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## **NURSING IN THE PERICONCEPTIONAL PERIOD ALTERS PLACENTAL GENE EXPRESSION AND SUBSEQUENT FOAL GROWTH**

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**Introduction:** To be profitable, equine breeders aim to produce a foal/mare/year, meaning that mares are bred while still nursing their previous foal. Most studies show that fertility as embryo/fetal mortality is increased in nursing mares. Long-term effects, however, have not been investigated.

**Objectives:** This project aimed to analyze the effect of nursing on placenta and foal growth.

**Methods:** Multiparous Saddlebred mares aged 10-16 years were bred with the semen of one unique stallion on the 2<sup>nd</sup> heat after foaling (Nursing, N, n=6) or after having been left barren for 1 year (Barren, B, n=14). They were then managed as one herd in pasture and stalled individually in the same barn from around 6<sup>th</sup> month of gestation until foaling (11 months gestation). Placentas were recovered at foaling, measured and weighed. Stereology was performed using MercatorPro software on HES-stained paraffin embedded samples. Paired-end RNA-sequencing was performed on frozen placenta (Illumina, NextSeq500). Differential expression was analyzed (DESeq2) using a false discovery rate (FDR) <0.05 cutoff. Gene Set Enrichment Analysis was performed using KEGG, GO BP and REACTOME databases. A Frequently Sampled IV Glucose tolerance test was performed in mares at 300d of gestation and in foals at 6, 12 and 18 months of age. Lactation and foal growth were monitored until, respectively, weaning ( $\approx$ 6 months) and 18 months of age. Other data related to foal growth, metabolism and placenta were analyzed using a linear model with permutations using R software.

**Results:** N were more sensitive to insulin at 300 days of gestation (in median, 2.54 for N vs 0.78L/(mUI\*min) for B,  $p < 0.05$ ). Gestation length was reduced (-8d) in N vs B. Although no morphological nor structural difference were observed, 38 genes were differentially expressed (23 and 15, respectively, over and under-expressed,  $FDR < 0.05$ ) in N placentas, among which 4 were directly involved in Wnt signaling pathway (WNT7, SFRP1 and LRP6 over-expressed and TRABD2A under-expressed in N placentas). Among the 226 perturbed pathways (183 GO BP, 16 KEGG and 27 REACTOME), only 13 were enriched in N placentas, mainly related with protein synthesis. Gene sets enriched in B placentas were involved in cell division and the regulation of inflammation and innate immunity. Although foal weight was similar at birth, post-natal growth was reduced in N, foals being lighter by 24kg at 18 months of age ( $p < 0.05$ ). No difference in carbohydrate metabolism was observed. Milk production, as analyzed through individual milking, was reduced at the end of the lactation period in N (N: 212.80  $\pm$  92.41 vs B: 401.62  $\pm$  100.64g,  $p < 0.05$ ) although milk quality was similar.

**Conclusion:** In conclusion, nursing at the beginning of gestation is associated with reduced gestation length and functional placental adaptation probably compensating for the increased insulin sensitivity in N mares and enabling the birth of a normal weight foal. Reduced lactation potential, however, impaired long-term foal growth.

**Disclosure of Interest:** None Declared