Removal of circulating nucleosomes/neutrophil extracellular traps (NETs) reduces ex-situ reperfusion injury in porcine DCD livers preserved with normothermic machine perfusion (NMP

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INTRODUCTION

DCD and extended-criteria donor (ECD) livers are extremely susceptible to reperfusion injury, even in the context of normothermic machine perfusion (NMP) occurring ex-situ.

This **ex-situ reperfusion injury (ERI)**, driven by the release of damage-associated molecular patterns (DAMPs) including nucleosomes/NETS, into the circuit upon reperfusion and can result in poor functional metrics ex-situ with subsequent organ discard.

AIM

We aimed to assess the impact of removing circulating nucleosomes/NETS during NMP on ex-situ function and subsequent reperfusion in a large animal DCD liver perfusion model..

METHODS

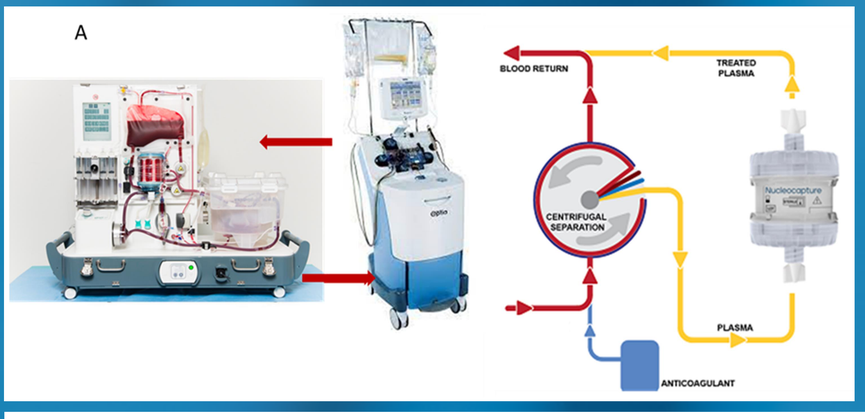
N = 12 DCD pig livers were included in the study.

Nucleosomes/NETS were removed from the circulating perfusate using the **NucleoCapture column** that was integrated into the perfusion circuit and these livers were compared to NMP controls.

Perfusate Nucleosomes/NETs, free histone and cell-free DNA (cfDNA) was measured sequentially during perfusion (including pre and post-column).

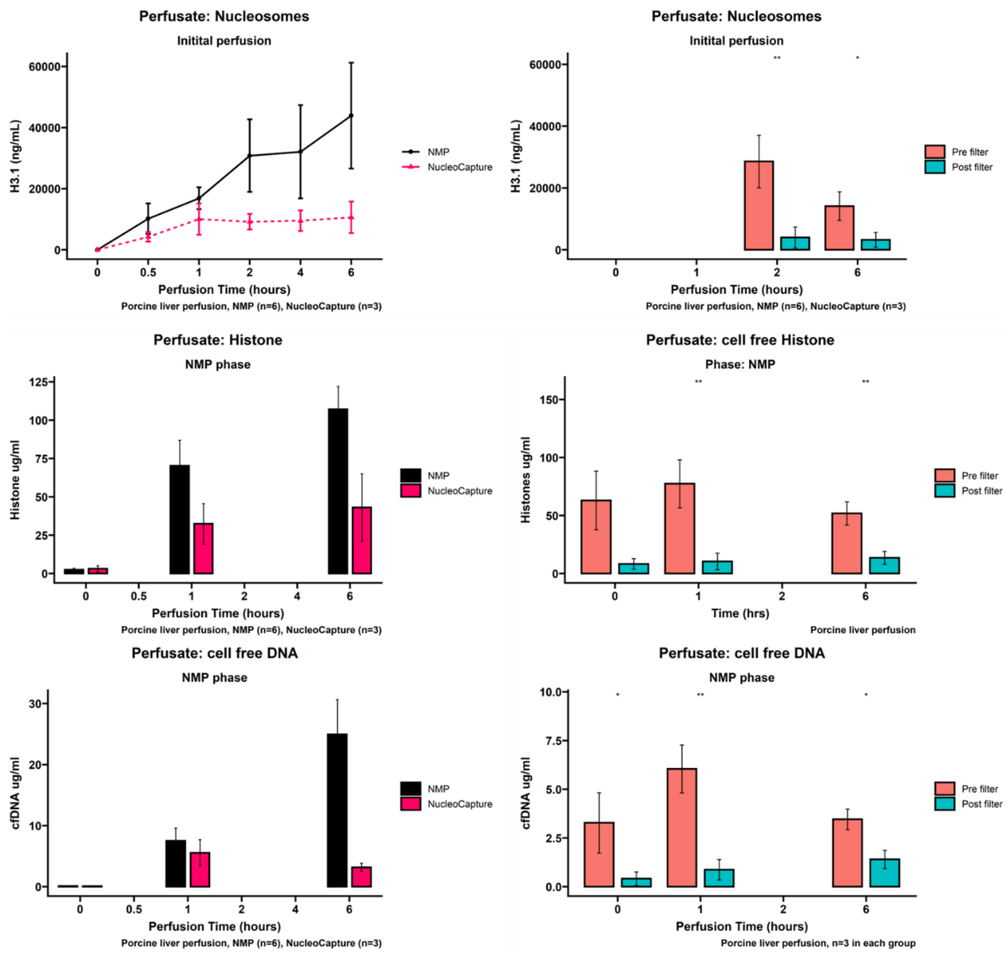
Perfusion parameters, functional assessment of the livers and histological features were assessed between groups.

Statical analysis was performed using a repeated measures ANOVA and t-test/Wilcoxons-test



RESULTS

NucleoCapture significantly reduced early circulating DAMPs across the column: cfDNA p=0.0087(1hr), Histone p=0.0087(1hr) and Nucleosomes p=0.033(2hr). This also corresponded with a significant improvement in early lactate clearance (0.5hrs p=0.033, 1hr p=0.013, 2hr p=0.043) supported by improved haemodynamic perfusion metrics and no neutrophil infiltration on histological assessment. Warm and cold ischaemic times were comparable between groups. All livers produced bile and metabolised glucose.



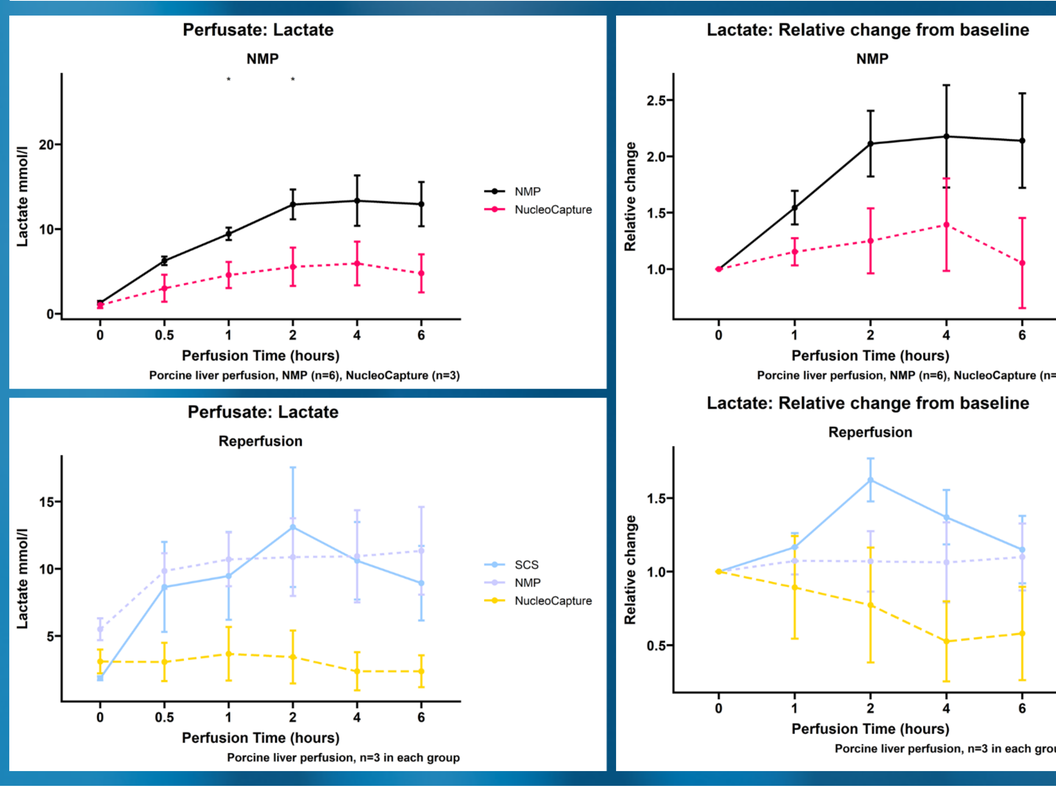
NucleoCapture during NMP depletes Nucleosomes, Histone and cfDNA.

Top row: NMP alone is associated with a progressive rose in nucleosomes, yet with NucleoCapture we observe a plateauing of nucleosomes. Samples taken pre and post filter confirm NucleoCapture is removing nucleosomes, with significant reductions across the filter.

Middle row: Cell free histone follows a similar course with rises through perfusion in the NMP group yet in the NucleoCapture group rises to lesser extent and efficient clearance by the column/filter.

Bottom row: cell free DNA also rises during NMP in line with nucleosomes and histone, but actually falls when NucleoCapture is adopted, with significant reductions across the column at every timepoint.

Nucleosomes/NETs are detected using the Nu.Q assay (Volition, Belgium), Histone was quantified using western blots (H3 antibody) and cfDNA quantified using a SYTOX® Green Dye fluorometric assay. NMP = nomothermic machine perfusion, cfDNA = cell free DNA.



Ex situ liver function (lactate clearance) during NMP and Reperfusion.

Top: Perfusate lactate during NMP. NucleoCapture is associate with significantly lower lactate levels at 1 and 2 hours and with lower levels evident early after reperfusion.

Top right: The relative change in lactate, taking the lactate level within the first 30 mins as a baseline,

Bottom: Perfusate lactate during RP. NucleoCapture is with low levels of lactate throughout reperfusion.

Bottom right: The relative change in lactate, taking the lactate level within the first 30 mins as a baseline. NMP = normothermic machine perfusion (n=6), NucleoCapture = NMP with the NucloCapture column in circuit (n = 3) , SCS = static cold storage, RP = Reperfusion (allogenic whole blood reperfusion)

CONCLUSIONS

NucleoCapture effectively removes circulating nucleosomes/NETs from the perfusate during NMP, improving graft function and mitigating ERI. Application of this technology during NMP of DCD and extended-criteria donor livers could reduce organ discard due to poor function ex-situ and be pivotal in organ optimisation for transplantation

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