

Effects of dietary arginine supplementation to primiparous mares in the last third of gestation on foal birthweight and placental function

Emilie Derisoud, Morgane Robles, Audrey Geeverding, Josiane Aioun, C. Dubois, Christophe Richard, Michèle Dahirel, Julianne Calvez, Delphine Rousseau-Ralliard, Laurence Wimel, et al.

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Methods: Placenta, ovaries and testicles of conceptus at 25, 30, 40 and >50 days of gestation (DG) (Full term) (n=3 males and 3 females at each period) were analyzed through immunohistochemistry and immunoblot for enzymes 3-β-HSD, 17-β-HSD, cytochrome P450c17, P450aromatase and 5-α reductase.
Results: There was an increase in testosterone concentration in the maternal circulation from 25DG (122.44±65.79 pg/mL) until the end of gestation (>50DG) (718.55±67.40 pg/mL). Estradiol had lower levels than testosterone (5.45±1.59 pg/mL at 25 DG and 132.19±15.96 pg/mL at >50DG). Enzymes 3-β-HSD, 17-β-HSD and cytochrome P450c17 were present in the placenta throughout gestation and could participate in the production of androgen hormones. Presence of cytochrome P450 enzyme in placenta was not verified. In fetal gonads, 17-β-HSD, 3-β-HSD and cytochrome P450aromatase enzymes were present. Finally, the enzyme 5-α-reductase was present in the testes
Conclusion: Testosterone concentration is greatly increased from 25 DG until the end of gestation and that the same does not occur with estradiol. The Results for the detection of steroidogenic enzymes suggest that the placenta may be the organ that acts in the production of androgen hormones and may not perform the conversion of these hormones into estrogens due to the absence of the enzyme responsible for this process. Finally, the testicles and ovaries can also contribute to the production of the main androgens and the ovary also has the enzyme necessary for the production of estrogens.

P1.24.
EFFECTS OF DIETARY ARGININE SUPPLEMENTATION TO PRIMIPAROUS MARES IN THE LAST THIRD OF GESTATION ON FOAL BIRTHWEIGHT AND PLACENTAL FUNCTION

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Objectives: Foals born to primiparous dams are smaller and lighter than foals born to multiparous dams, with reduced placental development. L-arginine supplementation was shown in other species to improve placental efficiency and increase fetal growth. We aimed to evaluate if L-arginine supplementation to primiparous mares improved feto-placental biometry and placental function at birth.
Methods: Twenty-two saddlebred mares of similar body condition and fed according to needs were artificially inseminated with the semen of one stallion. At 215 days of gestation, they were allocated to one of 3 groups: multiparous controls (MC, N=8), primiparous controls (PC, N=6) and primiparous arginine (PA, N=8). PA mares received daily supplementation with 100g L-arginine until parturition. Plasma amino-acid concentrations were measured in mares during gestation and in foals at birth before suckling. Placental structure was studied by stereology. The expression of genes involved in vascularization and nutrient transport was analysed by RT-qPCR. Data were analysed using Kruskal-Wallis tests.
Results: Plasma L-arginine (p=0.02) and ornithine (synthetized in-vivo from arginine) concentrations were increased (p<0.01) and lysine concentrations decreased (p<0.0001) in A mares, possibly reflecting competitive intestinal absorption between lysine and arginine. At birth, PC foals were lighter than MC foals (p=0.03) with PA being intermediate. Plasma amino-acid concentrations did not differ between foals. PC and PA placentas tended to be lighter than MC placentas (p=0.07). Placental efficiency did not differ. Placental structure was not different but for relative surface and volume of allantochorion in the pregnant horn that were reduced in PT vs MT (p<0.05) with PA being intermediary. The expression of H19, sFLT1 and VEGF were significantly reduced and that of CD36 increased in the placenta of PA vs MC (p<0.05).
Conclusion: L-arginine supplementation affected amino-acid absorption in pregnant mares but not in foals and increased the birthweight of foals. Work is ongoing to explore placental structure by electron microscopy.

P1.25.
MATERNAL DIETS ENRICHED IN OLIVE OIL PREVENT LIPID ACCUMULATION IN THE FETAL LIVER

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Objectives: In GDM rats, the liver of male fetuses accumulates lipids, an alteration possibly involved in the programming of metabolic diseases in later life. We aim to evaluate whether a maternal diet enriched in olive oil is capable of regulating lipid accumulation in the fetal liver, through the regulation of PPARs and their target genes involved in lipid metabolism.
Methods: Mild diabetic rats (F0) were obtained through neonatal streptozotocin administration, mated in the adulthood (F0 pregnancy) and their female offspring mated with control males (F1 pregnancy). In their F1 pregnancy GDM was developed through intrauterine programming. The liver of male fetuses from GDM rats and controls were explanted on day 21 of pregnancy.
Results: Triglycerides and cholesterol were increased in the fetal livers from GDM rats (53% and 48%, respectively; p<0.05 vs C), an alteration prevented by the maternal diet enriched in olive oil (p<0.05 vs GDM). Increases in PPARgamma and PPARdelta (62% and 25%, respectively; p<0.05 vs C), in the fetal livers from GDM rats were prevented by the maternal diet enriched in olive oil (p<0.05 vs GDM). No changes were observed in PPARalpha levels in the experimental groups. The PPAR target genes involved in lipid synthesis *Fas*, *Acc1* and *Scd-1*, were increased in the fetal liver from GDM rats (fold change 1.44, 2.51 and 2.04, respectively; p<0.05 vs C) and decreased when the GDM mothers were treated with the olive oil-supplemented diet (p<0.05).
Conclusion: A diet enriched in olive oil prevents accumulation of lipids and altered PPAR pathways in the fetal liver from GDM rats, probably improving the liver function in the fetus and later in the offspring.

P1.26.
IMPAIRED DECIDUAL PPAR SIGNALING IN DIABETIC RATS AT EARLY PREGNANCY

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Objectives: Maternal diabetes impairs embryo and placental development, an alteration that may start at early pregnancy. Histotrophic nutrition is relevant at the early postimplantation stage. Peroxisome proliferator activated receptor gamma (PPARgamma), a ligand activated transcription factor essential in development and a master regulator of lipid metabolic pathways, may be involved in histotrophic nutrition. Our objective was to evaluate PPARgamma and its target genes perilipin 2 (PLIN2) and fatty acid binding protein 4 (FABP4) in the decidua from 9-day-pregnant control and diabetic rats.
Methods: Diabetes was induced by streptozotocin administration (50 mg/kg) in female Wistar rats two weeks before mating with control males. On day 9 of pregnancy the decidua was explanted and prepared for evaluation of PPARgamma by Western blot and PLIN2 and FABP4 by immunohistochemistry.
Results: PPARgamma was increased in the decidua from diabetic rats compared to controls (30%, p<0.05). Levels of PLIN2 and FABP4 were also increased in the decidua from diabetic rats compared to controls (39% and 33%, p<0.01, respectively). PLIN2 and FABP4 immunostaining were also increased in the uterine glands in the diabetic group compared to controls. Increased PLIN2 immunostaining was observed in the yolk sac in the diabetic group compared to controls, although FABP4 was weakly stained in the yolk sac in both control and diabetic groups.
Conclusion: Maternal diabetes increases the levels of PPARgamma and its target genes in the decidua and uterine glands, suggesting their involvement in an altered histotrophic nutrition that may alter embryo development from a very early postimplantation stage.