Placenta 64 (2018) 44–52

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Placental function and structure at term is altered in broodmares fed with cereals from mid-gestation

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ARTICLE INFO

Article history:
Received 8 August 2017
Received in revised form
6 February 2018
Accepted 7 February 2018

ABSTRACT

Introduction: Feeding pregnant broodmares with cereal concentrates has been shown to increase maternal insulin resistance and affect foal metabolism in the short and long-term. These effects are likely to be mediated by the placenta. Here, we investigated feto-placental biometry and placental structure and function at term in mares fed with or without cereals concentrates.

Material and methods: From 7 months of gestation, 22 multiparous mares were fed forage only (group F (n = 12)) or received forage and cracked barley (group B (n = 10)) until foaling. Foals and placentas were weighed and placental samples were collected above the umbilical cord insertion at birth. Placental histological structure was studied by stereology. A RNAseq analysis was performed on 9 placentas of each group. Enrichment of gene sets was analysed using the Gene Set Enrichment Analysis (GSEA) software using the KEGG and GO databases.

Results: No difference in feto-placental biometry was observed between groups. The volume of microcotyledonary vessels was decreased in B placentas and the vascular wall of allantoic arterioles was thickened. Gene sets involved in neutral amino acids, folate and anions transport and fatty acids, cholesterol and folate degradation were down-regulated while gene sets involved in RNA expression, inflammation and vascularisation were up-regulated in B placentas.

Conclusion: Feeding pregnant mares with concentrates from mid-gestation alters the placental function and structure as observed in other species in cases of maternal insulin resistance.

1. Introduction

Since the early nineties, epidemiological studies in the Human species and experimental studies in model animals demonstrated that alterations of the maternal environment from periconception affects offspring development and health at adulthood [1]. This concept, called Developmental Origins of Health and Diseases (DOHaD), has also been demonstrated in the equine species [2].

In the horse, placentation is diffuse and epitheliochorial. The haemotrophic exchanges between maternal and fetal blood occur through the placental microcotyledons, that are intensely branched vascular structures covered with a haemotrophic trophoblast. The microcotyledons form interdigitations with the maternal endometrium to maximize nutrient exchanges. Additionally, histotrophic exchanges are mediated by pseudo-stratified trophoblastic cells specialized in the transfer of nutrients secreted by the uterine glands and take place across areolae located in-between the microcotyledons [3–5].

In the equine industry, breeders often feed broodmares with cereals in late pregnancy. In two previous studies, we demonstrated that feeding broodmares with cereals from mid-gestation increases maternal insulin resistance and the development of osteochondrosis in the foals, with potential detrimental effects on the horse industry economy [6,7]. Nevertheless, no studies have yet been conducted to understand the effect on the structure and function of the placenta. The present study follows up these previous works and investigates the effects of feeding broodmares with a moderate

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https://doi.org/10.1016/j.placenta.2018.02.003
0143-4004/

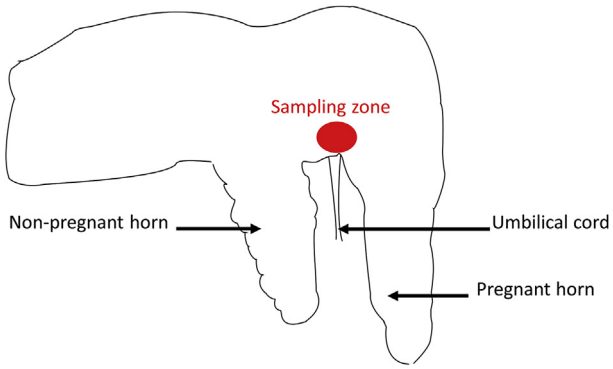

Fig. 1. Schematic representation of placental sampling zone.

amount of cereals from mid-gestation on the structure and function of the placenta at term. Placentas were measured at delivery and their structure was analysed using the stereology method and the ImageJ software. Placental function was assessed through the analysis of gene expression after RNA sequencing.

2. Materials and methods

2.1. Animals

The animal studies received ethical approval from the local ethics committee (« Comité Régional d'Ethique pour l'Expérimentation Animale du Limousin ») under protocol number 5-2013-5.

Twenty-two multiparous Saddlebred mares (median age 9y; range 6-19y) were fed cracked barley and forage (B, n = 10) or forage only (F, n = 12) from the 7th month of gestation until foaling. Management of mares from insemination to parturition has been previously described by Peugnet et al. [6]. In the present work, compared to previously presented [6], 2 mares (1 of each group) were removed from the results analysis due to pathology (metabolic syndrome) and age (25y old).

2.2. Placental biometry

Before suckling, foals were weighed. After delivery, the placenta (allantochorion) was immediately weighed. Placental efficiency was also determined by calculating the foal birthweight to placenta weight ratio. Placentas were laid in the "F" configuration, revealing both uterine horns and the uterine body. A clear Plexiglas sheet marked with 10cmx10 cm squares was applied above the placenta and photographed. The placental gross surface was subsequently measured on the photographs using the ImageJ® software (National Institute of Health) as previously described [8]. Placental volume was evaluated using a graduated water container, by measuring the volume of water displaced after immersing the placenta into the water.

Placental samples including the chorionic villi and the underlaying allantoic membrane were collected within 30 min of delivery above the umbilical cord insertion (Fig. 1). Aliquots (N = 3 for each procedure) were either fixed in 4% formaldehyde for histological analysis (1 cm^2 samples) or snap frozen in liquid nitrogen and stored at −80 °C for transcriptomic analysis (3 mm^2 samples).

2.3. Histological and stereological analyses

Placental samples were embedded in paraffin. Sections (7 μm) were stained with hematoxylin/eosin for stereological analysis and scanned using NanoZoomer Digital Pathology® (Hamamatsu Photonics).

Surface densities (Sv) and Volume fractions (Vv) of the different components of the allantochorion, *i.e.*, microcotyledons and allantois, were quantified by One stop stereology using the Mercator® software (ExploraNova, France, [9]) for 11 placentas in the F group and 9 placentas in the B group.

As shown in Fig. 2, the components measured were:

- **In the microcotyledonary region:** haemotrophic trophoblast, microcotyledonary vessels, connective tissue and microcotyledons as the sum of all microcotyledonary components.
- **In the allantoic region:** histotrophic trophoblast and allantoic vessels. The chorionic mesoderm and allantoic connective tissue were merged as allantoic connective tissue.

Vv and Sv were multiplied by the total volume of the placenta to obtain an estimation of the absolute volume (cm^3) and surface (cm^2) of the components of the allantochorion.

For each placenta, 12 allantoic arterioles were randomly selected. The surface area of the lumen (calculated using transverse

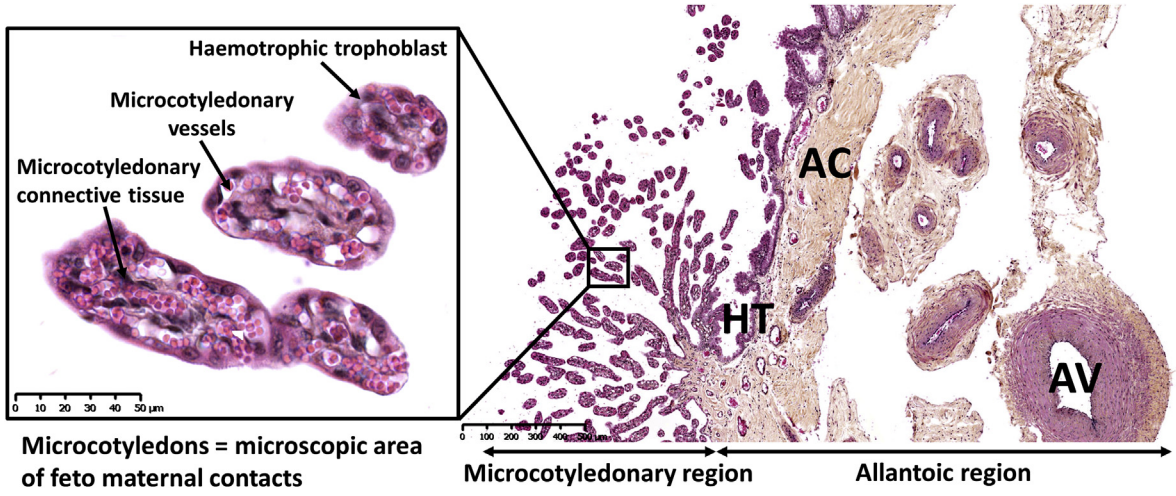

Fig. 2. Structure of the horse placenta at term and placental components measured by stereology.
AC: Allantoic connective tissue, AV: Allantoic vessel, HT: Histotrophic trophoblast.