

ABSTRACT

The vascular endothelial glycocalyx is a gel-like matrix, composed of proteoglycans and glycosaminoglycans, it is integral to homeostasis. Current visualisation protocols are technically challenging and there are limited methods to assess glycocalyx damage in clinical patients. This project pioneers glycocalyx visualisation and validation of biomarkers to assess glycocalyx breakdown in cats and dogs.

Glycocalyx in uterine and testicular artery samples from cats and dogs was stained by Alcian blue perfusion and fixed in 2.5% glutaraldehyde/ 0.1M sodium cacodylate for electron microscopy visualisation. Novel immersion fixation with 1% periodic acid/ 0.75g lysine/ 4% paraformaldehyde prior to Bismuth staining was also trialled. Quantification of glycocalyx breakdown was achieved through optimisation of an Alcian blue assay and use of hyaluronan ELISA to detect sulfated and non-sulfated plasma glycosaminoglycans respectively.

The glycocalyx was visualised by perfusion fixation in three of the eight samples collected; testicular artery from one dog and uterine artery from one dog and one cat. Immersion fixation techniques yielded inconclusive results. Despite substantial improvements to the Alcian blue assay, suspected interference from factors in the plasma prevented reliable results. The reference interval for hyaluronan established from 54 healthy dogs was 9.02-145.29 ng/mL.

This research is the first to definitively visualise the GCX in cats and dogs using Alcian blue perfusion. The generation of normal hyaluronan reference intervals for dogs is an essential step towards investigation into glycocalyx degradation in diseased populations. Research efforts must increase if veterinary medicine is to benefit from the diagnostic and therapeutic potential of glycocalyx study.