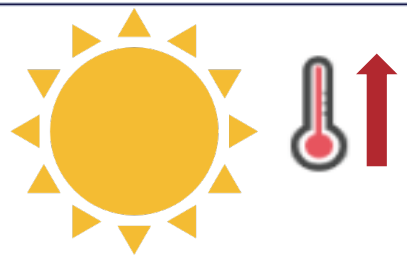
divas
DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

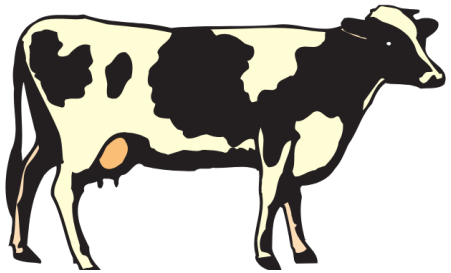
IMMUNOMODULATORY EFFECT OF SMALL EXTRACELLULAR VESICLES FROM THE PLASMA OF HEAT-STRESSED COWS ON BOVINE IMMUNE CELLS

UNIVERSITÀ DEGLI STUDI DI MILANO

Introduction

Heat stress





Cytokines → Immune system

- TLRs
- Cytokines
- Leucocytes trafficking
- Humoral immunity
- Inflammation

- Respiratory rate ↑
- Pulse rate ↑
- Conception rate ↓
- Milk production ↓

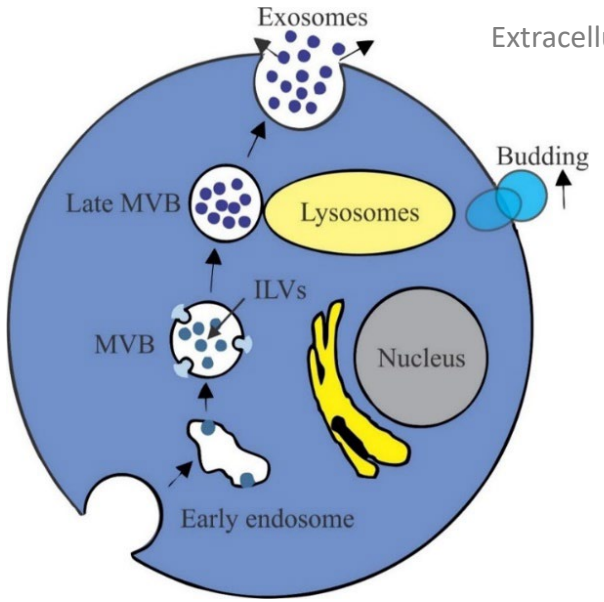
=

Animal's welfare

and

Economic losses

Introduction
Extracellular vesicles



Extracellular vesicles

All cell types

30-150 nm

- Cellular communication
- Regulation of immune response

↓

Carry RNA, proteins, metabolites, and DNA fragments

↓

Profound change in cellular response

Outer stimuli and stress

From: Patil et al., 2020

From: Mathivanan et al., 2010

Aim of the study

To examine the immunomodulatory impacts of plasma sEVs isolated from Holstein (HF) and Brown Swiss (BS) cows, which experienced two distinct conditions: thermal comfort (TC) and hyperthermia (HS) on bovine monocytes and PMNs.

Experimental design

sEVs Purification

Thermal Comfort Condition
(THI = 68)

3 Holstein cows
3 Brown Swiss cows

Hyperthermia
(THImax=84, THImin=69)

Cooling system switched off

Physiological parameters were measured

Centrifugation

Size Exclusion Chromatography

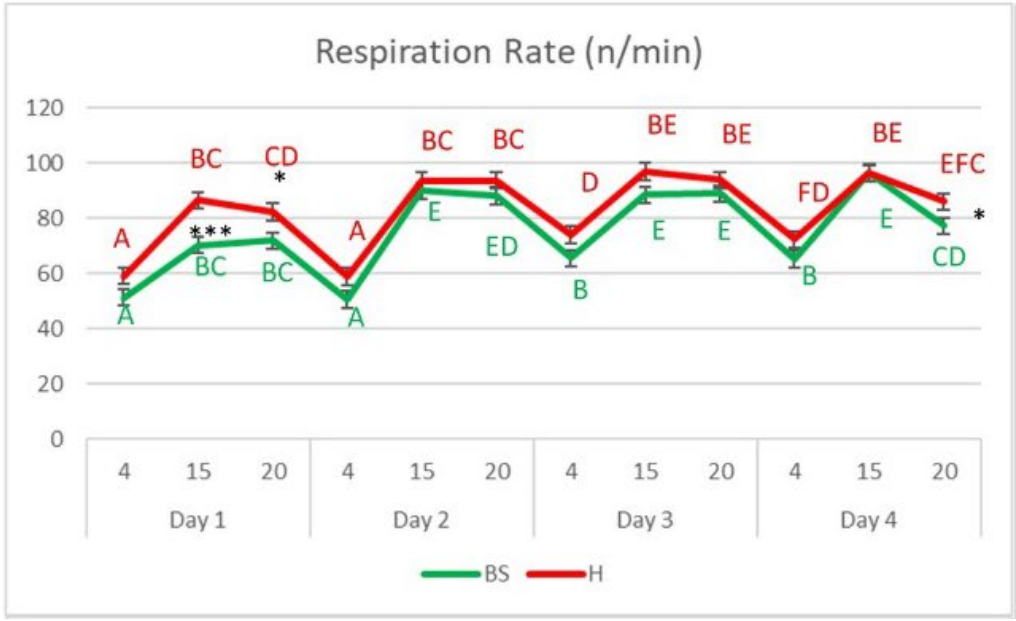
LPS depletion/LPS test

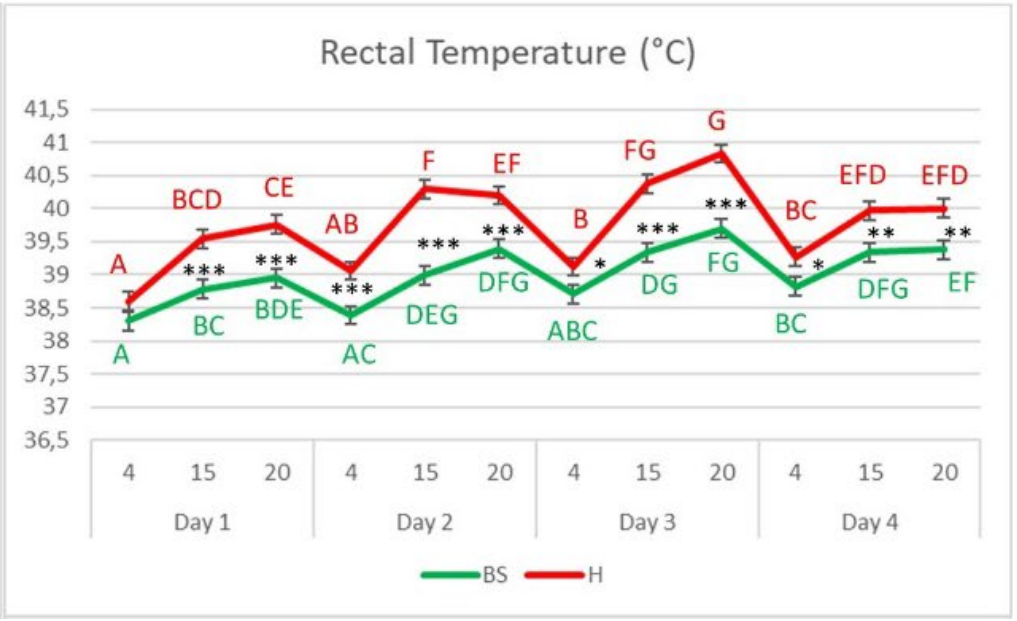
TC Exosomes

HS Exosomes

Results

Physiological parameters





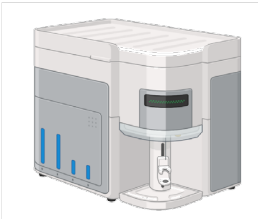
Experimental design

sEVs Characterization

- **Nanoparticle tracking analysis (NTA) – Size and Counting**

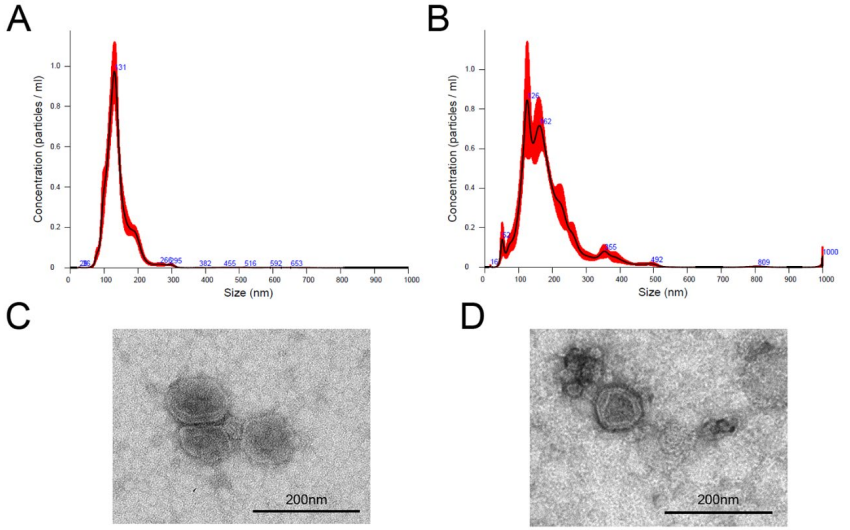
- **Transmission Electron Microscopy (TEM) – Integrity identification**

- **Flow cytometry and dot blot- Identification of exosomes**
  - **mouse anti-human CD9**
  - **mouse anti-bovine CD63**



Thermal Comfort Condition EVs

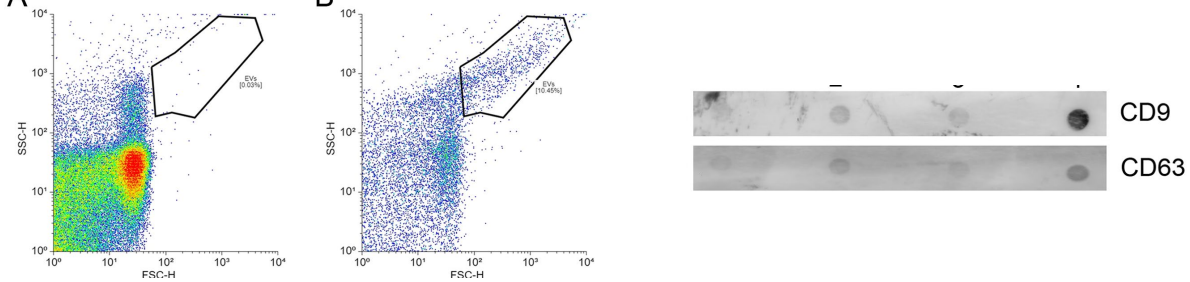
Heat Stressed EVs



Control

Bonded exosomes

Dot blot positivity

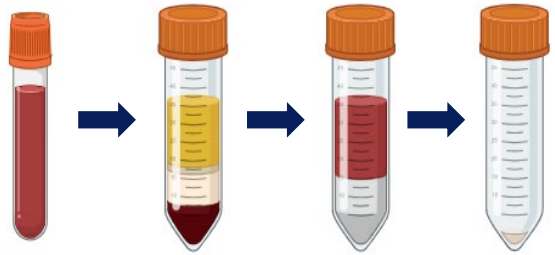


Experimental design

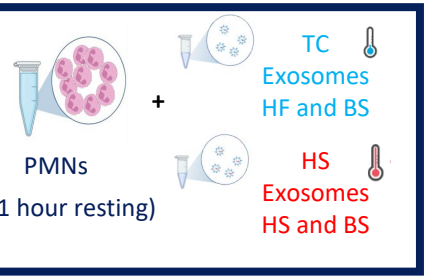
PMNs Purification and viability

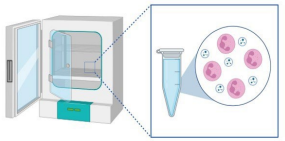
Holstein Cows in Thermal Comfort Condition

PMNs Purification



Percoll Gradient Centrifugation





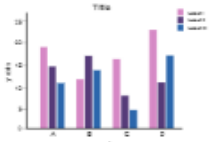
2 hours
37°C
5% CO_2

Viability Assay

Five different concentrations of protein (µg/ mL)

- 1
- 5
- 25
- 125
- 625

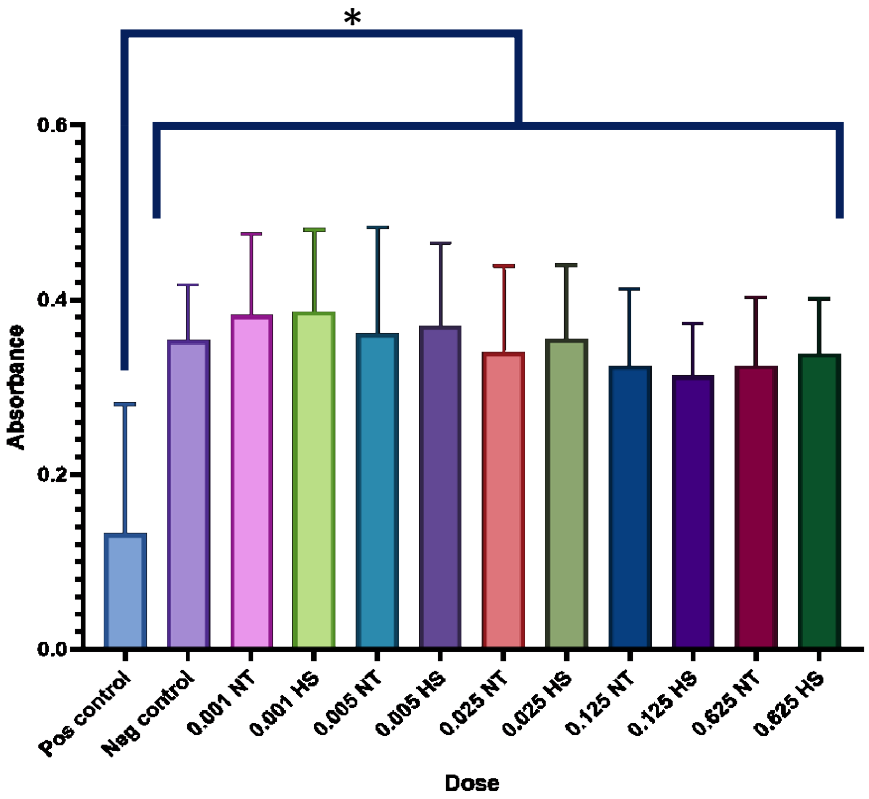
measurement



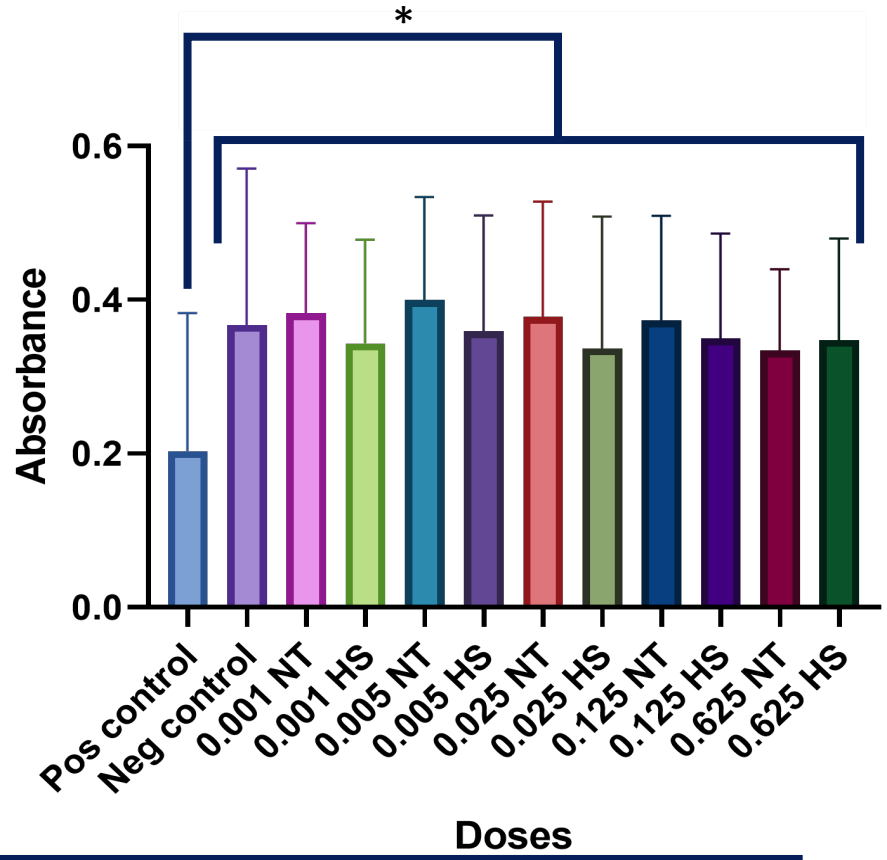
Statistical Analysis

Results

PMNs Holstein Viability



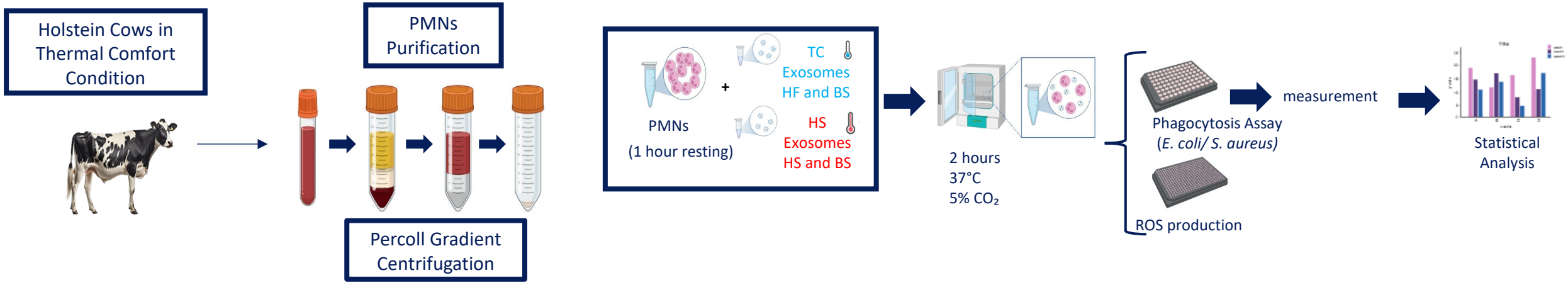
PMNs Brown-Swiss Viability



EVs did not affect the viability of PMNs at any of the concentrations analyzed ($P > 0.05$).

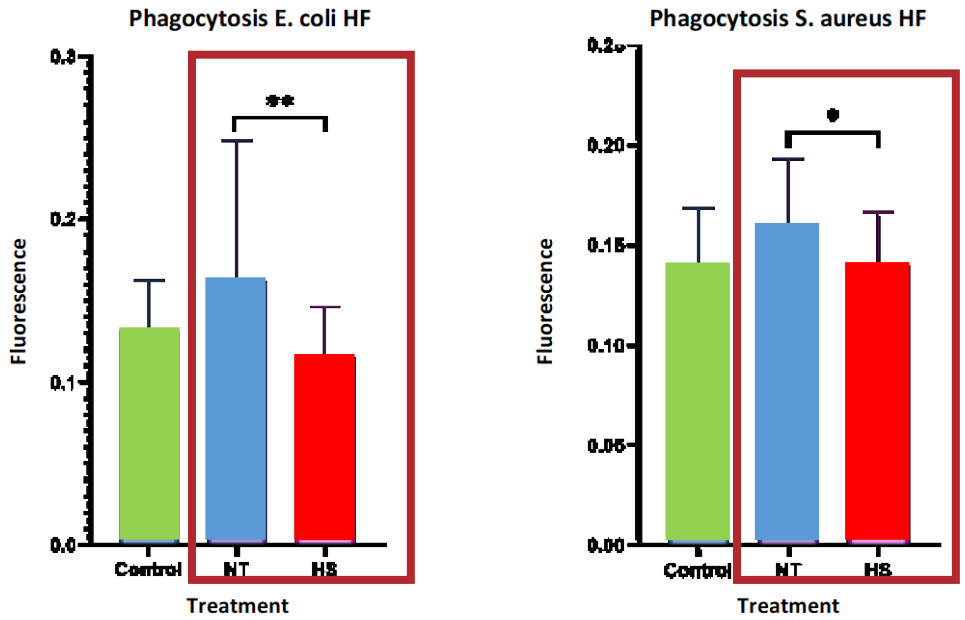
Experimental design

PMNs Purification, Phagocytosis, and ROS production assay.

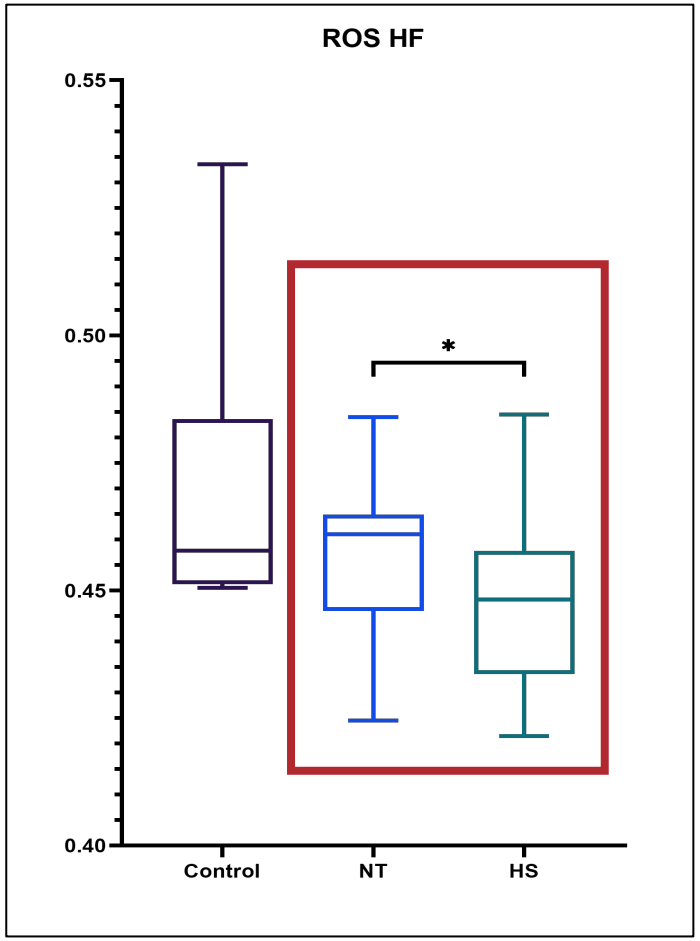


Results

Holstein PMNs Phagocytosis and ROS production



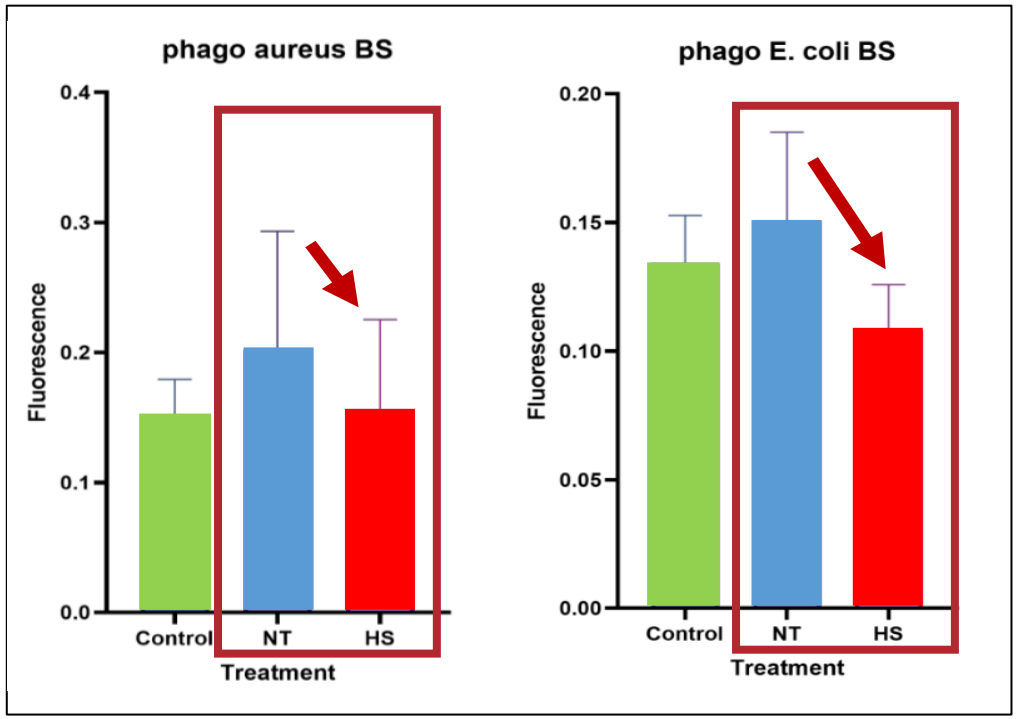
HS-EVs negatively affect the phagocytic capacity of PMNs, both against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*).



The production of ROS is modified by HS-Evs.

Results

Brown-Swiss PMNs Phagocytosis and ROS production



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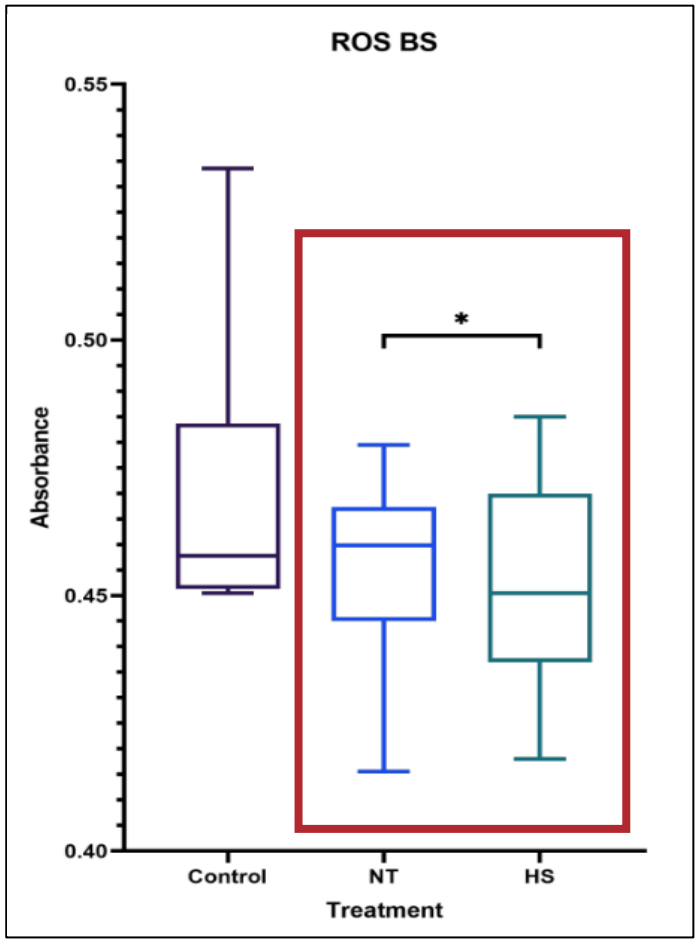


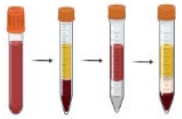
The production of ROS is modified by HS-EVs, resulting in a decrease in oxidative stress.

Experimental design

Monocytes Purification and assays

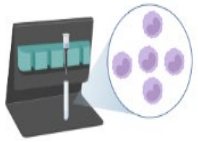
Holstein cows in Thermal Comfort Condition

PBMCs Purification

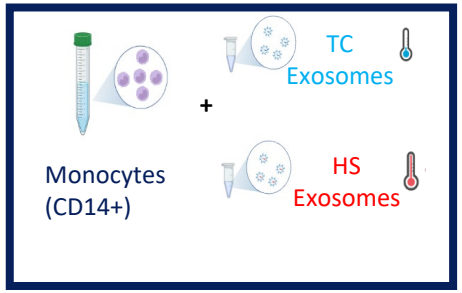


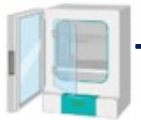
Ficoll Gradient Centrifugation

Monocytes Purification

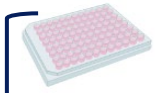


Magnetic Activated Cell Sorting (MACS)



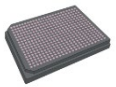


20h
37°C
5%
CO_2



Viability Assay

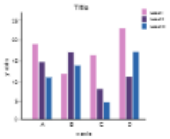
Phagocytosis Assay



Apoptosis Assay

Chemotaxis Assay

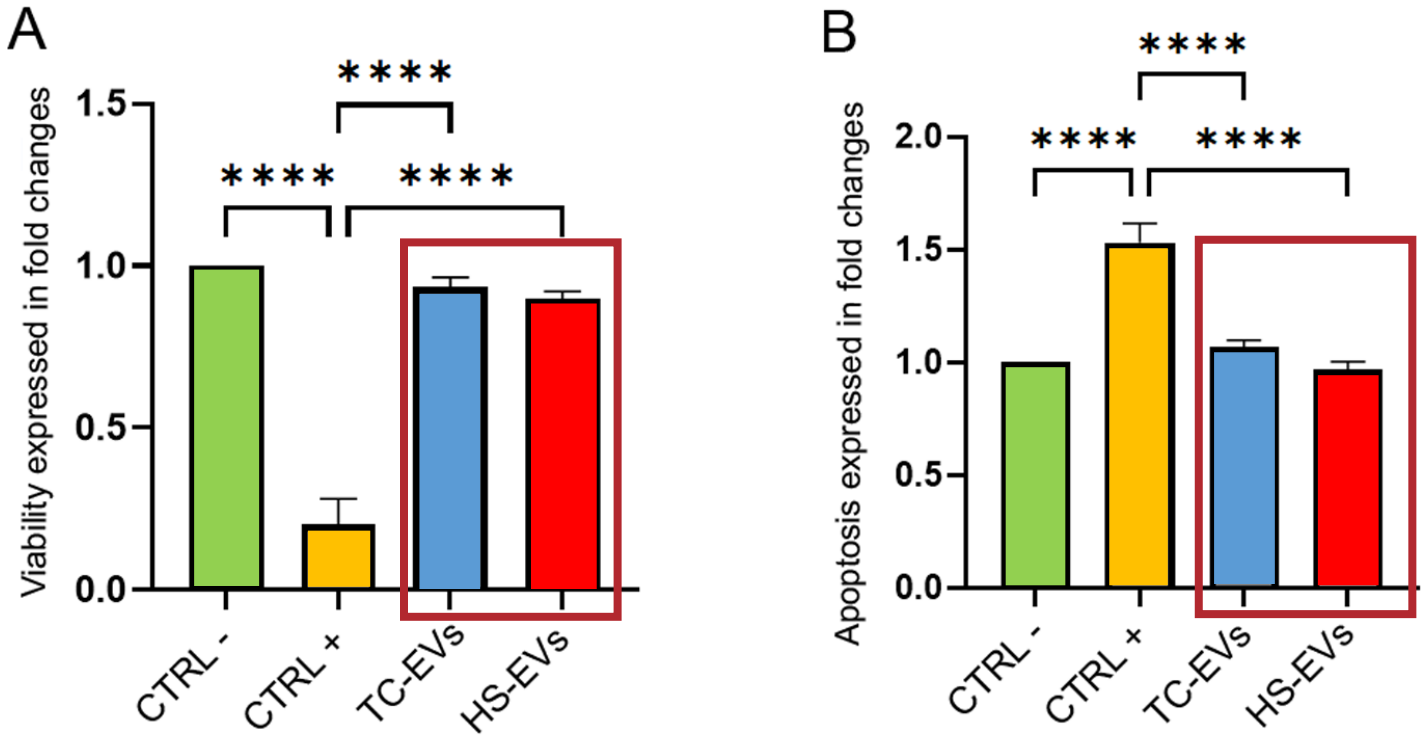
measurement



Statistical Analysis

Results

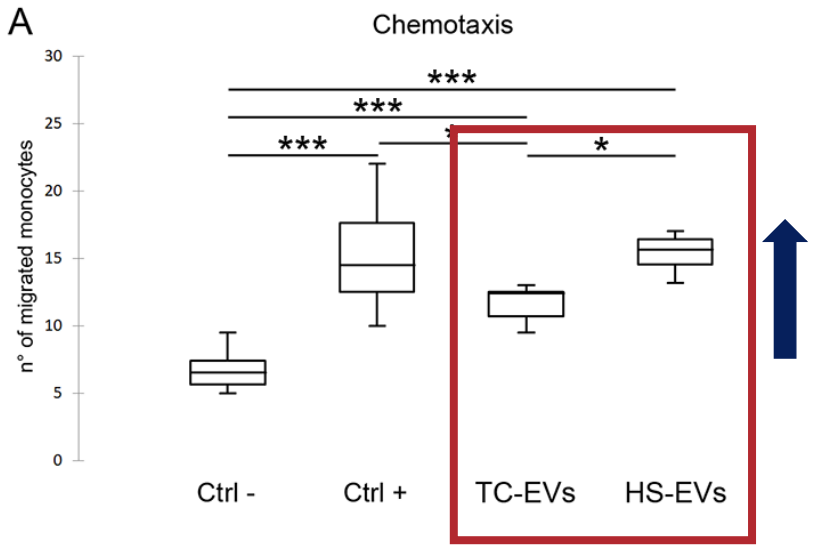
Monocytes death



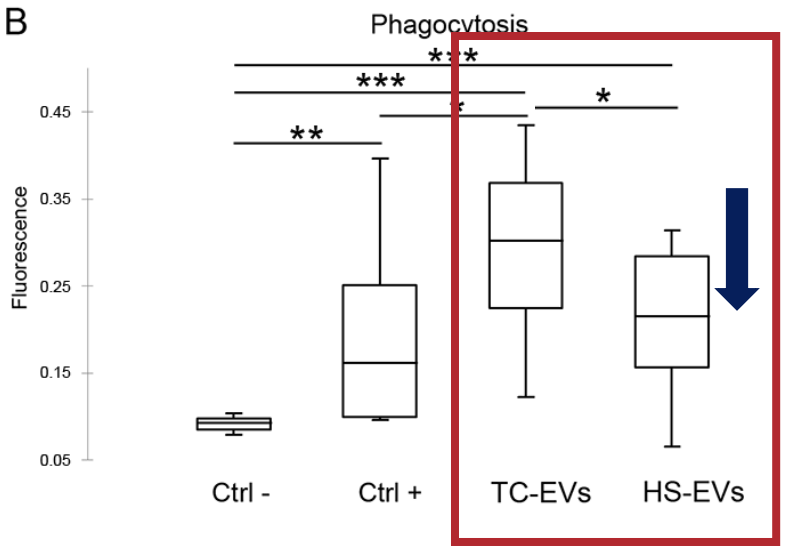
The results demonstrated that exosomes from both HS and TC cows were not toxic to cells.

Results
Monocyte response

Chemotaxis



Phagocytosis



The monocyte migration increased 32%.

The phagocytosis ability was affected by 24.79%

Take home message and Conclusion

HS-EVs increase the capacity of monocytes to migrate to the infection site;

HS sEVs hamper phagocytic capability on bovine monocytes and PMNs;

- ✓ Exosomes play an immunomodulator role;

Phagocytic capacity

↓

Oppose microorganisms

↓

This can lead to an increase in the necessity for antibiotics

- ✓ Further investigations should be done to understand if only the phagocytic capability is affected or also the capacity to kill MO;
- ✓ Increase the number of biological replicates;
- ✓ Do the same assays for both cell types;

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Thank you for your time!

Luiz Gustavo de Matos

Contact: luiz.dematos@unimi.it