Presence of **neutrophil myeloperoxidase** in lamellar tissue of horses with **laminitis** induced using a hyperinsulinemic model

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Introduction Laminitis is a pathology of the equine digit resulting in failure of the dermo-epidermal interface[1]. Inflammation is likely a central player in its pathophysiology. Myeloperoxidase (MPO) is a neutrophilic enzyme producing oxidizing species capable of inducing cell damage. Neutrophil activation and MPO release have been observed in the black walnut heartwood extract model, used to investigate sepsis-related laminitis[2,3]. However, less is known about the role of neutrophil activation in the prolonged euglycemic hyperinsulinemic clamp (pEHC) model, mimicking endocrine disturbances like hyperinsulinemia encountered in pituitary pars intermedia dysfunction and equine metabolic syndrome.

Objectives

To determine the implication of neutrophil activation, in particular MPO release, following laminitis induction using the pEHC model.

Materials & Methods

Archived samples from a previous experiment were used. Five horses were randomly assigned to the pEHC-treated (n=3) or control group (n=2). Laminitis was induced using the pEHC model as described by Asplin and coworkers[4]. Histological sections of lamellar tissue were obtained and immunohistochemically stained for MPO and counterstained with hematoxylin-eosin (Fig 1). The sections were examined for histopathological evidence of laminitis and MPO.

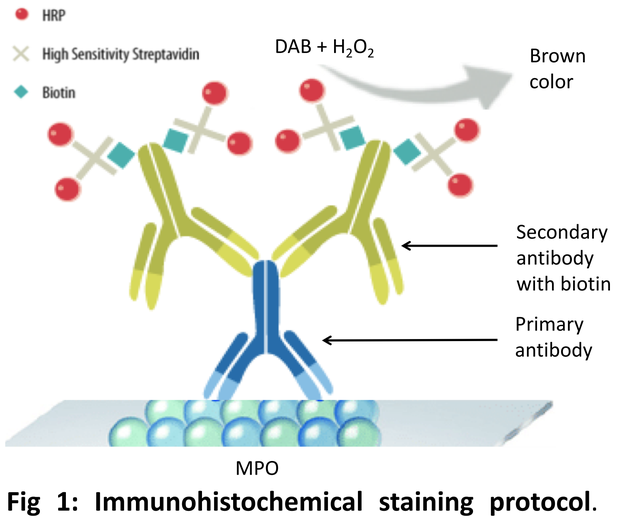

Fig 1: Immunohistochemical staining protocol. First, a rabbit anti-MPO antibody obtained against purified equine MPO was applied. Next, the anti-rabbit antibody conjugated with biotin was added, followed by the streptavidin-peroxidase solution. Then the chromogen diaminobenzidine (DAB)/substrate solution was applied and brown coloration was monitored.

Results

In addition to the general histopathological features of laminitis, an intense and diffuse MPO labelling was observed in the dermal lamellae of all pEHC-treated horses (Fig 2B, 3B), whereas only focal, mild MPO labelling was observed in control horses (Fig 2A, 3A).

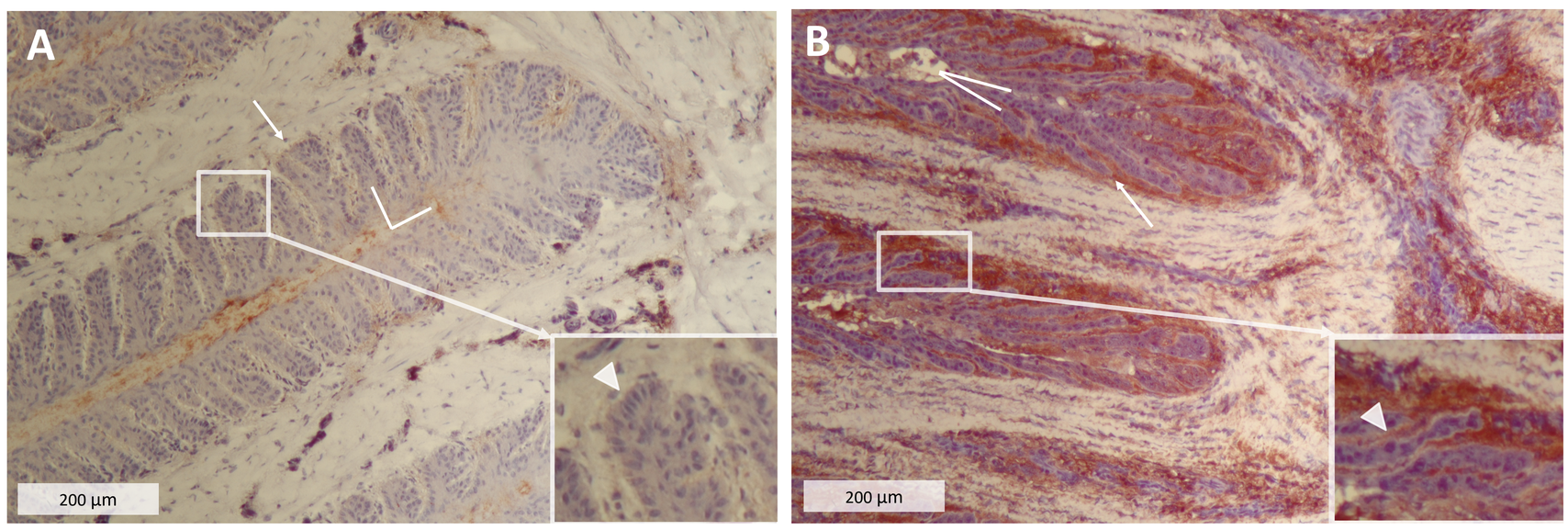

Fig 2: Photomicrograph of hoof lamellae stained with anti-MPO and HE.
In the control horse (A) the secondary epidermal lamellae are short, perpendicularly oriented on the primary epidermal lamellar axis (lines), have rounded tips (arrow) and an oval nucleus (arrowhead). Whereas in the pEHC-treated horse (B) intense, diffuse MPO labelling is observed in the dermal lamellae. In addition, the secondary epidermal lamellae are elongated, placed in an acute angle on the primary epidermal lamellae (lines), have tapered tips (arrow) and a round nucleus (arrowhead).

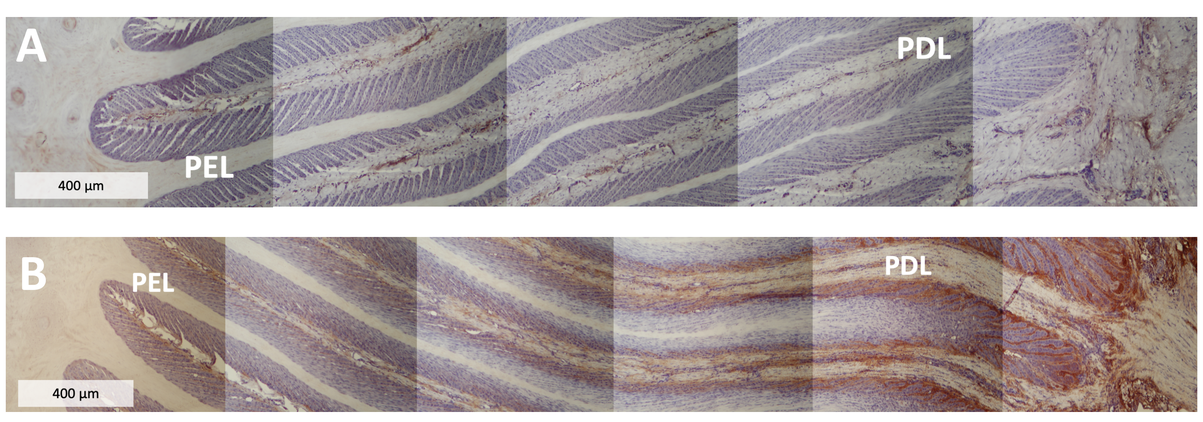

Fig 3: Reconstruction of photomicrographs of the dermo-epidermal interface stained with anti-MPO and HE. Minimal MPO labelling is observed in the dermal tissue of the control horse (A), whereas diffuse and intense MPO labelling is visible in the dermal tissue of the pEHC-treated horse.
PDL: primary dermal lamellae, PEL: primary epidermal lamellae

Discussion

Neutrophil activation with MPO release in pEHC-treated horses suggests involvement of inflammation in the pathophysiology of endocrinopathic laminitis. MPO can chlorinate, nitrate and oxidize organic molecules, resulting in tissue damage[5]. In addition, MPO and its products can damage endothelial cells. Furthermore, hypochlorous acid, generated by MPO, interferes with insulin signaling in the human metabolic syndrome and patients suffering from insulin resistance[6].

Conclusion. Neutrophil activation with myeloperoxidase release may play a role in the pathophysiology of endocrinopathic laminitis. Further studies are needed to investigate the link between hyperinsulinemia and neutrophil activation in equine laminitis.

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