# Investigating Plasma Inflammatory Cytokine Levels' Relationship to Pregnancy Outcomes in *Bos Taurus* Heifers

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Anytime a heifer (a bovine female from weaning until the first parturition event) or cow (bovine female which has already calved at least once) fails to calve, it is costly to the individual producer and the beef industry as a whole. Open beef heifers are one of the costliest inefficiencies on an operation because the producer has already expended resources to raise her, and the heifer will fail to produce progeny. If a producer pregnancy checks their herd at weaning, an open cow is less costly compared to an open heifer because the cow can be culled before any extra feed is invested in her. However, the producer still incurs the cost of replacing the cow and many producers do not pregnancy check their cattle. Only 34.5% of all beef producers in the United States use pregnancy detection as a management tool to make culling decisions (Lamb et al, 2014). For nearly two-thirds of all United States' beef producers, open cows are first diagnosed at the end of the subsequent calving season. While the producer can opt to cull open cows at the end of the calving season, the cost of maintaining her without a return has already been incurred for a year (Lamb et al, 2014). In the Southeast, it typically takes a raised replacement heifer six successful parturition events to pay for the cost of her development and maintenance in the herd. If a cow fails to calve in one of her first six opportunities and the producer opts to keep her, it takes nine parturition events to pay for the cost of her development and maintenance in the herd (Boyer et al, 2020). The time it takes to receive a profitable return from an individual beef cow emphasizes the importance of fertility in beef production. In 2014, Lamb et al estimated the total cost of infertility to the entire United States beef industry to be about $2.8 billion annually (Lamb et al, 2014).

The goal of this research project is to test possible biomarkers as predictors for future reproductive potential in beef heifers. The ultimate goal is to find a reliable biomarker in the plasma and a test which accurately quantifies that biomarker. The test could then be used as a predictor of future reproductive success, or lack thereof, in beef heifers. The specific objective of this project is to investigate the relationship between plasma inflammatory cytokine levels and pregnancy results in heifers.

Estrous synchronized Angus and Angus-cross heifers were utilized for this project. Following estrous synchronization, fixed-time artificial insemination (FTAI) was performed on the heifers and fourteen days later a fertile bull was introduced for three consecutive estrous cycles. Heifers conceiving from FTAI were labelled as high-performing heifers, while those heifers which remained open following both FTAI and bull exposure were labelled as low-performing heifers. Previous research conducted in our lab identified metabolites linked to inflammation in the blood plasma which were at different levels at weaning in high-performing and low-performing heifers (Phillips et al, 2018). Furthermore, mRNA transcript levels for the inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), differed in the white blood cells between these two groups (Phillips et al, 2018). In the current project, we evaluated the levels of TNF-α and IL-6 proteins in the plasma using Western blotting. The levels of the two proteins were compared in high-performing (n=9) and low-performing (n=6) heifers in blood samples drawn at weaning and at the time of AI. Target proteins were quantified using ImageJ densitometry and compared using a t-test in GraphPad Prism software.

Based on the previous findings in our lab of significantly different mRNA transcript levels for IL-6 and TNF-α in the

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white blood cells between high-performing and low-performing heifers (higher transcript levels in the low-performing heifers) (Phillips et al, 2018), the hypothesis was the low-performing heifers would have higher plasma protein levels for IL-6 and TNF-α. However, no statistical difference was found in either of the cytokine levels in the samples taken at the time of weaning (Figures 1 and 2) or the samples taken at the time of artificial insemination. Several possible explanations could explain this observation. The obvious conclusion to draw from this study is neither IL-6 nor TNF-α are adequate as a potential predictive biomarker for future reproductive success in beef heifers. An explanation for the difference in the transcript levels and the protein levels could be a post-transcription regulation factor. Oftentimes, mRNAs function as a regulatory factor by preventing the translation of mRNA to protein. There are many other regulators of translation as well, and one or more of these regulatory factors could potentially cause the differences seen in this protein study compared to the white blood cell transcript study. While this study focused on comparing the cytokine levels based on a per volume of plasma basis, the protein levels could also be compared on a total plasma protein basis. Work was devoted to this thought during the project; however, running the Western blots on an equal total protein load did not result in any significant difference in the cytokine levels between the low-performing and high-performing heifers.

## Statement of Research Advisor

Over the past year Gavin has worked to establish and test a replicable method in the lab to compare inflammatory cytokines, in bovine blood plasma. This assay will permit examination of other blood parameters in heifers at various stages of development.
- *Paul Dyce, College of Agriculture*

## References

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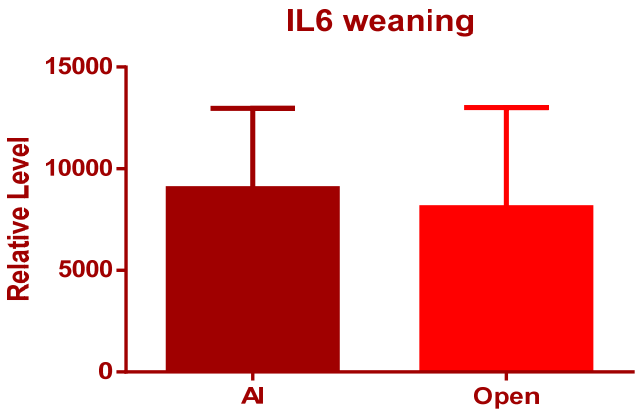



**Fig. 1** Comparison of the relative levels of IL-6 in equal volumes of blood plasma at weaning in high-performing (bar labelled AI) and low-performing (bar labelled Open) heifers.

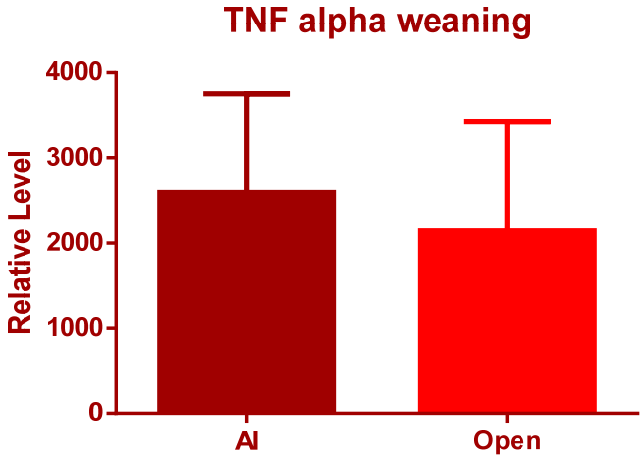
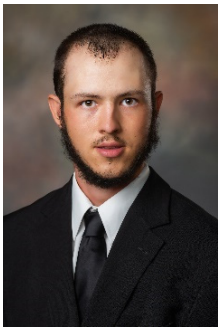
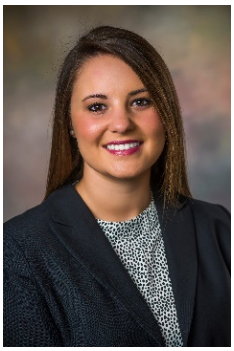



**Fig. 2** Comparison of the relative levels of TNF-α in equal volumes of blood plasma at weaning in high-performing (bar labelled AI) and low-performing (bar labelled Open) heifers.

## Authors Biography



Gavin L. Rankins is a May 2022 graduate of Auburn University with a B.S. degree in Animal Sciences – Animal/Allied Industries. He has been member of the Auburn University Reproductive Biology Lab from May 2021 until graduation with a research focus on proteins.



Rachel A. Hollingsworth is pursuing an M.S. degree in reproductive biology in the Department of Animal Sciences of the College of Agriculture at Auburn University. She received a B.S. degree in Animal Sciences at Auburn University. Her current research is focused on utilizing molecular biology tools and techniques to validate potential targets which might serve as indicators of beef heifer fertility.

Erin E. Mahoney is a May 2020 graduate of the University of Tennessee at Martin. She received a B.S degree in animal science and a B.S degree in veterinary technology at UTM. She is in her second year of her masters studying reproductive biology and bovine fertility. She hopes to receive a spot at a U.S. vet school upon graduation.

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