Innovation in wildlife endocrinology: LA-REIMS metabolic fingerprinting for early pregnancy testing in giant pandas

Cools T^{1,2}, Li, D.³, Plekhova, V.¹, Bouts, T.⁴, Wilson, K⁵., Vancsok, C.⁴, Mulot, B⁶., Leclerc, A.⁶, Kok, J.⁷, Li, R.³, Zhang, H.³, Vanhaecke L^{1,8} and Wauters J^{1,2,4}

¹Laboratory of Chemical Analysis, Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, Ghent University, Belgium.

²Department of Reproduction Biology, Leibniz Institute for Zoo and Wildlife Research, Germany.

³China Conservation and Research Centre for Giant Panda (CCRCGP), People's Republic of China.

⁴Pairi Daiza Foundation - Pairi Daiza, Belgium.

⁵MRC Centre for Reproductive Health, University of Edinburgh, Scotland, United Kingdom.

⁶ZooParc de Beauval and Beauval Nature, France.

⁷Ouwehands Dierenpark Rhenen, The Netherlands.

⁸Institute for Global Food Security, School of Biological Sciences, Queen's University, Northern Ireland, United Kingdom.

E-mail: Tom.Cools@ugent.be; Jella.Wauters@ugent.be

Pregnancy diagnosis in giant pandas is challenged by a delay in the luteal phase (diapause in pregnant cycles) and pseudopregnancy (similar progestogen profile in bred and non-bred animals). Nevertheless, optimal management for pregnant animals and timely planned logistics to monitor birth are crucial for successful breeding. A straightforward pregnancy test is so far not existing: with current state-of-the-art methodologies, pregnancy can only be confirmed in the last three weeks.

Our aim was to investigate the potential of laser-assisted rapid evaporative ionization mass spectrometry (LA-REIMS) as an early hands-on rapid method for pregnancy testing.

Samples of 7 cycles (3 pregnant, 4 non-pregnant) of 3 giant pandas were included with 12 samples per cycle equally distributed over 4 stages: anestrus, late corpus luteum dormancy phase (CLD), early active luteal phase (EAL) and late active luteal phase (LAL). 50 μ L of undiluted urine was loaded without sample pretreatment on a 96-well plate for automated LA-REIMS analysis using an Nd:YAG laser and Xevo G2-XS QToF-MS in negative scanning mode. A total of 2791 molecular features were obtained after preprocessing the data with Progenesis. Subsequently, data was transferred to SIMCA for multivariate analysis, showing good repeatability based on QC clustering (PCA-X). Significantly different models (7-fold validation) between pregnant and non-pregnant cycles were demonstrated by OPLS-DA for the first time during EAL (Q²(Y)=0.711, R²(Y)=0.971, p<0.01) and confirmed during LAL (Q²(Y)=0.742, R²(Y)=0.998, p=0.01). Moreover, after exclusion of bred, but non-birth cycles (potential fetal losses, n=2), discriminative fingerprinting between pregnant and pseudopregnant cycles was shown during CLD (Q²(Y)=0.81, R²(Y)=0.99, p=0.01). In conclusion, this study clearly illustrates the applicability of LA-REIMS as an alternative fast diagnostic tool in wildlife endocrinology. Further research should reveal whether there is potential for pregnancy confirmation in the early stages of diapause.