Characterization of Extracellular Vesicles at Parturition in Dairy Cows with Late Gestation Heat Stress

L.T. Casarotto[1], L. Galio[2], C. Henry[3] , P. Chavatte-Palmer[2], and G.E. Dahl[1]

[1]*Department of Animal Sciences, University of Florida, Gainesville.*

[2]*Université Paris-Saclay, UVSQ, INRAE, BREED, Jouy-en-Josas, France.*

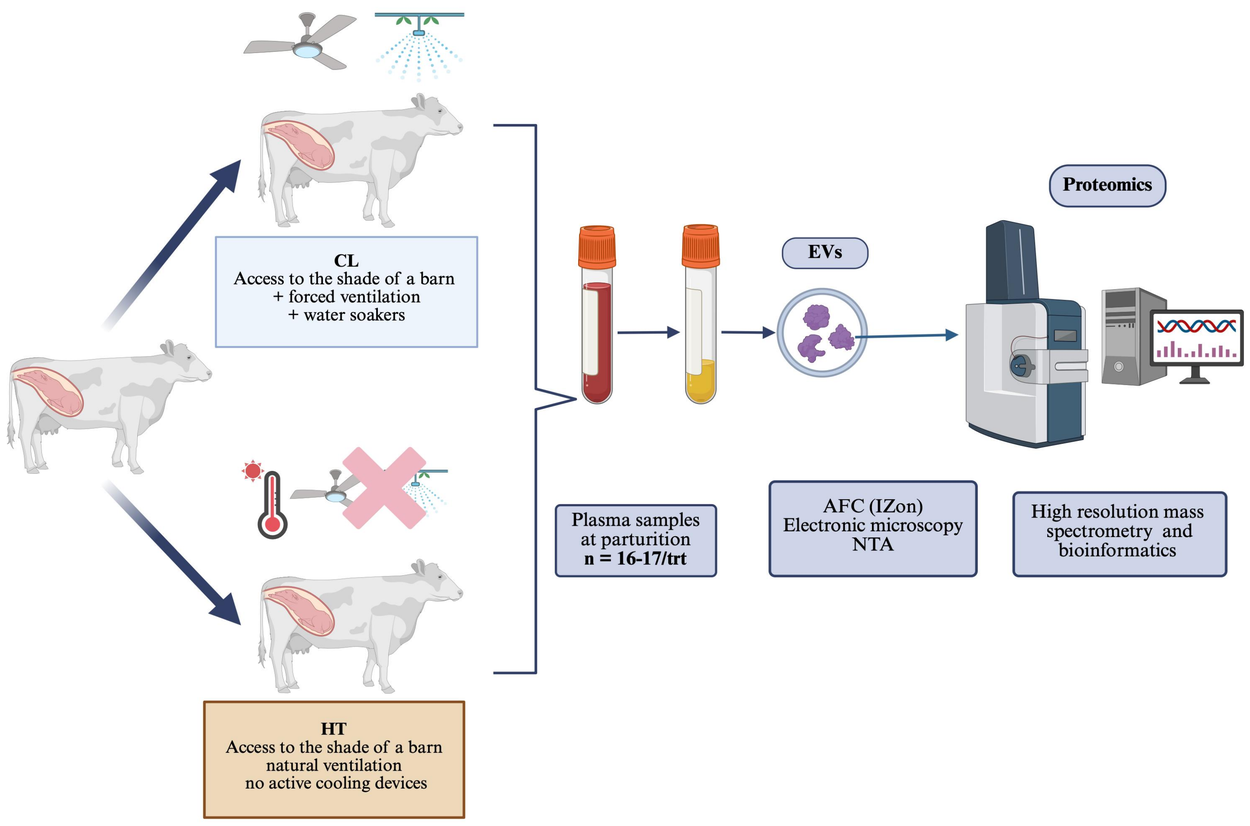
[3]*Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, PAPPSO, Jouy-en-Josas, France.*

INTRODUCTION

- Environmental heat stress (**HT**) significantly impacts lactating and dry dairy cows, leading to a range of negative effects. Specifically, it disrupts the cows normal autonomic and behavioral thermoregulatory processes, which are vital for maintaining their body temperature in hot conditions (West, 2003).
- Extracellular vesicles (**EVs**) are recognized as important mediators of cell-cell communication, capable of transferring various cargos, including proteins, nucleic acids, metabolites, and even entire organelles, between cells (Kalluri and LeBleu, 2020).
- Thus, EVs are potential biomarkers of health status.

We hypothesized that heat stress during late gestation would alter the secretion profile of EVs at parturition in dairy cows. This study aimed to assess and characterize the protein changes in EVs cargo at parturition from maternal plasma of dairy cows exposed to late gestation heat stress.

MATERIALS AND METHODS



Bioinformatic analysis was performed to identify proteins with a significance q-value < 0.01. Descriptive and statistical analyses were conducted using the results from DIA-NN with the Shiny proteom_IC tool (https://github.com/MarjorieLeduc/Shiny_PROTEOM_IC/tree/main). A two-sided, unpaired Welch t-test was applied to proteins that had at least three valid values in one group and at least 70% valid values in the other group, using log2-transformed LFQ intensity. The significance threshold was set at a p-value of ≤ 0.05. Proteins with a log2-fold change (log2FC; CL/HT) of 1.2 or greater, or 0.66 or less, were considered significantly affected.

RESULTS

EVs biomarkers from bovine plasma have been detected including several proteins significantly affected when comparing protein cargo of CL vs. HT. A total of 684 proteins were detected in total, where **20 proteins** met the significant threshold of $p \leq 0.05$ comparing **CL (n = 17) vs. HT (n = 16)** treatments.

Protein	Gene	logFC	P-value	Function
Basal Membrane and Extracellular Matrix				
Laminin subunit gamma 1	LAMC1	0.65	0.001	Cell migration; ECM disassembly; tissue development
Laminin subunit alpha 2	LAMA2	0.67	0.006	Cell migration and adhesion
HGF activator	HGFAC	-0.58	0.01	ECM space; serine protease; blood coagulation
Protein HP-20 homolog	.	-0.58	0.02	COL trimer
Collagen alpha-1(IV) chain	COL4A1	0.48	0.03	ECM component and organization; COL trimer
Collectin subfamily member 10	COLEC10	-0.95	0.04	COL trimer, ECM space
Laminin subunit beta 1	LAMB1	0.44	0.05	Cell adhesion
Pro-coagulator Factors				
Fibrinogen alpha chain	FGA	0.66	0.04	Fibrinogen complex; blood coagulation; plaques aggregation
Amine oxidase 3	AOC3	-0.65	0.04	Oxidoreductase; metal binding (Ca, Co)
Fibrinogen gamma-B chain	FGG	0.63	0.05	Fibrinogen complex; blood coagulation; plaques aggregation
Fibrinogen beta chain	FGB	0.66	0.05	Fibrinogen complex; blood coagulation; plaques aggregation
vonWillebrand factor	VWF	1.08	0.05	Blood coagulation; cell adhesion
Immunity and Cell Surface Receptors				
Ig-like domain-containing protein	.	-1.00	0.01	ECM space; immune response
Ig lamda chain variable region	.	-1.22	0.02	.
Ig heavy chain variable region	.	-0.68	0.05	Immunoglobulin complex
Transferrin receptor protein 1	*TFRC*	0.64	0.02	Iron receptor
TFG protein	*TFG*	-0.98	0.04	Vesicle transport
SPN protein	*SPN*	0.43	0.05	Cell surface receptor
A0A3Q1LVJ5	.	-0.77	0.02	.
A0A3Q1NKM0	.	0.49	0.007	.

CONCLUSIONS

In conclusion, this study demonstrates that late gestation heat stress induces significant changes in the protein cargo of maternal EVs at parturition, particularly in proteins associated with extracellular matrix remodeling, coagulation, and immune responses. These alterations may compromise maternal recovery and immune function during the transition to lactation, potentially increasing susceptibility to postpartum health disorders. The identified EV proteins may serve as biomarkers for assessing maternal adaptation and recovery, emphasizing the importance of environmental management during the dry period to safeguard dairy cow health and productivity.

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