Development of Research Tools: In Search of Heifer Fertility Biomarkers

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Often in the cowcalf industry, a proportion of selected heifers (bovine females that have not yet calved) fail to become pregnant following their first breeding season. When a producer raises a heifer to full reproductive maturity or purchases a reproductively mature heifer and she is unable to calve, the cost that it took to raise or purchase that heifer is not fully recovered. This is detrimental to the producer and the overall beef industry. Infertility in the beef industry is estimated to cost over $4.7 billion annually (Prevatt et al, 2018). When replacement heifers are selected, they are chosen based on many genetic and phenotypic factors including body condition, age, disposition, reproductive tract score, and the performance of their parents. Even after taking these factors into account, a proportion fails to become pregnant in their first breeding season.

The objective of this research project is to investigate different candidate antibodies to potential biomarkers that were previously identified as different in fertile and sub-fertile heifers, at the transcript level. In this project we are investigating differences in protein expression in the endometrial tissue of fertile and subfertile heifers. The overall goal is to develop fertility-based biomarkers in heifers. For this specific project, only protein levels in the endometrial tissue will be investigated.

A group of Angus and Angus-crossed heifers were put through an estrous synchronization and artificial insemination (AI) program. This was then followed by natural service (NS), by placing them with a fertile bull, for two additional estrous cycles. After completion of the program, the heifers were grouped as either fertile (pregnant from AI), pregnant from NS, or subfertile (failed to become pregnant). In previous research from our lab, using samples from these heifers, RNA based molecular targets were identified that were expressed differently between the fertile and subfertile groups of heifers. In this current project, six biomarkers were evaluated including pannexin 1 (PANX1), connexin 43 (CX43), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), delta and notch-like epidermal growth factor-related receptor (DNER), and growth arrest and DNA damage inducible gamma (GADD45G). The protein level expression for each marker was evaluated using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting techniques. The antibodies for the markers were applied to the blots and the bound quantities were analyzed and com-pared between the two groups of heifers. Image Lab software was used to determine the relative quantity of the proteins using densitometry, then raw values were statistically compared using a t-test and GraphPad Prism software.

In previous research done in our lab, it was found that there is a significant difference between fertile and sub-fertile heifers for the biomarkers chosen. For IL-6 and TNF-α, there were significantly different mRNA transcript levels in the white blood cells between the two groups (Phillips et al, 2018). The granulosa expression profiles between fertile and sub-fertile heifers were significantly different (p value < 0.05) for DNER and GADD45G (Hollingsworth et al, 2023). Due to these previously detected differences, we were interested in investigating whether any of these markers were different at the protein level in endometrial tissue. However, there was no significant statistical difference between the fertile and subfertile groups for any of the markers we tested (Figures 1, 2, and 3). Based on these results, we can conclude that Panx 1, Cx43, IL-6, TNF-α, DNER, and GADD45G are not expressed differently at the protein level in the endometrial tissues of heifers with differing fertility. This study focused specifically

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on protein expression in endometrial tissue, but the Dyce Laboratory is currently investigating other tissues and targets.

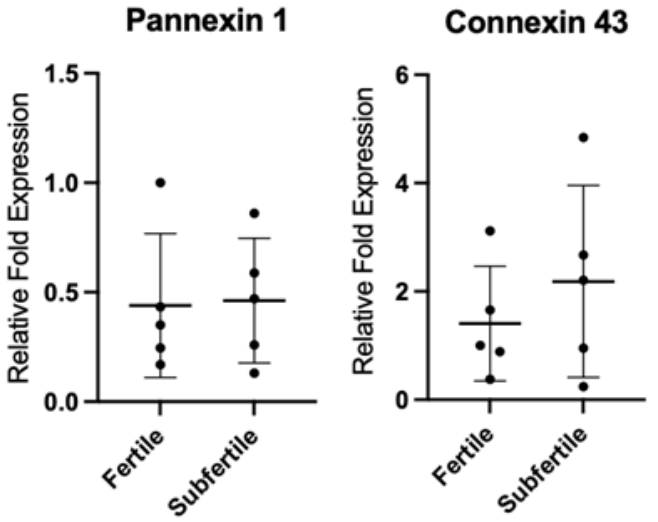

Fig. 1 Comparison of protein level expression of Pannexin 1 and Connexin 43 in endometrial tissue between fertile and sub-fertile heifers.

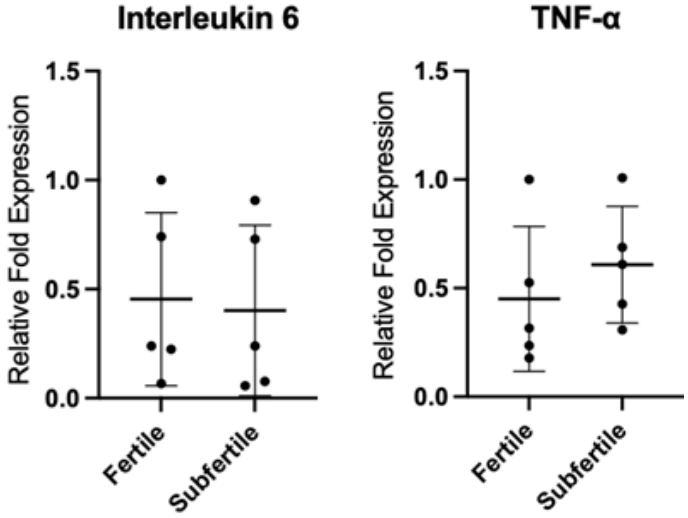

Fig. 2 Comparison of protein level expression of Interleukin 6 and TNF-α in endometrial tissue between fertile and sub-fertile heifers.

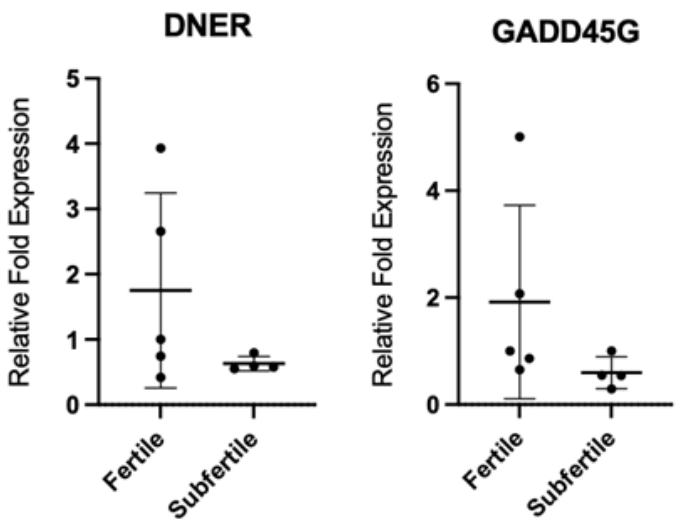

Fig. 3 Comparison of protein level expression of DNER and GADD45G in endometrial tissue between fertile and sub-fertile heifers.

Statement of Research Advisor

Over the past year Morgan has worked to gain protein isolation and analysis skills. Her work culminated in testing potential protein biomarkers in the endometrial layer of fertile and sub fertile beef heifers. This highlight will permit examination of other tissues in heifers at various stages of development.

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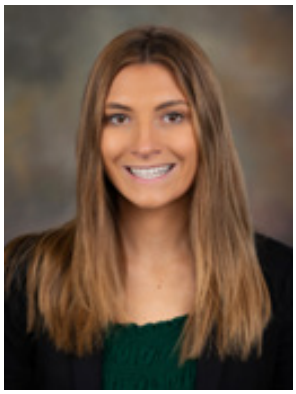
Authors Biography



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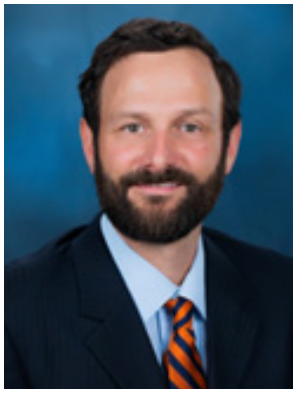

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