metabolism during pregnancy in mares. A. Changes in plasma glucose concentration after a meal in non-pregnant (black, n=4) and pregnant mares at <270 days (green, n=6) or >270 days (red, n=5) pregnancy. Pregnant and especially late pregnant mares are more efficient in absorbing sugar ingested. **B. Changes in plasma insulin concentration after a meal in non-pregnant (black) and pregnant mares at <270 days (green) or >270 days (red) gestation.** Pregnant mares produce more insulin in response to plasma glucose increase, but this response does not change between early and late gestation. **C. Insulin sensitivity in early (<155 days, n=12) and late (>280 days, n=37) gestation in French-Anglo Arabian mares.** Insulin sensitivity decreases as gestation progresses. ([A,B] adapted from Fowden AL, Comline RS, Silver M. Insulin secretion and carbohydrate metabolism during pregnancy in the mare. *Equine Veterinary Journal*. 1984;16(4):239-246. doi:10.1111/j.2042-3306.1984.tb01919.x; [C] from Robles M, Couturier-Tarrade A, Derisoud E, et al. Effects of dietary arginine supplementation in pregnant mares on maternal metabolism, placental structure and function and foal growth. *Scientific reports*. 2019;9(1):1–19; with permission.)

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Figure 3. Evolution of the mare's metabolism during gestation. Changes in carbohydrate, protein and lipid metabolism allow the mare to provide for the needs of the fetus while having limited food availability in winter.

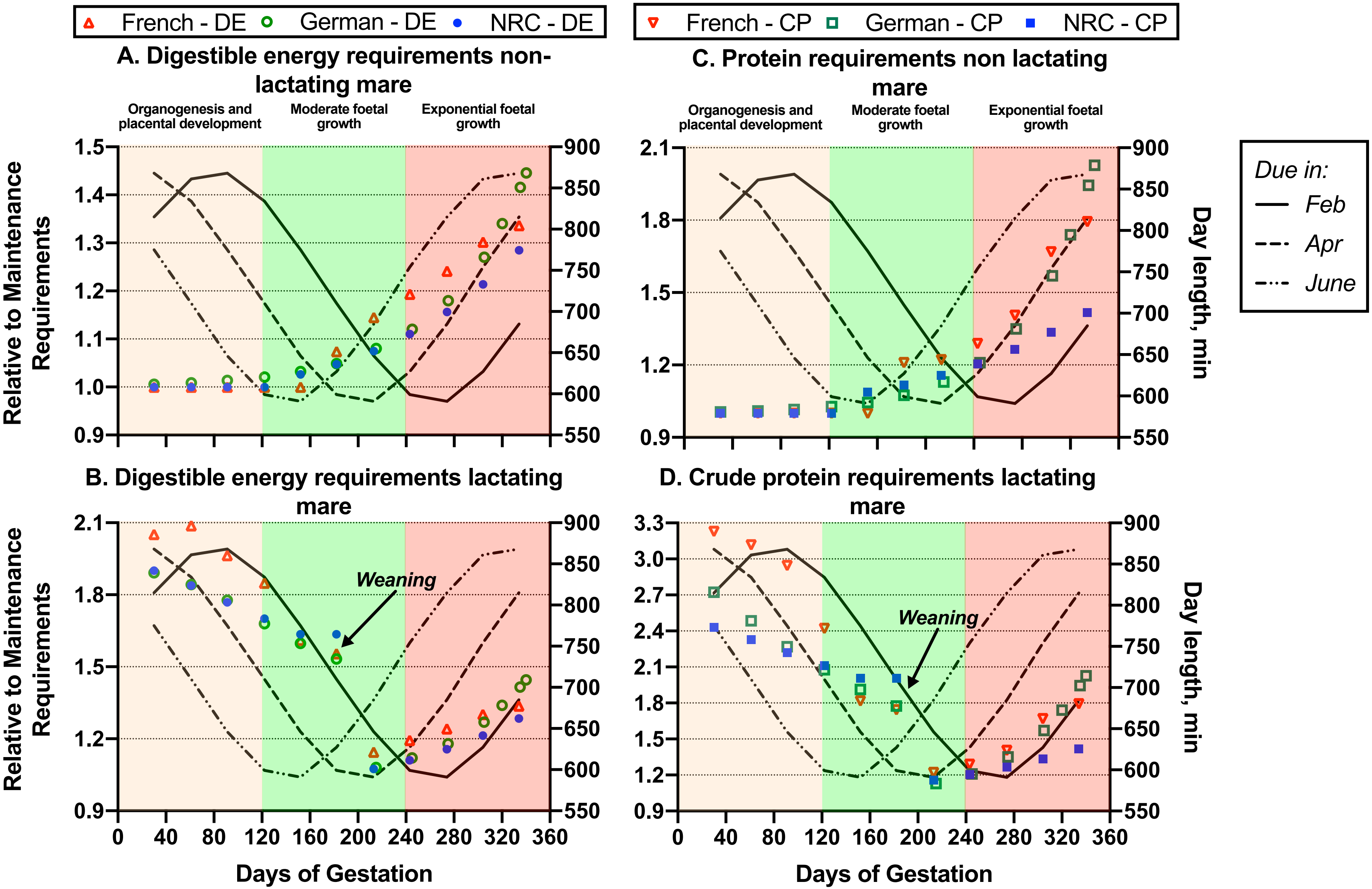
Figure 4. Transient invasive and definitive non-invasive placentas. **A. Development and roles of the chorionic girdles. B. Structure of the definitive chorioallantois.** **([A] adapted from** Allen WR, Wilsher S. A Review of Implantation and Early Placentation in the Mare. *Placenta*. 2009;30(12):1005-1015; and Allen WR, Stewart F. Equine placentation. *Reproduction, Fertility and Development*. 2001;13(8):623–634; and Wooding FBP, Burton G. *Comparative Placentation: Structures, Functions and Evolution*., Springer, 2008; [B] from Steven DH, Samuel CA. Anatomy of the placetal barrier in the mare. *J Reprod Fert*. 1975;Suppl. 23:579-582; with permission.)

Figure 5. The timing of organ development in the equine embryo and fetus. Horizontal lines indicate specific day of gestation while vertical arrows indicate periods of gestation where observations were made.

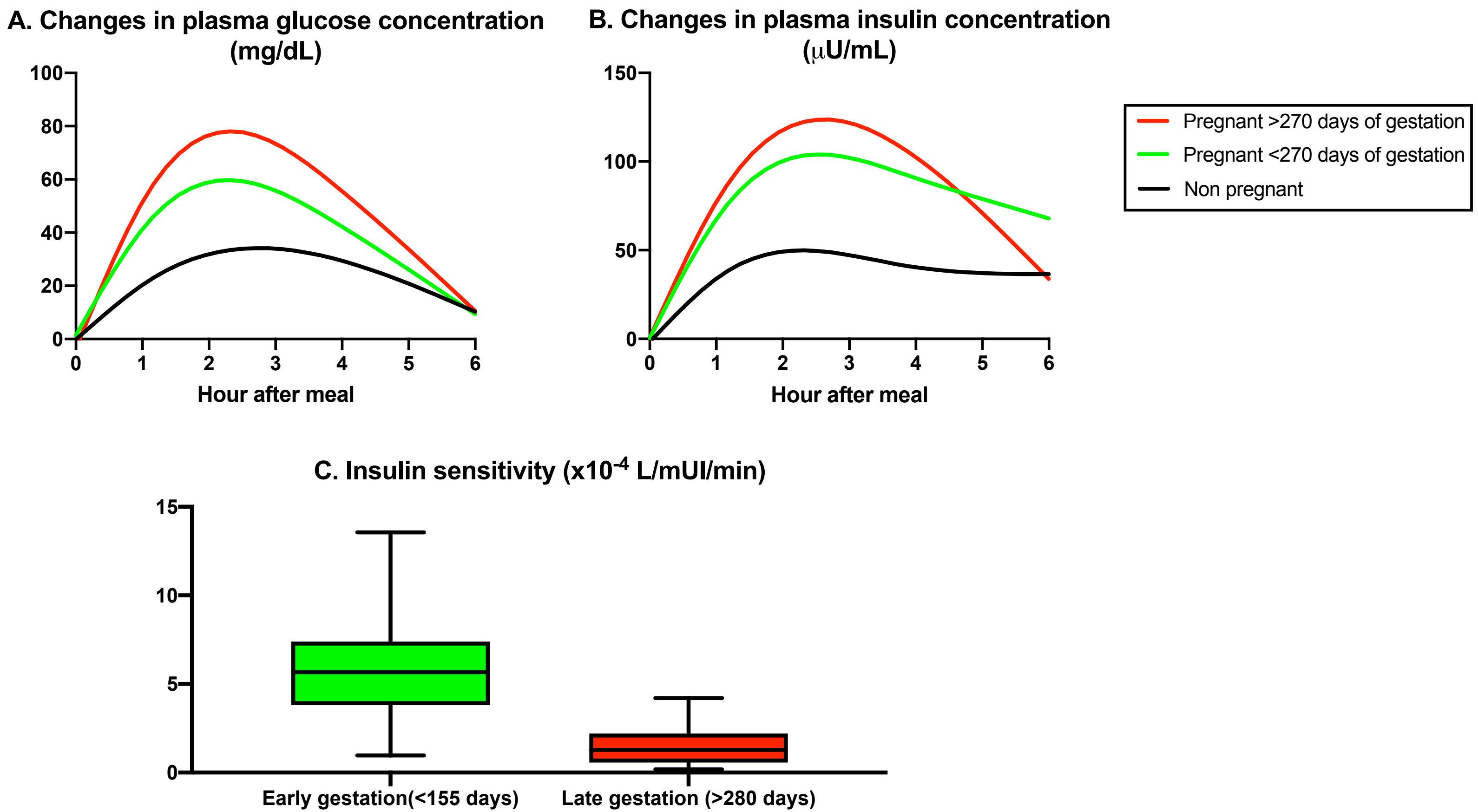
Figure 6. The timing of organ development in the equine embryo and fetus, detailed version. Each organ is separated by horizontal lines and represented by an icon. Page 1 features teeth, pancreas, liver and heart. Page 2 features bones and cartilage, muscles, gonads and a summary of neurons, lungs, pituitary gland, mammary glands and kidneys. For each organ, a timeline expressed in days post-ovulation presents the major developmental events, described in brown boxes. Detailed results in fetal insulin production are also provided for pancreas (tables and figures). For muscles, the blue line over the timeline highlight a period more than a set point. References are written directly in the figure for clarity.

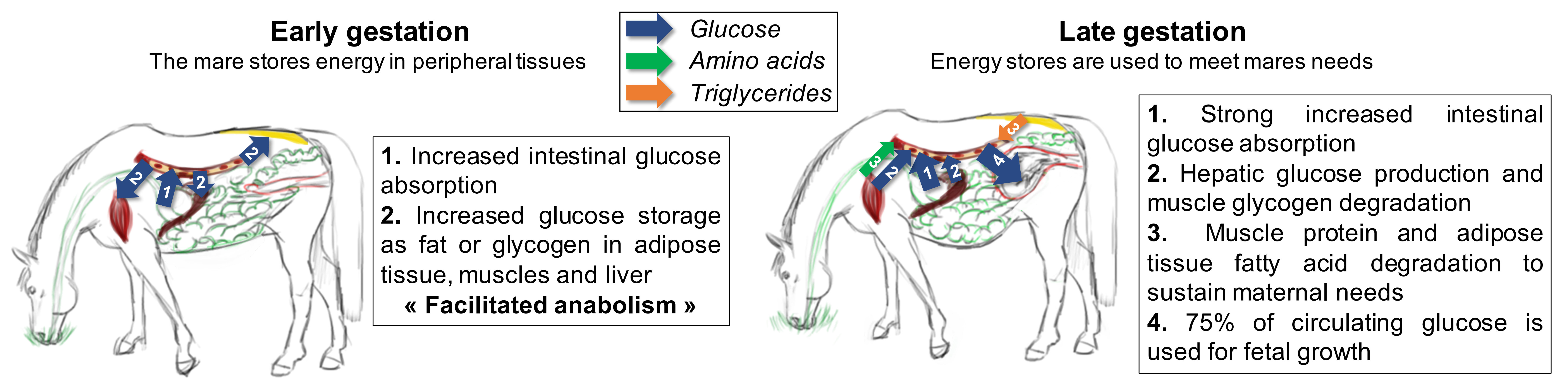
Figure 7. The principles of the Developmental Origins of Health and Disease in the horse.

Figure 8. The incidence of osteochondrosis at 12 months of age was higher in foals born to mares fed with high-starch meals (>110g/100kg BW) compared to foals born to mares fed with low-starch meals (<75g/100kg BW) (*MR, PCP personal communication*).

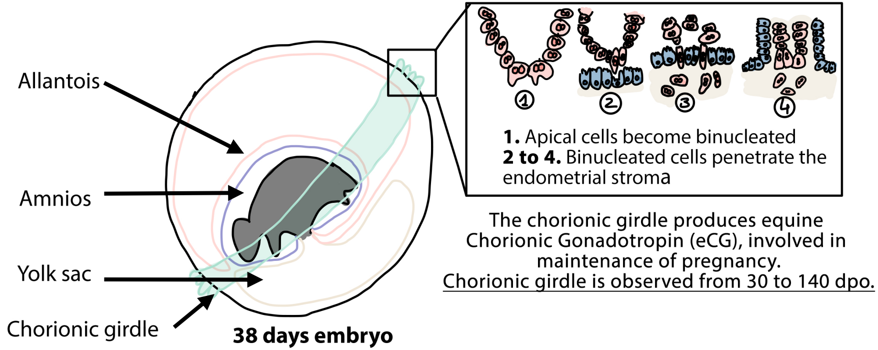


French - DE
German - DE
NRC - DE
French - CP
German - CP
NRC - CP
A. Digestible energy requirements non-lactating mare
Organogenesis and placental development
Moderate foetal growth
Exponential foetal growth
C. Protein requirements non lactating mare
B. Digestible energy requirements lactating mare
D. Crude protein requirements lactating mare
Weaning
Relative to Maintenance Requirements
Day length, min
Days of Gestation
Due in:
Feb
Apr
June

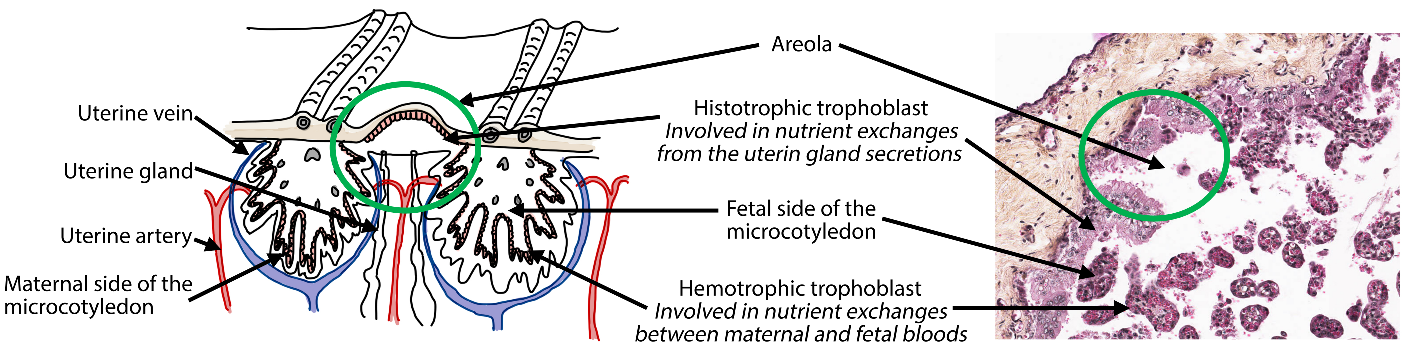

A. Changes in plasma glucose concentration (mg/dL)
100
80
60
40
20
0
0 1 2 3 4 5 6
Hour after meal
B. Changes in plasma insulin concentration (μU/mL)
150
100
50
0
0 1 2 3 4 5 6
Hour after meal
Pregnant >270 days of gestation
Pregnant <270 days of gestation
Non pregnant
C. Insulin sensitivity (x10-4 L/mUI/min)
15
10
5
0
Early gestation(<155 days)
Late gestation (>280 days)

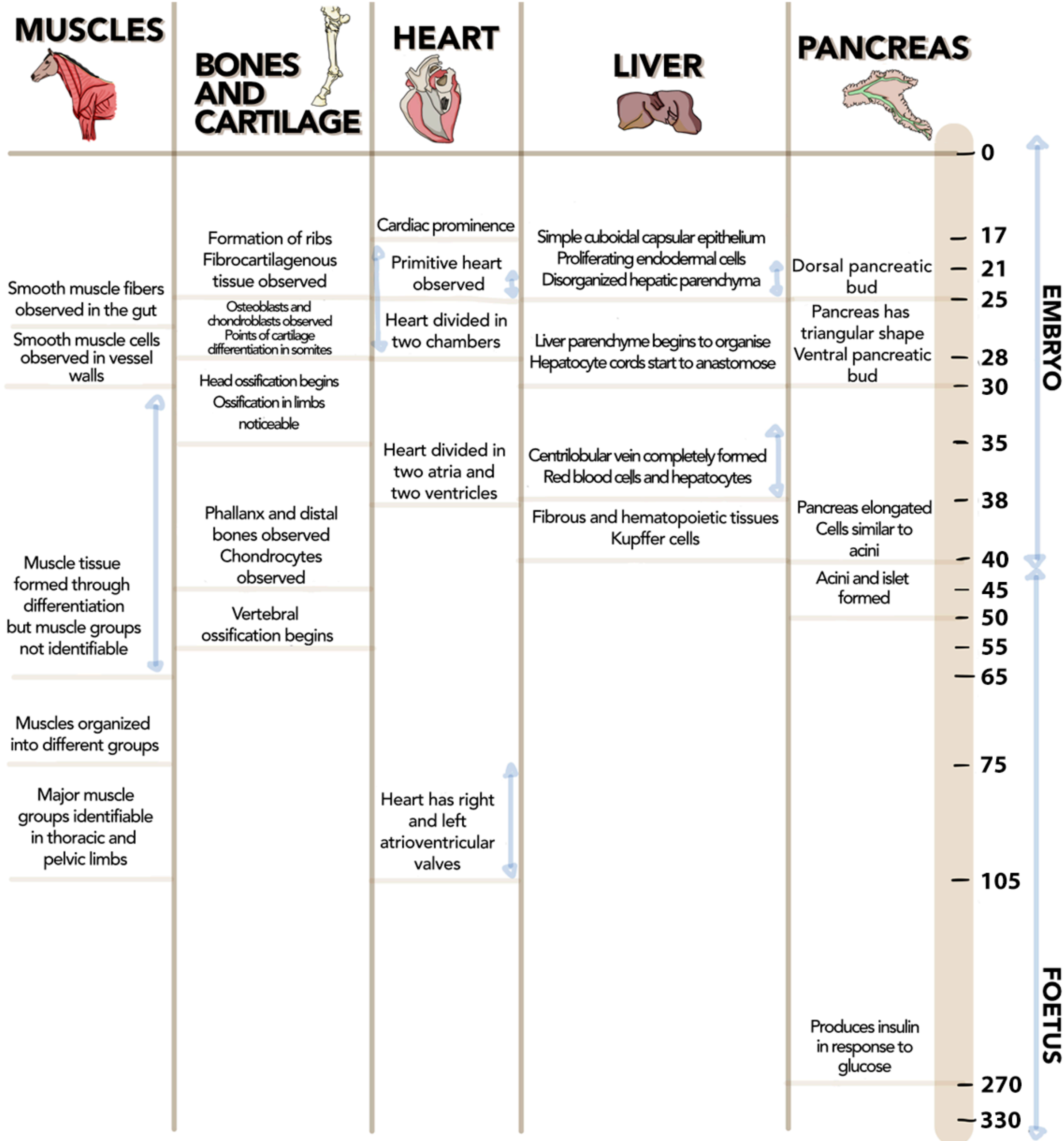

Early gestation
The mare stores energy in peripheral tissues
Glucose
Amino acids
Triglycerides
Late gestation
Energy stores are used to meet mares needs
1. Increased intestinal glucose absorption
2. Increased glucose storage as fat or glycogen in adipose tissue, muscles and liver
« Facilitated anabolism »
1. Strong increased intestinal glucose absorption
2. Hepatic glucose production and muscle glycogen degradation
3. Muscle protein and adipose tissue fatty acid degradation to sustain maternal needs
4. 75% of circulating glucose is used for fetal growth

A. The transient invasive placenta



B. The definitive non-invasive placenta

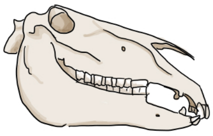




MUSCLES
BONES AND CARTILAGE
HEART
LIVER
PANCREAS
0
17
21
25
28
30
35
38
40
45
50
55
65
75
105
270
330
EMBRYO
FOETUS
Smooth muscle fibers observed in the gut
Smooth muscle cells observed in vessel walls
Muscle tissue formed through differentiation but muscle groups not identifiable
Muscles organized into different groups
Major muscle groups identifiable in thoracic and pelvic limbs
Formation of ribs
Fibrocartilagenous tissue observed
Osteoblasts and chondroblasts observed
Points of cartilage differentiation in somites
Head ossification begins
Ossification in limbs noticeable
Phallanx and distal bones observed
Chondrocytes observed
Vertebral ossification begins
Cardiac prominence
Primitive heart observed
Heart divided in two chambers
Heart divided in two atria and two ventricles
Heart has right and left atrioventricular valves
Simple cuboidal capsular epithelium
Proliferating endodermal cells
Disorganized hepatic parenchyma
Liver parenchyme begins to organise
Hepatocyte cords start to anastomose
Centrilobular vein completely formed
Red blood cells and hepatocytes
Fibrous and hematopoietic tissues
Kupffer cells
Dorsal pancreatic bud
Pancreas has triangular shape
Ventral pancreatic bud
Pancreas elongated
Cells similar to acini
Acini and islet formed
Produces insulin in response to glucose

EQUINE FETAL DEVELOPMENT

TEETH



0 — **120** — **140** — **160** — **220**

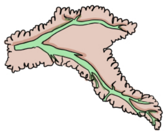
120
- Ossification of alveolar cavities
- Dental germs in the alveolar groove
- Alveolar septa development

140
- Early appearance of P3 dental germ (140)
- Cusps of P3 visible
- P2, P3, P4 germs visible (146)

160–220
Appearance of incisor teeth germ (160-224)

Soana et al (1999). The teeth of the horse: evolution and anatomo-morphological and radiographic study of their development in the foetus

PANCREAS



0 — **25** — **30** — **40** — **50**

25
- Appearance of the dorsal pancreatic bud
- Strong Oct4 staining

30
- Pancreas as a triangular shape
- Located between intestinal loops
- Ventral pancreatic buds form parts of pancreatic heads

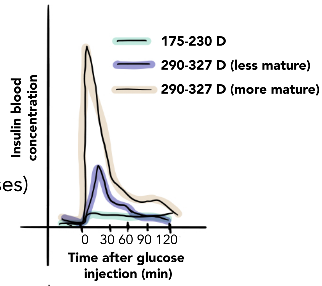
40
- Pancreas elongated
- Cells similar to acini

50
- Acini and pancreatic islets formed

Rodrigues et al (2014). Prénatal development of the digestive system in the horse

	175-230 DAYS	290-327 DAYS
Basal insulin (μU/mL)	6 ± 1.1	9.05 ± 1.4
Basal glucose (nmol/L)	2.79 ± 0.36	3.09 ± 0.22
Insulin after glucose infusion	No effect	Increased insulin release (even more in more mature fetuses)

INSULIN RELEASE AFTER GLUCOSE INJECTION

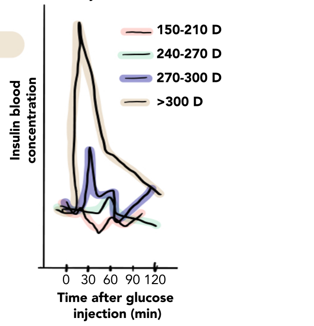


Less mature => cortisol < 15ng/mL

More mature => cortisol > 15ng/mL

Fowden et al (2005). Maturation of pancreatic b-cell function in the foetal horse during late gestation

	150-210 DAYS	240-270 DAYS	270-300 DAYS	>300 DAYS
Basal insulin (μU/mL)	8.0 ± 1.0	7.0 ± 1.5	6.5 ± 1.0	9.0 ± 2.0
Basal glucose (nmol/L)	2.41 ± 0.19	3.09 ± 0.24	2.59 ± 0.21	2.77 ± 0.33
Insulin after glucose infusion	No effect	No effect	Small rise	High rise



Fowden and Silver (1980). Pancreatic b-cell function in the foetal foal and mare

+ From 260 days, equine pancreatic α cells are functional but are unresponsive to variations in glycaemia until after birth

Fowden et al (1999). Pancreatic a-cell function in the foetal foal during late gestation

LIVER

0 — **21-25** — **30** — **35-38** — **40**

21-25
- Simple cuboidal capsular epithelium (21)
- Proliferating endodermal cells (21)
- Disorganized hepatic parenchyma (25)
- Hepatoblasts (25)
- Strong Oct4 staining

30
- Liver parenchyma begins to organise
- Hepatocyte cords start to anastomose

35-38
- Centrilobular vein completely formed
- Red blood cells and hepatocytes

40
- Fibrous and hematopoietic tissues
- Kupffer cells
- Oct4 restricted to the hepatocytes

Rodrigues et al (2014). Prenatal development of the digestive system in the horse
Franciolli et al (2011). Characteristics of the equine embryo from days 15 to 107 of pregnancy

The liver is an active site of hematopoiesis at 100 days (but maybe before)

Battista et al (2014). Haematopoiesis in the equine foetal liver suggests immune preparedness

HEART

0 — **17-19** — **24-25** — **21-28** — **38** — **75-115**

17-19: Cardiac prominence

24-25: Primitive heart observed

Beating and discernible from 20 days in the ventral quadrant

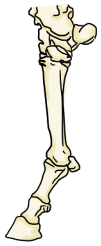
21-28: Heart divided in two chambers

38: Heart divided in two atria and two ventricles

75-115: Heart has right and left atrioventricular valves

Rodrigues et al (2014). Prenatal development of the digestive system in the horse
Franciolli et al (2011). Characteristics of the equine embryo from days 15 to 107 of pregnancy
Allen and Wilsher (2009). A review of implantation and early placentation in the mare
Rodrigues et al (2014). Embryonic and fetal development of the cardio respiratory apparatus in horses from 20 to 115 days of gestation

BONES AND CARTILAGE



0 — 25 — 30 — 35 — 45 — 55

- **25:**
  - Formation of ribs
  - Fibrocartilagenous tissue observed
- **30:**
  - Osteoblasts, chondroblasts and fibroblasts observed
  - Points of cartilage differentiation in somites
- **35:**
  - Head ossification begins
  - Ossification in limbs noticeable
- **45:**
  - Phallanx and distal bones observed
  - Chondrocytes observed
- **55:** Vertebral ossification begins

Barreto et al (2016). Organogenesis of the musculoskeletal system in horse embryo and early fetuses
Franciolli et al (2011). Characteristics of the equine embryo and fetus from days 15 to 107 of pregnancy
Acker et al (2001). Morphologic stages of the equine embryo proper on day 17 to 40 after ovulation

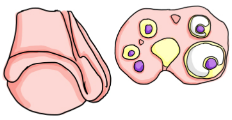
MUSCLES

0 — 28 — 30 — 65 — 75 — 105

- **28:** Smooth muscle fibers observed in the gut
- **30:** Smooth muscle cells observed in vessel walls
- **30–65:** Muscle tissue is formed through differentiation but muscle groups are not identifiable
- **75:** Muscles organised into different groups
- **105:** Major muscle groups identifiable in thoracic and pelvic limbs

Barreto et al (2016). Organogenesis of the musculoskeletal system in horse embryo and early fetuses

GONADS



0 — 25 — 270

- **25:** Ovary containing primordial follicles and unorganized primordial cells is observed
- **270:** Inguinal migration begins

+ Fetal gonads grow rapidely and peak between the 6th and 8th months of gestation when they reach the weight of adult gonads before regressing

Cole and Hart (1933). The development and hormonal content of fetal horse gonads.
Sakai (1955). Studies on the development of the embryonic ovary in swine, cattle and horse.
Franciolli et al (2016). Characteristics of the equine embryo and fetus from days 15 to 107 of pregnancy
Bergin et al (1970). A developmental concept of equine cryptorchism

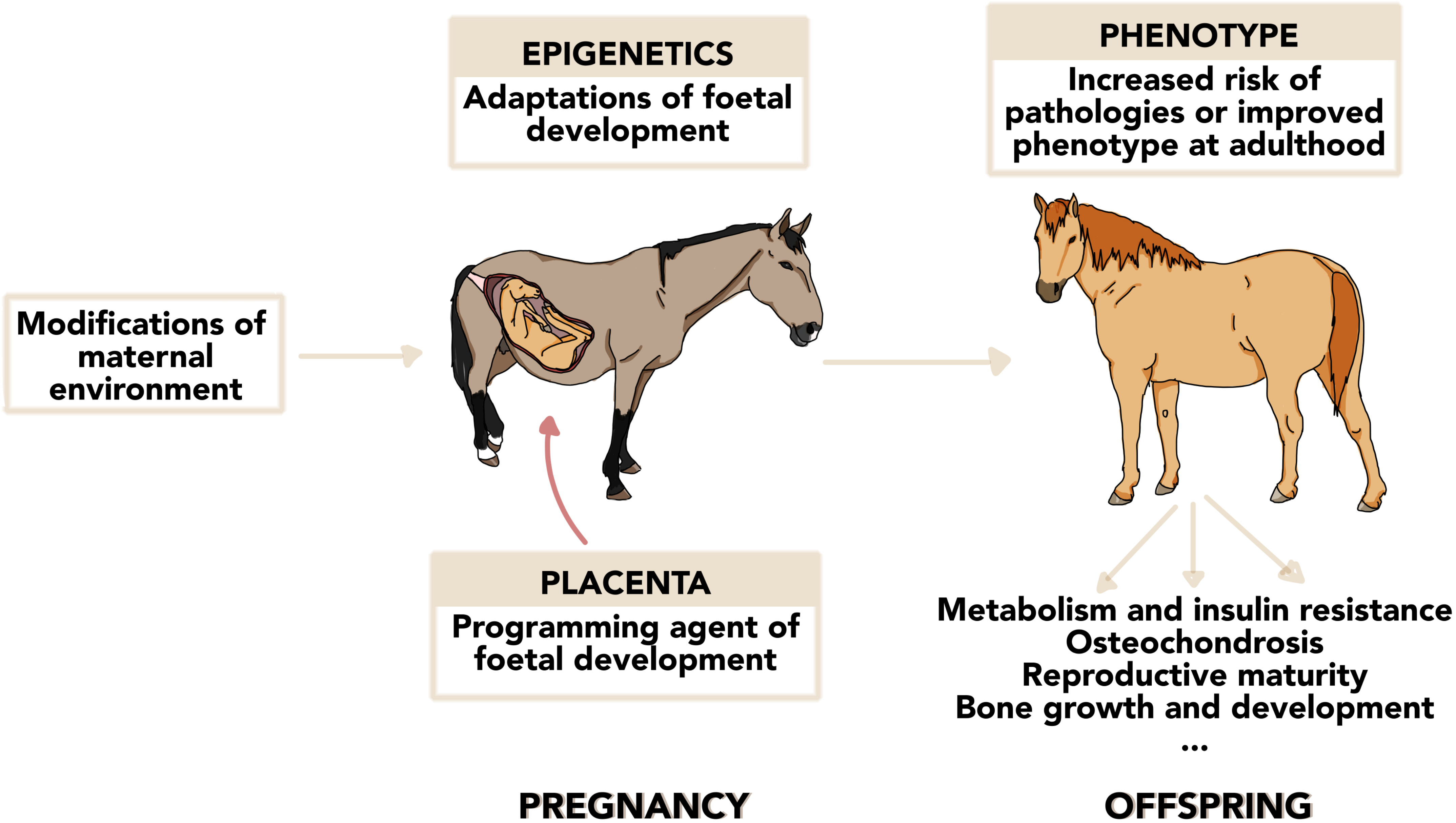
MISCELLANEOUS

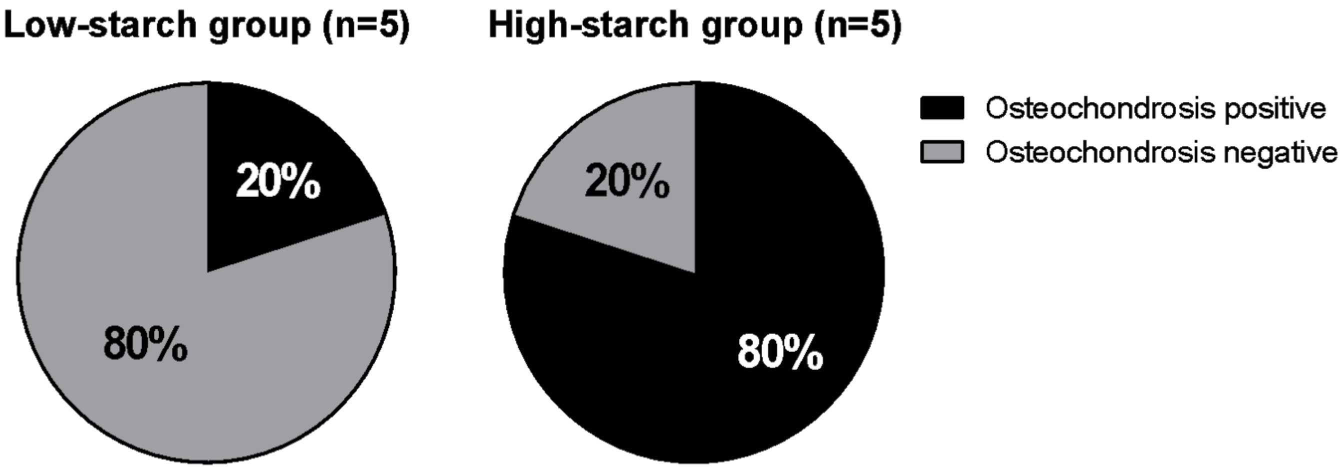
- Onset of neurulation at 13 days
- Lung buds are observable at 24 days
- Formation of the pituitary gland between 31 and 48 days (depending on the study)
- Formation of the mammary gland at 80 days

0 — 22 — 26 — 28 — 30

- **22:**
  - Urogenital ridges observed
  - Pronephric gut observed
  - Nephric vesicles contain tubules
- **26:** Glomeruli observed
- **28:**
  - Ureter identified
  - Mesonephric vesicles opened in mesonephric ducts
- **30:** Mesonephroi fully formed

Acker et al (2001). Morphologic stages of the equine embryo proper on days 17 to 40 of ovulation
Franciolli et al (2016). Characteristics of the equine embryo and fetus from days 15 to 107 or pregnancy
Gaivao et al (2014). Gastrulation and the establishment of the three germ layers in the early horse conceptus


EPIGENETICS
Adaptations of foetal development
PHENOTYPE
Increased risk of pathologies or improved phenotype at adulthood
Modifications of maternal environment
PLACENTA
Programming agent of foetal development
Metabolism and insulin resistance
Osteochondrosis
Reproductive maturity
Bone growth and development
...
PREGNANCY
OFFSPRING


Low-starch group (n=5)
20%
80%
High-starch group (n=5)
20%
80%
Osteochondrosis positive
Osteochondrosis negative