divas
DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

Immunomodulatory effect of exosomes from plasma of heat-stressed cows on bovine monocytes

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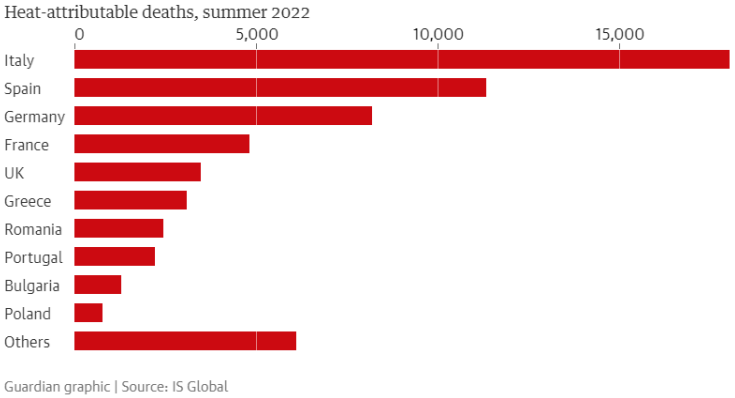
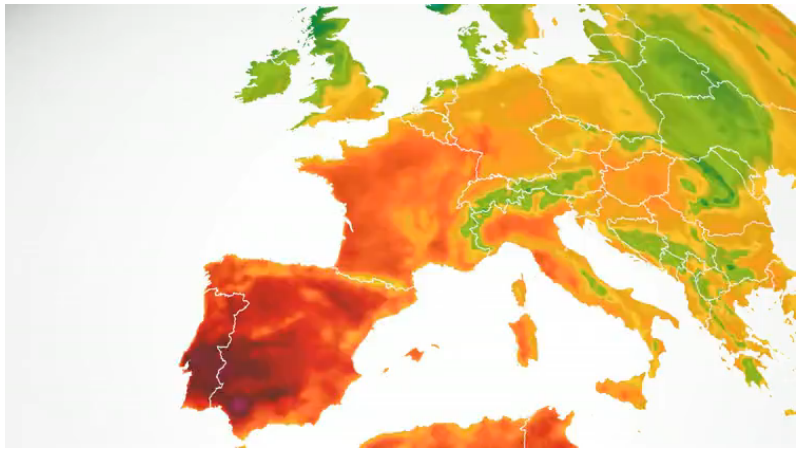
UNIVERSITÀ DEGLI STUDI DI MILANO

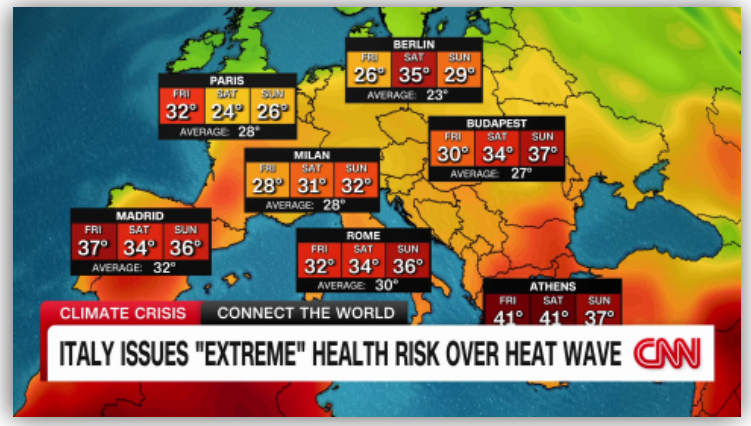
Introduction
Climate change



"Summer heat killed 61,672 people in Europe in 2022" – The Guardian, 2023

"July 2023 confirmed as hottest month on record" - World Meteorological Organization (WMO), 2023

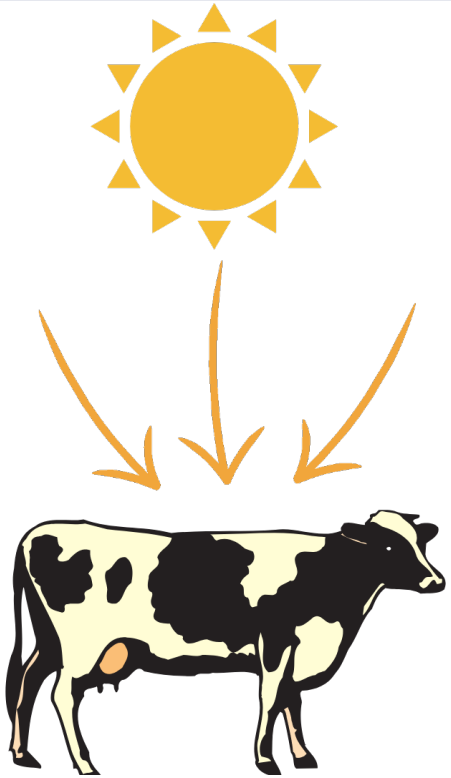




"CNN Meteorologist says world must get used to records being broken "day after day" CNN, 2023

Introduction

Heat stress



Cytokines → Immune system

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

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

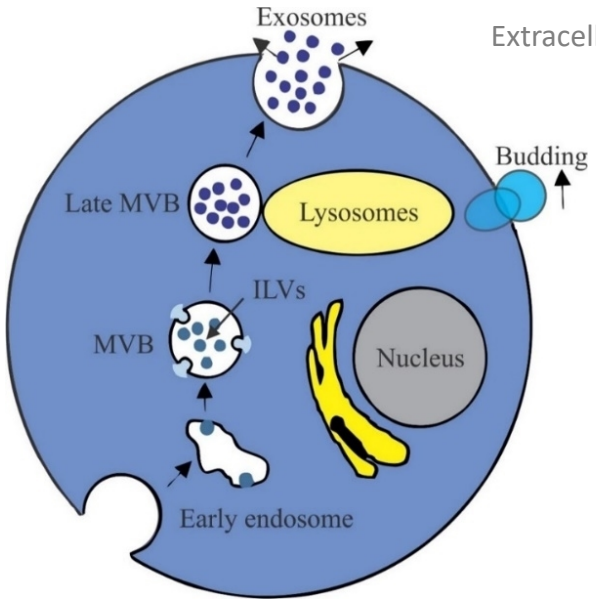
=

Animal's welfare

and

Economic losses

Introduction
Exosomes



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

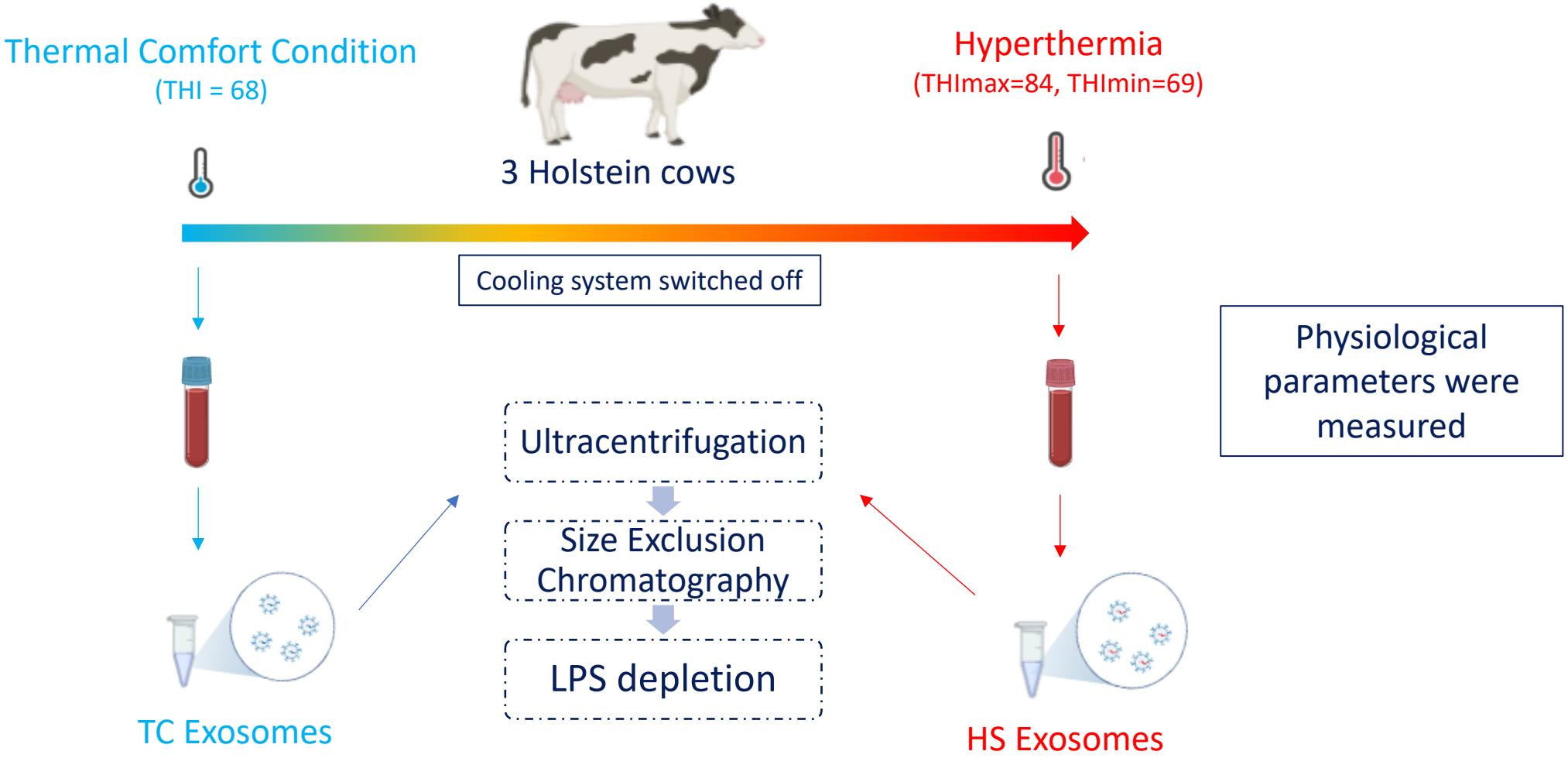
From: Mathivanan et al., 2010

Aim of the study

Assess whether exosomes isolated from cows who experienced hyperthermia *in vivo* modulated the immune response of CD14+ monocytes *in vitro.*

Materials and Methods

Exosomes Purification



Materials and Methods

Exosomes Characterization

Nanoparticle tracking analysis (NTA) - Size and Counting

Transmission Electron Microscopy (TEM) - Integrity identification

Flow cytometry and dot blot- Identification of exosomes

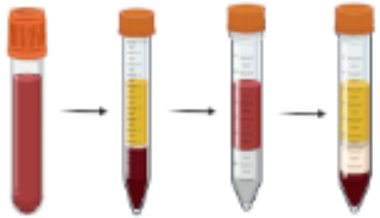
- mouse anti-human CD9
- mouse anti-bovine CD63

Material and Methods

Monocytes Purification

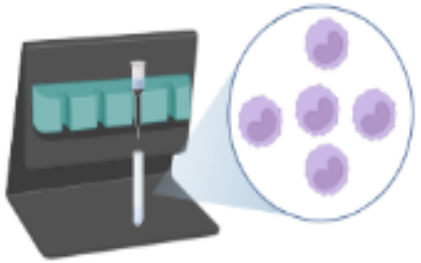
Holstein cows in Thermal Comfort Condition

PBMCs Purification



Ficoll Gradient Centrifugation

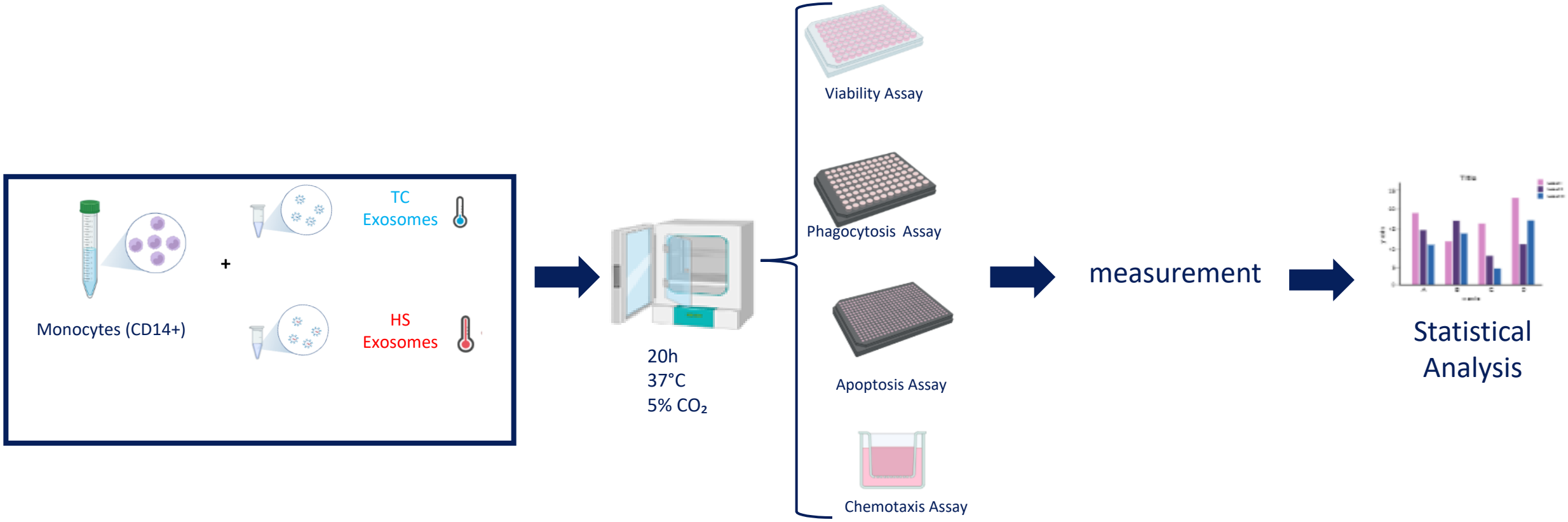
Monocytes (CD14+) Purification



Magnetic Activated Cell Sorting (MACS)

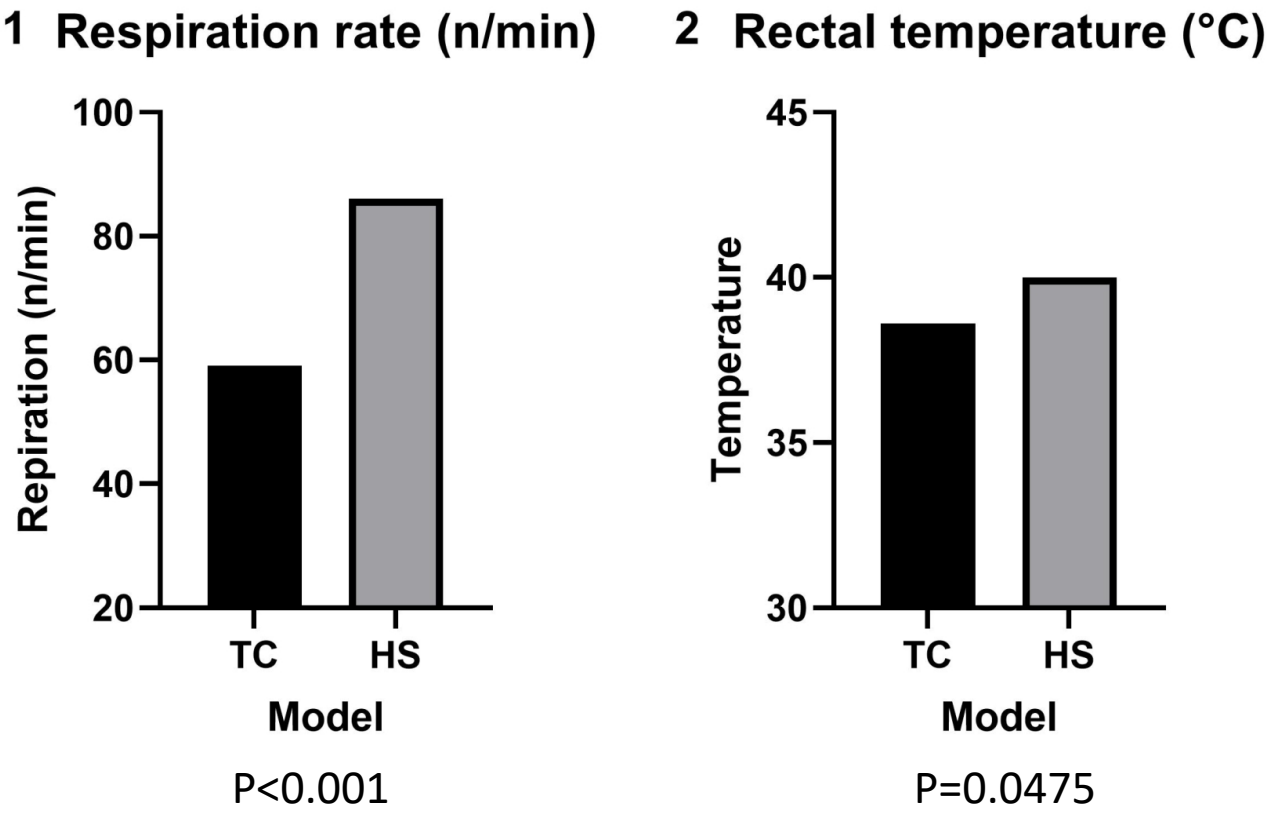
Materials and Methods

Assays



Results

Physiological parameters



Temperatures and respiration rate in Holstein dairy cows during 4 days of heat stress. ***(1)*** *Respiratory rate;* ***(2)*** *Rectal temperature;*

Results

Exosomes characterization

Nano tracking

Thermal Comfort Condition EVs

Heat Stressed EVs

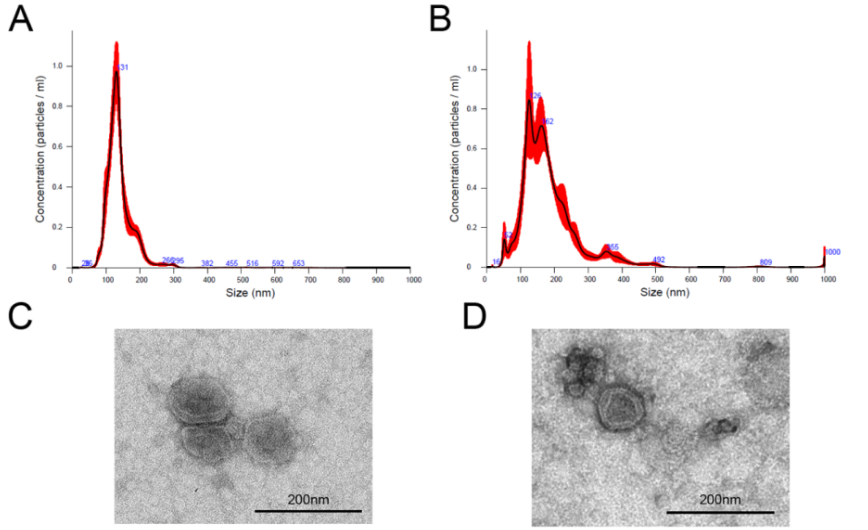
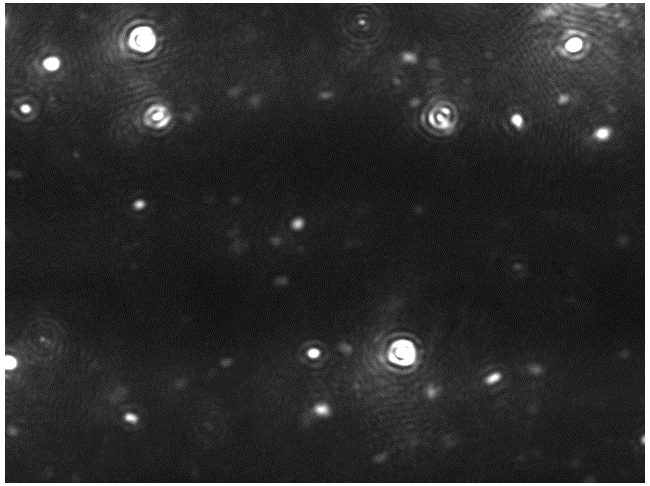


Figure 1. The mode average of TC and HS EVs was 123.06 nm ± 10.98 nm.

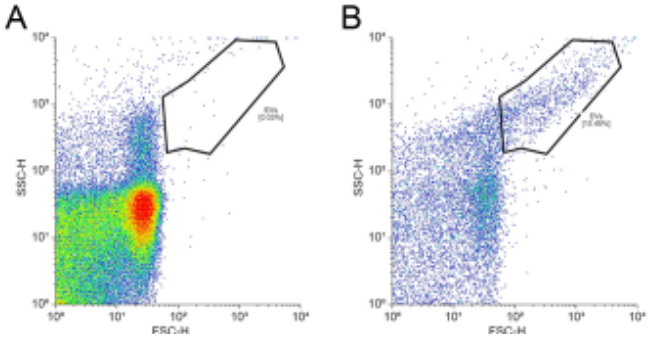


Nano tracking particle analysis (NTA)

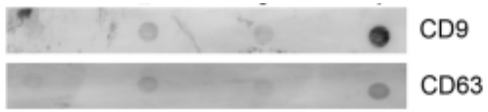
Flow cytometer and dot blot identification

Control

Bonded exosomes



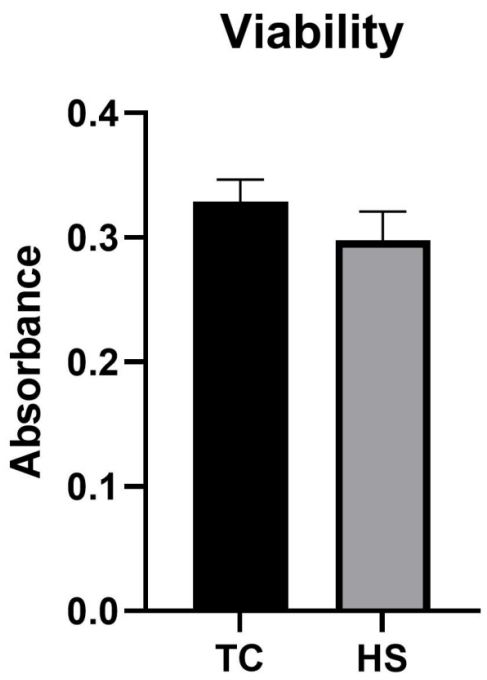
Dot blot positivity



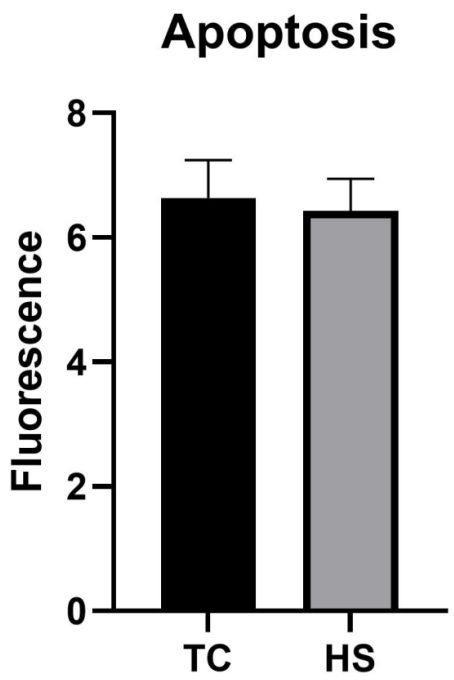
A and B show the control and exosomes bonded to the proteins CD9 and CD63 consecutively. Figure E shows the dot blot test for the proteins CD6 and CD63

Results
Cell death

A



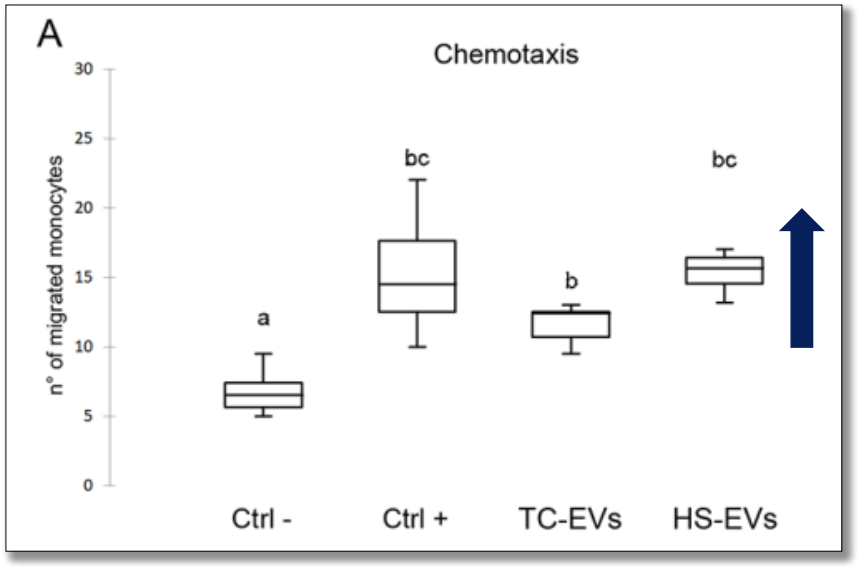
B



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

Results
Cell response

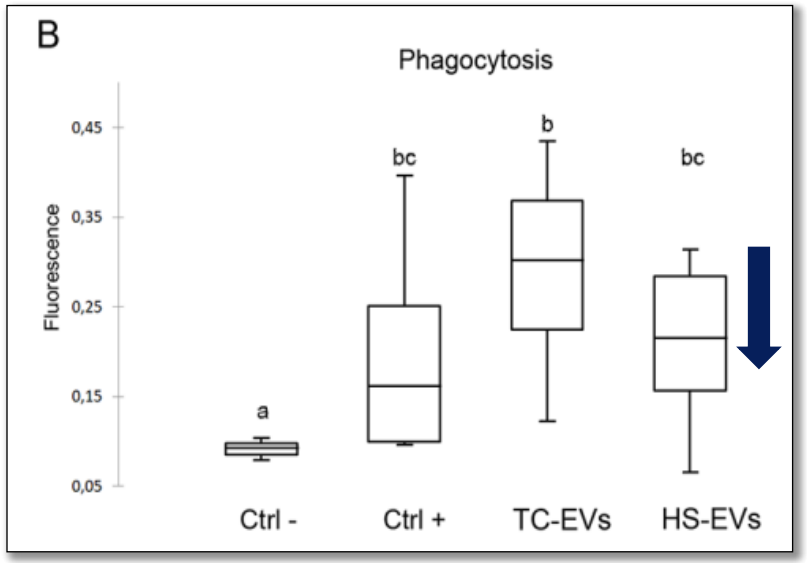
Chemotaxis



The monocyte migration increased 32%.

P = 0.0204

Phagocytosis



The phagocytosis ability was affected by 24.79%

P= 0.008

Take home message and Conclusion

HS-EVs increase the capacity of CD14+ monocytes to migrate to the infection site;

HT exosomes hamper phagocytic capability on bovine monocytes (CD14+);

✓ 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;

↓

ROS production and Killing assays/ Molecular tests

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Thank you for your time!

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