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Equine saliva metabolome analysis during anestrus, estrus cycle and early gestation for the identification of salivary biomarkers of reproductive stages

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Precision livestock farming using metabolomic to acquire precise and real-time data can help farmers in individual animal management and decision making. Moreover, saliva collection is a non-invasive, painless and easy sampling method. Thus, this study proposes a metabolomic analysis in mare saliva during reproductive stages to identify salivary biomarkers to detect their reproductive stage in a welfare friendly production system. Saliva samples from 6 mares were collected in the seasonal anestrus, in the follicular phase 3, 2 and 1 day before ovulation and the day when ovulation was detected, in the luteal phase 6 days after ovulation and in gestation 18 days after ovulation and insemination. Metabolome analysis was performed by ¹H-nuclear magnetic resonance spectroscopy. We identified 59 discriminant metabolites in saliva after repeated-measures one-way ANOVA tests. Creatine concentration significantly decreased from 2 days before ovulation until the day when ovulation was detected. Thus, a drop in salivary creatine concentration could allow detection of ovulation. Alanine concentration during anestrus was significantly higher than during follicular phase. Thus, alanine could be a candidate salivary biomarker of anestrus to estrus cycle transition. We showed previously that pregnenolone concentration was significantly higher during early gestation than during anestrus or follicular phase (Goudet et al., 2022, Frontiers in Animal Science, 3: 1055179). In conclusion, salivary biomarkers of ovarian cyclicity, ovulation and early gestation can be proposed. Further studies with a greater number of animals are in progress to confirm the reliability of these candidate biomarkers.