

Light microscopy

Small tissue samples were dissected from the placenta of each animal placenta and immediately fixed in 10% neutral buffer-buffered formalin 10% for 24 hours. Samples-Tissue samples were then rinsed; and stored in 70% ethanol 70% until processing for light microscopy. Samples were dehydrated through by a sequence series of ethanol concentrations (70%–100%), cleared using xylene, and embedded in paraffin¹⁸. Paraffin blocks were cut with at 5 μm thick thickness using a rotary microtome, mounted on gelatin-coated slides coated with gelatin, deparaffinized using histolene, and rehydrated through descending ethanol concentrations of ethanol. Slides Sections were stained with either hematoxylin-eosin or the Masson's trichrome. Several-Two histochemical techniques were also used applied too. The Periodic Acid-Schiff-periodic acid-Schiff (PAS) reaction was used to identify neutral carbohydrates, and the Alcian blue 8GX (pH 2.5) was employed to detect identify acid mucosubstances¹⁹. The histological sections underwent dehydration were dehydrated in increasing graded ethanol, clearing-cleared using histolene, and were coverslipped with DPX mounting medium. Slides were viewed with using a light microscopy microscope and imaged with a digital camera and the software cellSens Standard software, version 1-11.

Commented [CSS1]: Na medida do possível, evite ser vago. O que **pequeno** significa? Seria possível fornecer um valor, ainda que uma variação aproximada? possível.

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Morphometrics

We estimated the thickness of the chorionic epithelium for each individual female. Measurements were taken directly on digital photos of the histological sections of each individual using the software ImageJ²⁰. For this, we measured the epithelial height of five epithelial cells were measured in at five different sites. Next, we took the average of the averaged all measured values for all measures to obtain an average a mean value per individual.

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Commented [CSS6]: ...different... aqui parece redundante. Se cinco as medidas foram feitas em cinco locais medidas, então parece claro que são pontos diferentes.