

## **Multiscale correlative imaging of horse and zebra placental villi**

Davis Laundon<sup>1</sup>, Neil Gostling<sup>2,3</sup>, Shelley Harris<sup>1</sup>, Pascale Chavatte-Palmer<sup>4,5</sup>, Rohan Lewis<sup>1,2</sup>

<sup>1</sup>*The Institute of Developmental Sciences, Human Development and Health, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, UK*

<sup>2</sup>*Institute for Life Sciences, University of Southampton, University Rd, Highfield, Southampton, SO17 1BJ, UK*

<sup>3</sup>*School of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, University Rd, Highfield, Southampton, SO17 1BJ, UK*

<sup>4</sup>*Université Paris-Saclay, UVSQ, INRAE, BREED, 78350, Jouy-en-Josas, France*

<sup>5</sup>*Ecole Nationale Vétérinaire d'Alfort, BREED, 94700, Maisons-Alfort, France*

Despite having a single evolutionary origin, the mammalian placenta exhibits wide interspecific morphological and structural variation. Equine (horses and their kin) placentas display branching villi which sit in apposition with maternal tissue and represent the site of fetomaternal nutrient and waste exchange. Three-dimensional imaging techniques have recently identified and quantified novel structures in human placental villi, however similar tools have yet to be broadly applied to other species. Such approaches have the potential to both expand our understanding of comparative placentation and better resolve the structural composition of the studied taxa. Using scanning electron microscopy (SBF-SEM) of horse and zebra placenta, we demonstrated the presence of stromal macrovesicles previously only observed in human placental villi. Here, we also present a workflow for correlative three-dimensional imaging of equine placental villi by combining x-ray microtomography (microCT) and SBF-SEM. This allows calculation of the total surface area of the equine placenta including microvilli. Through this workflow, we quantify the villus structure across multiple orders of magnitude in horse and zebra placentas. These morphometric data, including volume, surface area, and branching angle, help us to better resolve equine placental organization and contribute towards a holistic understanding of equine placental function.