University of Maribor

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5th Congress of the Society for Laboratory Animals of Slovenia and 3rd joint SLAS - CroLASA meeting

Extent of necrosis as a measure of acute pancreatitis severity in studies employing live cell imaging

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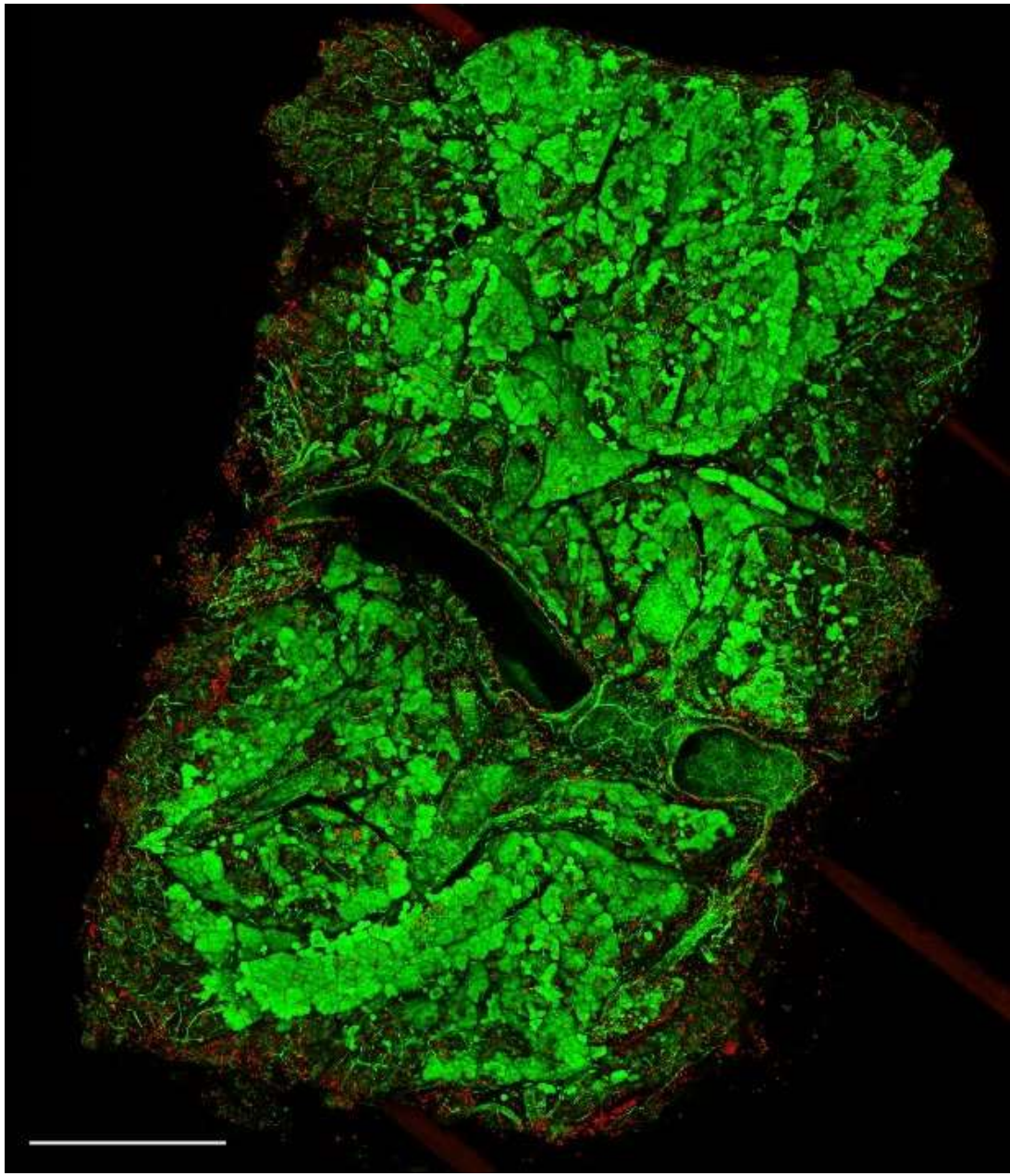
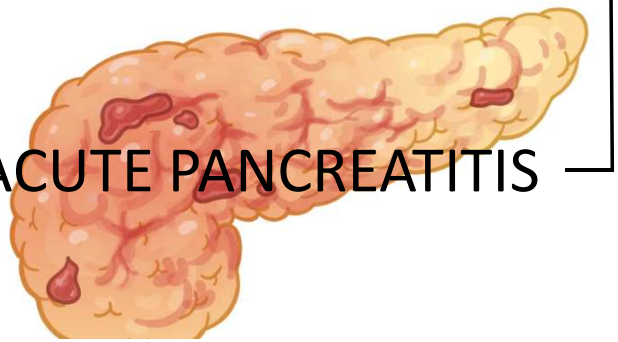
Ljubljana, 15. 6. 2023



Risk factors:

- Alcohol,
- gallstones, duct obstruction,
- metabolic diseases,
- ERCP, ERP,
- autoimmune origin,
- genetic factors,
- infections,
- drugs.

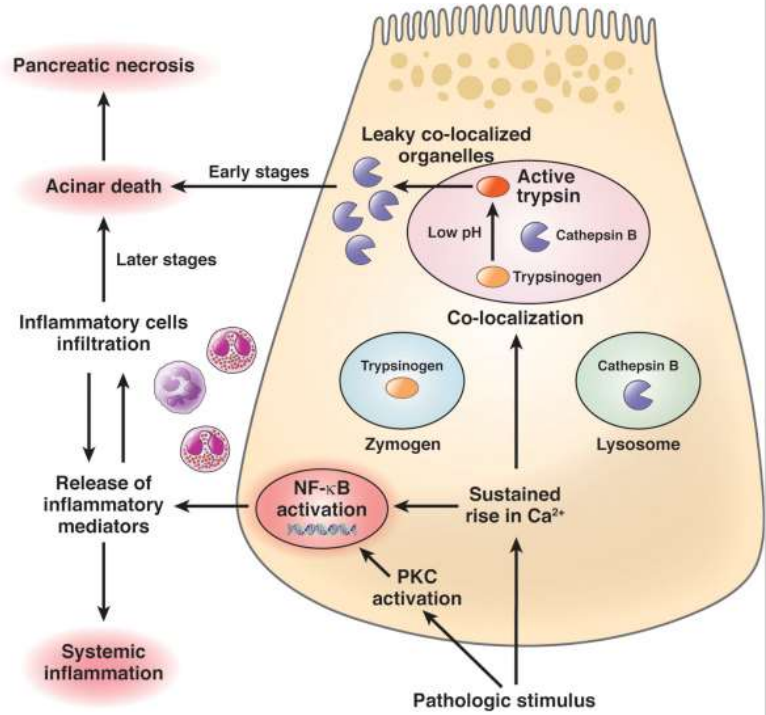
DIAGNOSIS
Clinical: sudden and very severe pain in the upper abdomen.
Biochemical: increase in pancreatic enzymes in the blood.



ACUTE PANCREATITIS

PATHOGENESIS

- Activated pancreatic proenzymes within acinar cells,
- oxidative and endoplasmic stress,
- impaired autophagy,
- impaired mitochondrial dysfunction.



AUTODIGESTION AND DESTRUCTION OF PANCREATIC TISSUE

- severe and very painful inflammatory response,
- multi-system organ failure,
- high mortality rate

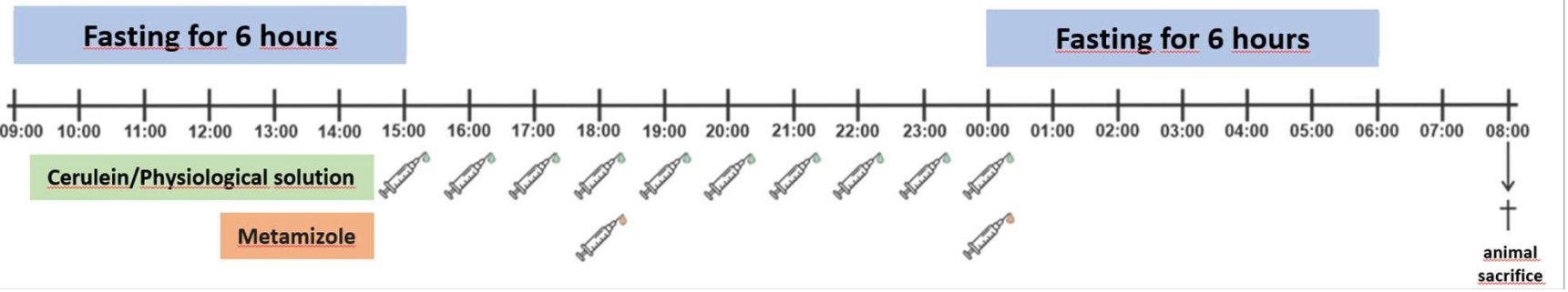
Saluja et al., Gastroenterology 2019

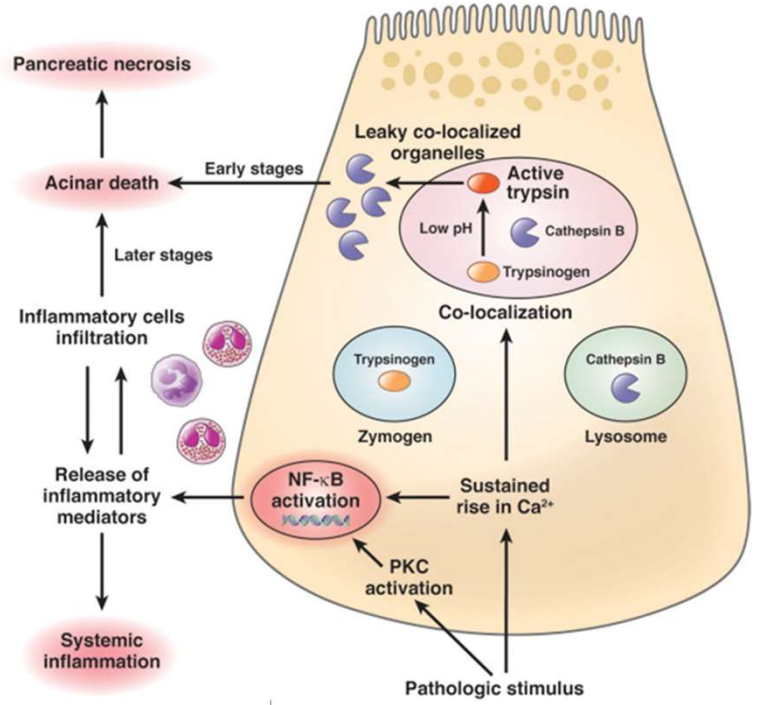
Cerulein-induced acute pancreatitis in mouse model

Intraperitoneal administration of **cerulein**

Supraphysiological pharmacological overstimulation of acinar cells

Control group: 6 mice
AP group: 6 mice

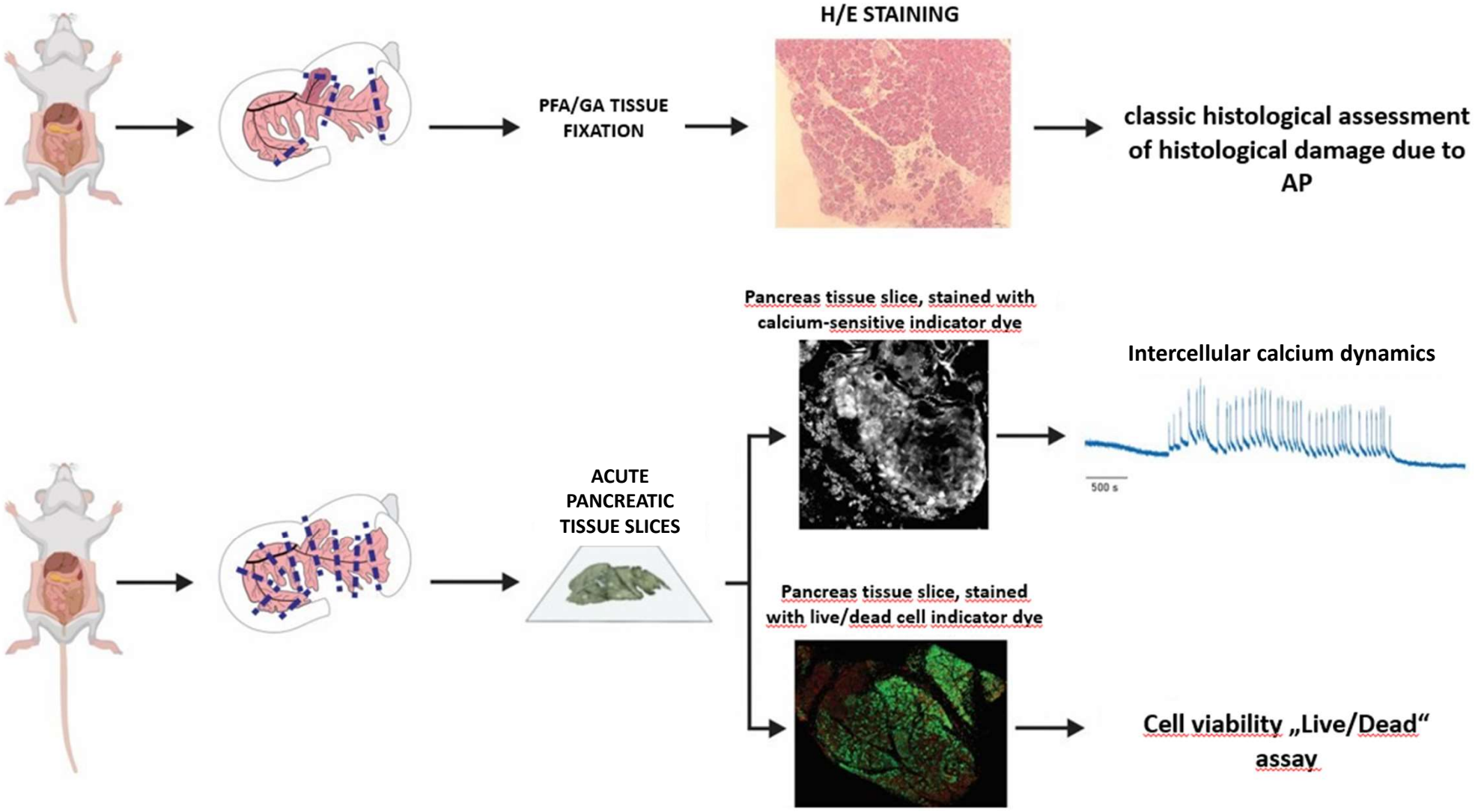




Saluja et al., Gastroenterology 2019

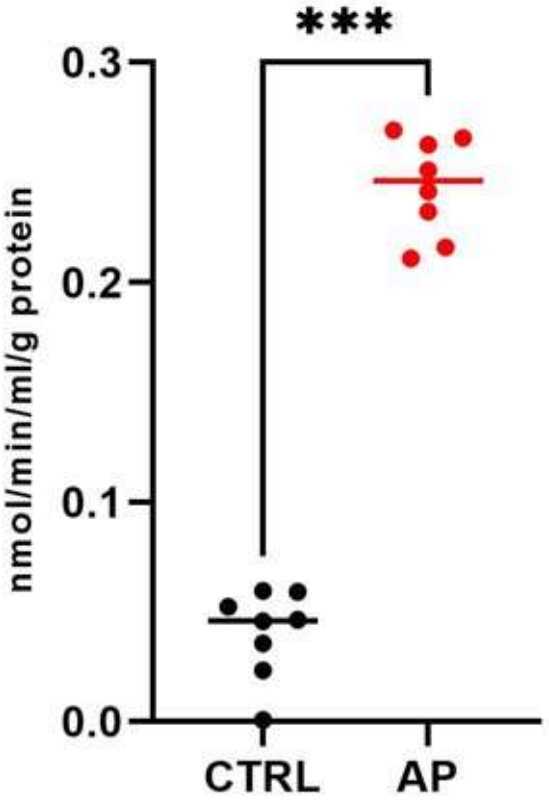
AIM OF THE STUDY

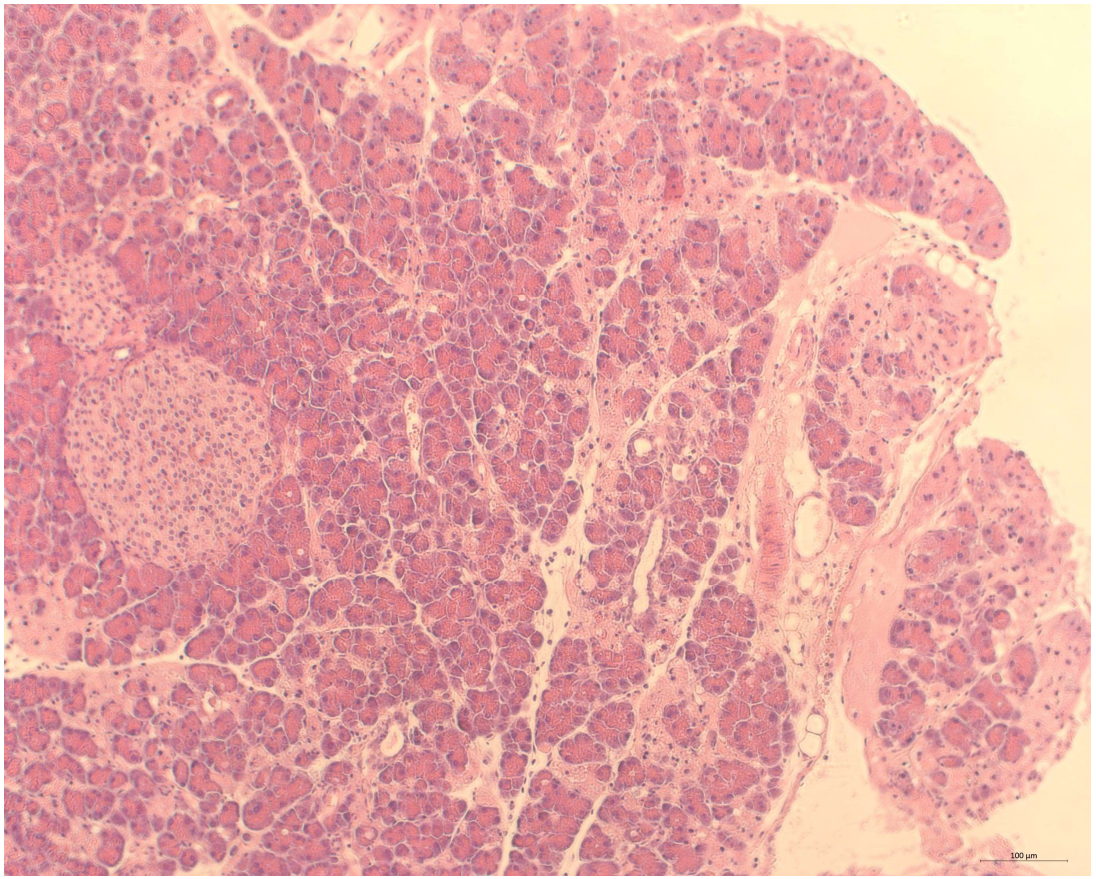
- Little is known about the AP-induced changes on the endocrine pancreas and its pathophysiology.
- Exocrine and endocrine functions of the pancreas strongly interconnected.
- Calcium imaging of endocrine cells in live acute mouse pancreas tissue slices = an effective approach to study the exo-endocrine interactions in the pancreas.
- **However**: inherent incompatibility between live cell calcium imaging in slices and the classic histological assessment of the severity of AP.
- A novel approach to enable assessment of AP severity in acute tissue slices.


H/E STAINING
PFA/GA TISSUE FIXATION
classic histological assessment of histological damage due to AP
Pancreas tissue slice, stained with calcium-sensitive indicator dye
Intercellular calcium dynamics
500 s
ACUTE PANCREATIC TISSUE SLICES
Pancreas tissue slice, stained with live/dead cell indicator dye
Cell viability „Live/Dead“ assay

RESULTS

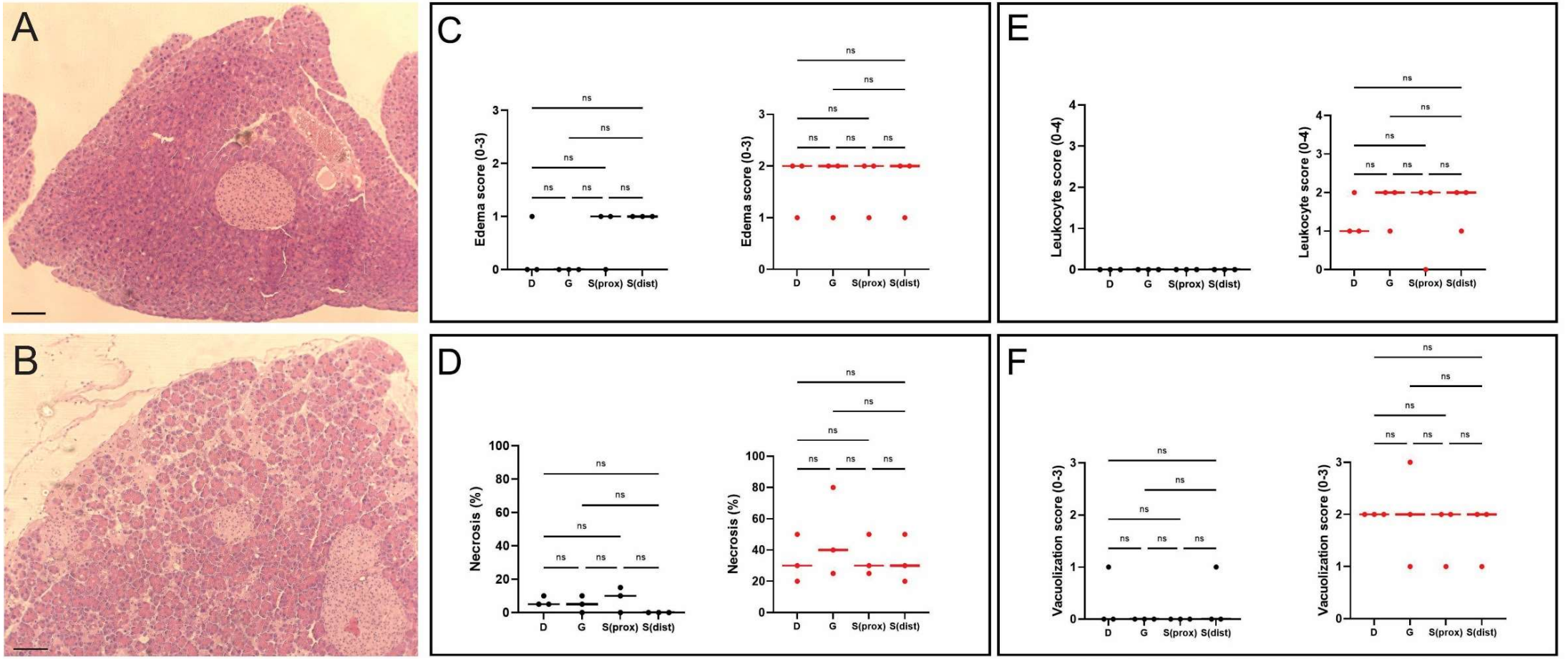
- **extensive damage to the acinar tissue** and **increased plasma levels of amylase** while preserving the morphology of the islets of Langerhans
- **pancreatic edema**, **necrosis**, **vacuolization**, and **inflammatory infiltration**





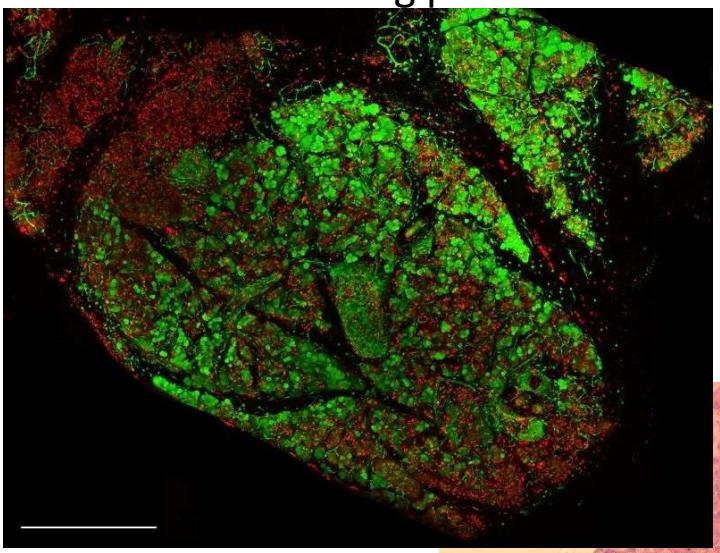
RESULTS

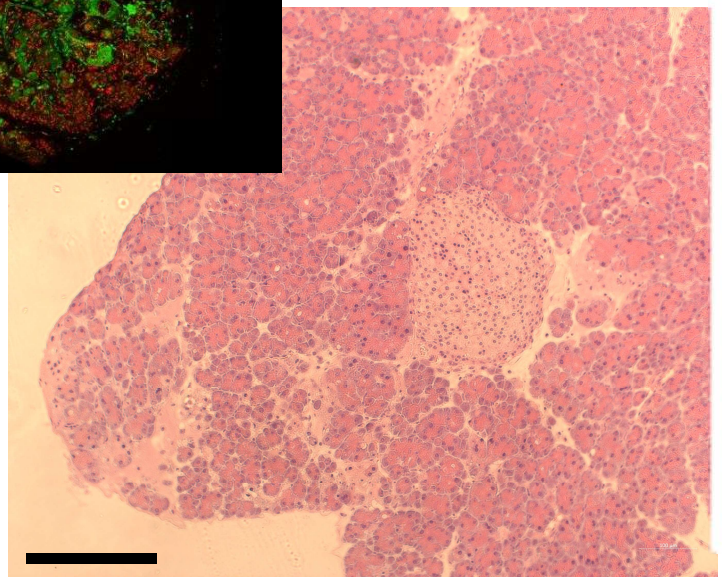
The cerulein-induced changes did not differ between the duodenal, gastric, and splenic parts of the pancreas.

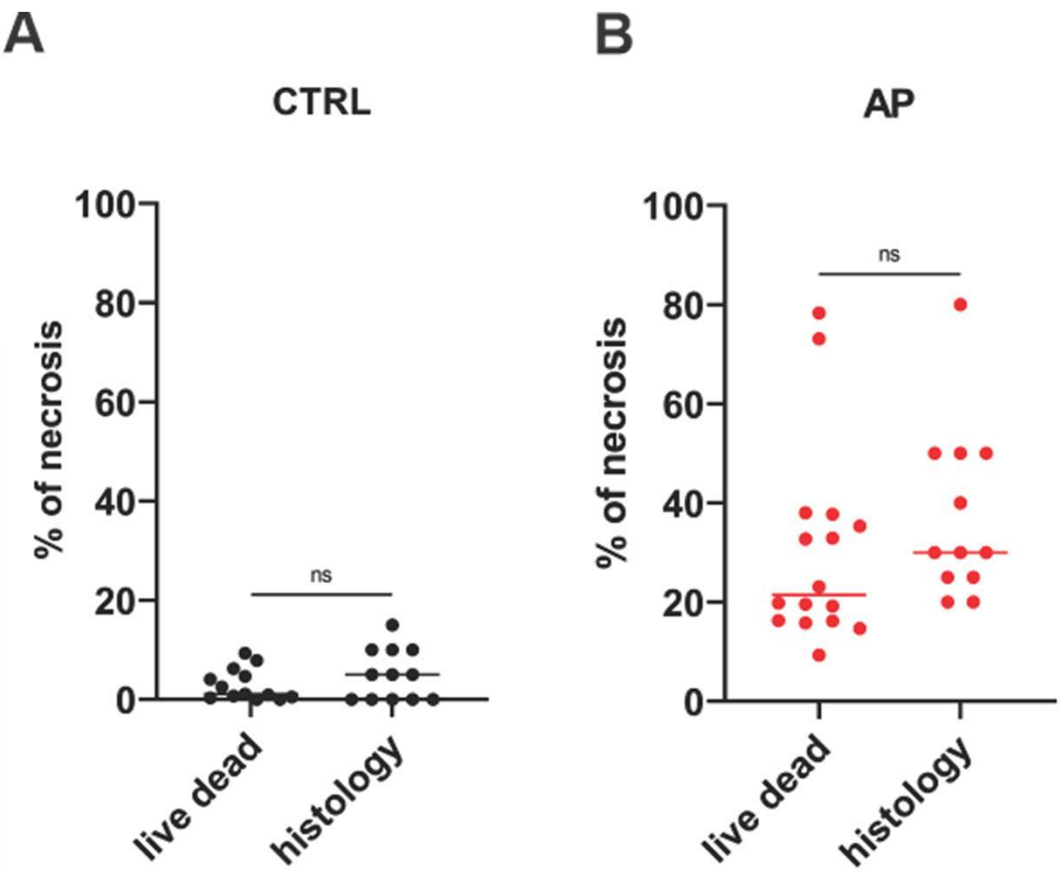


RESULTS

- good correlation between the classical histological grading of the AP and the extent of necrosis assessed on the living pancreatic slices

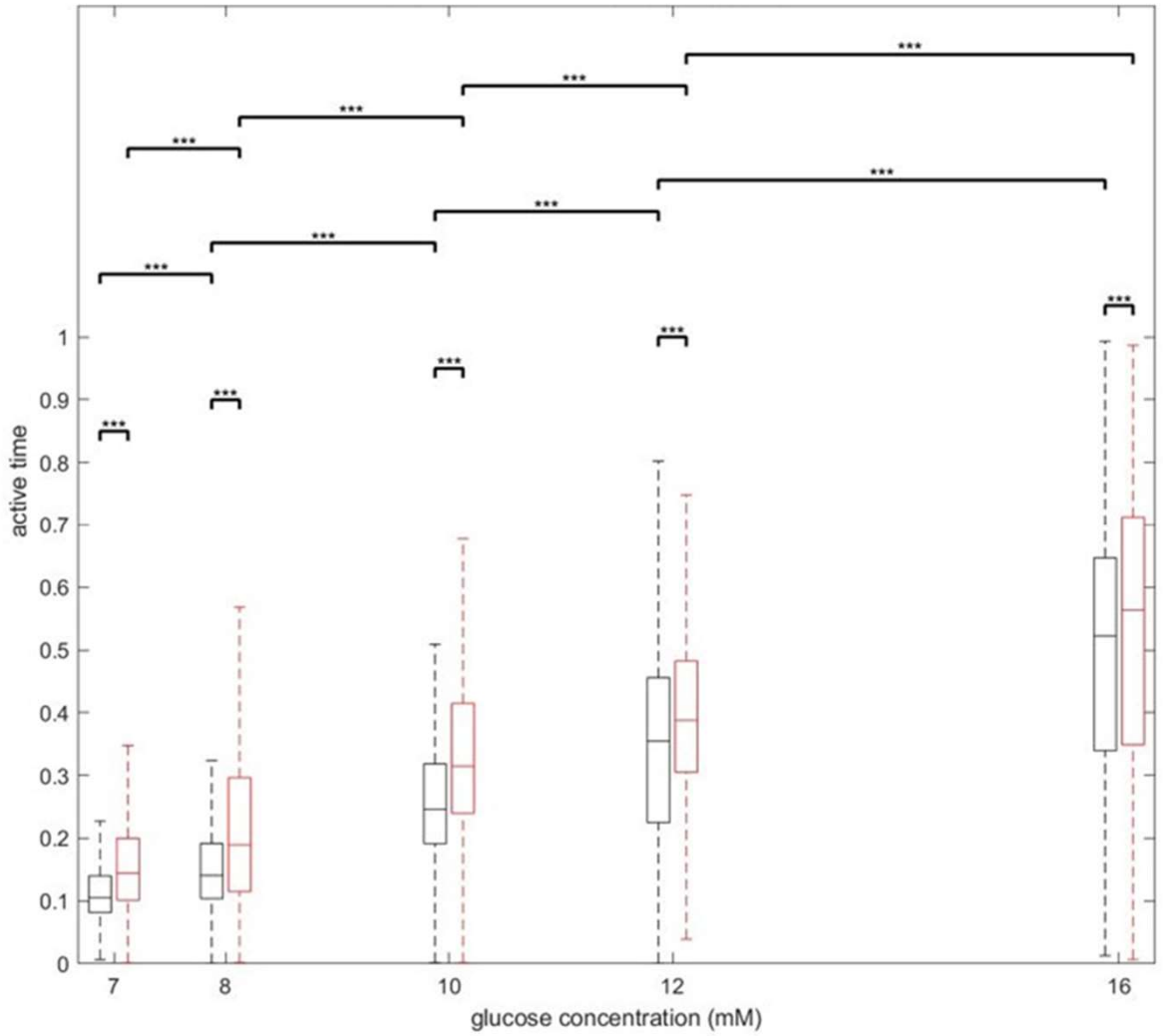






RESULTS

Increase in activity time with rising glucose concentrations in the AP group of animals.



CONCLUSION

- Commercially available LiveDead assay may be used as a measure to assess AP severity in studies employing live cell imaging, circumventing the need to fix the tissue to evaluate pancreatic damage.
- We validated our approach against the gold standard, i.e., classical histological grading of AP severity.
- The main advantage: the ability to simultaneously perform live cell imaging and assessing tissue damage in a quick and simple way on same specimens, decreasing biological variability and further decreasing the number of animals involved in a study.

Acknowledgements

Associate professor dr. Andraž Stožer
Assistant professor dr. Jurij Dolenšek
Assistant professor dr. Maša Skelin Klemen
Assistant professor dr. Lidija Križančić Bombek
Assistant dr. Viljem Pohorec
Assistant dr. Jasmina Kerčmar
Assistant Eva Paradiž Lietgeb
Assistant Jan Kopecky
Assistant Marko Šterk
Associate professor dr. Marko Gosak
Young researcher Nastja Sluga
Technical assistant Rudi Mlakar
Technical aasistant Maruša Plesnik Rošer
Technical aasistant Nika Polšak
Technical aasistant Jasmina Jakopiček

FUNDED BY

Slovenian Research Agency