Shackouls Honors College Undergraduate Research Scholars Program

Deliverables

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1.) Abstract

**Meeting**: ASAS/ADSA Joint Annual Meeting in Orlando, FL July 12-15, 2015. Abstract accepted for poster presentation as number W260.

**Title:** Examining peripheral activity of catechol-O-methyltransferase (COMT) in Holstein cows following artificial insemination

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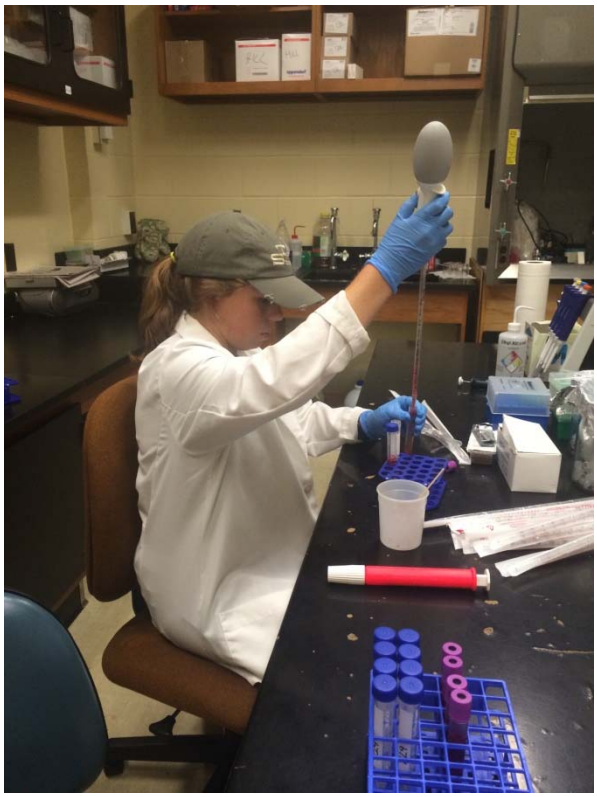
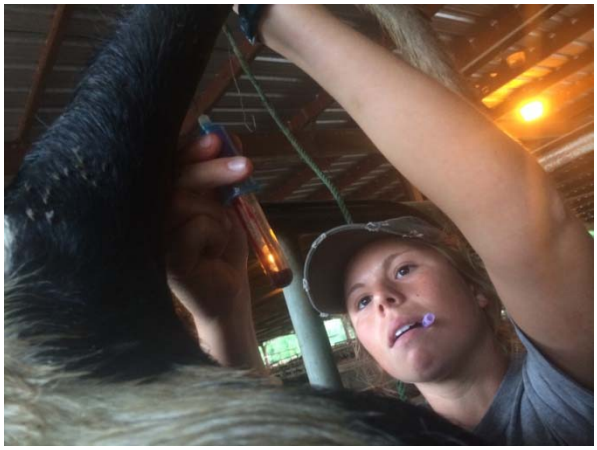
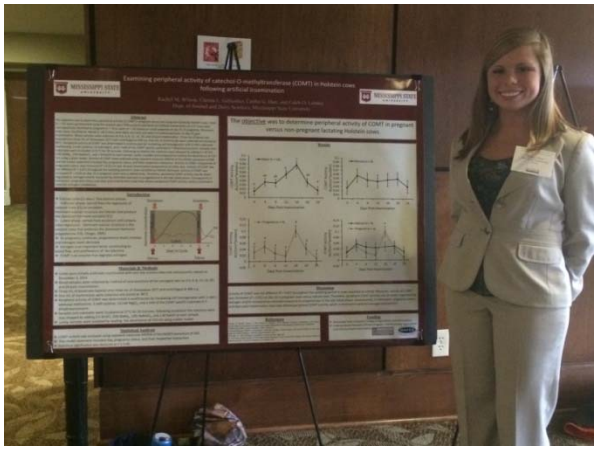
**Abstract:** The objective was to determine peripheral activity of COMT in pregnant versus non-pregnant lactating Holstein cows. Cows (n = 22) were synchronized using the ovsynch plus CIDR protocol and bred via artificial insemination on d 0. Cows were retrospectively classified as pregnant (n = 4) or open (n = 14) based on rectal palpation at day 35 of pregnancy. Moreover, cows were classified as rebred (n =4) if they came back into heat and were re-inseminated prior to day 25 post-insemination. Blood samples were collected on d 0, 4, 8, 12, 16, 20, and 24 post-insemination. Three mL of blood was layered onto three mL of Histopaque 1077 and centrifuged at 400 x *g*. One mL of erythrocytes were collected and stored at -80°C. Peripheral activity of COMT was determined in erythrocytes by incubating cell homogenates with 2 m*M* s-adenosyl methionine, 3 m*M* cysteine, 10 m*M* $MgCl_2$, and 1 m*M* of the COMT specific substrate 6-7-dihydroxycoumarin. Samples and substrates were incubated at 37°C for 30 minutes. Following incubation the reactions were stopped by adding 0.5 *M* HCl, 10% $NaN0_2$, 10% $NaMoO_4$, and 1 *M* NaOH to each sample. Lastly, samples were analyzed by reading the absorbance at 510 nm using a plate reader. Activity of COMT were analyzed using repeated measures ANOVA of the MIXED procedure of SAS and the model statement included day, pregnancy status, and their respective interaction. Activity of COMT, irrespective of pregnancy status, was increased ($P < 0.01$) on day 16 post-insemination compared to all other days. Activity of COMT was not different ($P = 0.87$) throughout the sampling period in cows classified as rebred. Moreover, activity of COMT was increased ($P < 0.01$) on day 16 in pregnant cows versus rebred cows. Therefore, peripheral COMT activity may be down-regulated by estrogen and/or increased by extended exposure to progesterone in the late luteal phase. Consequently, in retrospect, pregnancy status and days post-insemination may have altered peripheral COMT activity, which is involved in catechol-estrogen metabolism.

**Keywords:** catechol-O-methyltransferase, erythrocytes, pregnancy

2.) Pictures







3.) Report

During my research, I examined the peripheral activity of catechol-O-methyltransferase (COMT) in pregnant versus non-pregnant Holstein cows. The activity of COMT has not been previously studied in cattle; therefore, we are the first group to do so. However, it has been examined in humans and mice displaying that this specific enzyme's activity can be detrimental to pregnancy. Therefore, I was interested in researching the activity of COMT in cattle and examining if it was possibly correlated to early pregnancy maintenance.

The bovine estrous cycle is approximately twenty one days consisting of two distinct phases. First, the follicular phase is the period beginning with the regression of corpora lutea (CL) and ongoing until ovulation. The dominant ovarian structures during this phase are follicles that produce the hormone estradiol (E2). Secondly, the luteal phase is the period beginning at the onset of ovulation until regression of the corpus luteum. The dominant ovarian structure during this phase is the corpus luteum which produces the hormone progesterone (P4). However, as pregnancy continues, progesterone levels increase and estrogen levels decrease. Estrogen is an important hormone that has been implicated in regulating uterine blood flow and proliferation of the placenta. Progesterone helps to maintain pregnancy when it is established. As pregnancy becomes more established, estrogens role becomes more vital due to its facilitation in blood flow and growth to the placenta along with the fetus located inside the placenta.

As you can see, both hormones are critical during pregnancy. COMT is an enzyme that metabolizes estrogen. As I stated earlier, estrogens role as a hormone becomes more critical as pregnancy is established because it is facilitating growth, blood flow, and oxygen to both the placenta and fetus if pregnancy is established. Therefore, if COMT is present and subsequently degrading the vital hormone, estrogen, then possible detrimental pregnancies could occur. Consequently, I was interested in measuring the activity of COMT in pregnant versus open (non-pregnant) cows to investigate if COMT was possibly the basis of low reproductive efficiency in dairy cattle.

Initially, ovsynch plus CIDR (controlled internal drug release) protocol was used for estrus synchronization in the cows. Subsequently, they were bred by artificial insemination on day zero. Cows were then retrospectively classified as pregnant or open by rectal palpation on day thirty-five. Due to the typical trait of low reproductive efficiency associated with dairy cattle, fourteen out of the twenty-two total cattle were deemed open and four were consequently rebred prior to the end of blood sampling. These four cows were rebred on December 4, 2014. Artificial insemination breeding service method was implemented approximately at timing of when cows came back into heat, prior to day twenty-five post-insemination. Blood samples were collected by vena puncture method of the coccygeal vein (commonly referred to as tail-bleeding) on day 0, 4, 8, 12, 14, 16, 20, and 24 post insemination. Three mL of blood were layered over three mL of

histopaque 1077 solution and centrifuged at 400 x g. One mL of erythrocytes were collected and stored at minus eighty degrees Celsius. Peripheral activity of COMT was determined in erythrocytes by incubating cell homogenates with 2 mM s-adenosyl methionine, 3 mM cysteine, 10 mM magnesium chloride, and 1 mM of the COMT specific substrate 6-7-dihydroxycoumarin. Samples and substrates were then incubated at 37°C for thirty minutes followed by the reactions being haulted due to the addition of 0.5 M hydrogen chloride, 10% sodium nitrate, 10% sodium molybdate, and 1 M sodium hydroxide to each sample. Samples were then analyzed by reading the absorbance at 510 nanometers using a plate reader. Activity of COMT was analyzed using ANOVA of the mixed procedure of SAS. The model statement included day, pregnancy status, and their respective interaction. Statistical significance was declared at $P \leq 0.05$. ANOVA stands for Analysis of Variance which is a collection of statistical models used in order to analyze the differences between group means and their associated procedures. SAS stands for Statistical Analysis System which is a software system used for advanced analytics, business intelligence, data management, and predictive analytics.

Orientation towards the included following graphs (Figure 1 - 4) displays the x-axis signifying days post-insemination, along with the y-axis signifying COMT activity. In the figure 1, as stated earlier, fourteen cattle were deemed open on day thirty-five of pregnancy by rectal palpation. As you can see, the activity of COMT increased from day zero to day twelve and further increased at day sixteen (Figure 1). Day four and day eight are simply intermediates (Figure 1). Due to my project dealing with only twenty-two cows, the numerical values of enzyme activity had no significant connotation. I was solely interested in the fluctuating statistical values.

In Figure 2, pertaining to the four rebred cattle, there was no variation over time in COMT activity. Moreover, in Figure 3, pertaining to the pregnant cattle, COMT activity displayed no disparity over the time period from day zero to day twelve. However, at day sixteen enzyme activity increased significantly. This increase in activity could be caused from a phenomenon termed “Maternal recognition of pregnancy.” This occurrence takes place approximately at day sixteen in pregnant cattle. During maternal recognition of pregnancy the conceptus signals to the dam that she is pregnant, therefore estrogen levels increase and COMT activity subsequently increases.

Lastly, in Figure 4, displaying enzyme activity in both the open and pregnant cattle, COMT activity increased at day sixteen. Currently, my hypothesis is that a fetal presence in the open cattle caused this peak in enzyme activity at day sixteen. Therefore, it could have been possible that fetal loss occurred in the open cattle at day sixteen due to the increased COMT activity. However, COMT activity was also increased in the pregnant cows at day 16, so my interpretation is that increased COMT at day 16 does not cause embryonic loss

In conclusion, activity of COMT, irrespective of pregnancy status, was increased on day sixteen in pregnant cows versus rebred cows which displayed COMT activity that was

indifferent throughout the sampling period. Peripheral COMT activity may be down regulated by estradiol and or increased by extended exposure to progesterone in the luteal phase. Therefore, in retrospect, pregnancy status and days post-insemination may have altered peripheral COMT activity, which is involved in catechol-estrogen metabolism. To further investigate COMT activity and function, I am currently examining ten cattle in late pregnancy, collecting blood samples and examining both estrogen and enzyme activity in order to confirm my hypothesis of the function of this enzyme.

Figure 1.) Catechol-O-methyltransferase (COMT) activity in dairy cows classified as non-pregnant (open). Means with different letters represent significant differences across days post-insemination ($P < 0.05$).

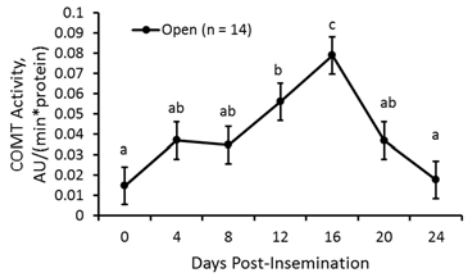



Figure 2.) Catechol-O-methyltransferase (COMT) activity in dairy cows classified as rebred and returning to estrus prior to end of sampling period. Activity of COMT was not different across days post-insemination ($P > 0.10$).

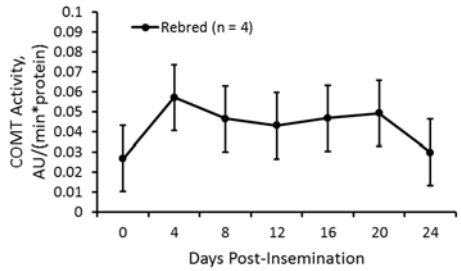


Figure 3.) Catechol-O-methyltransferase (COMT) activity in dairy cows classified as pregnant. Means with different letters represent significant differences across days post-insemination ($P < 0.05$).

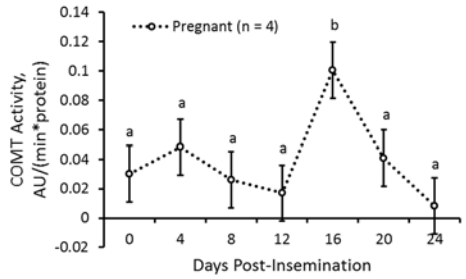
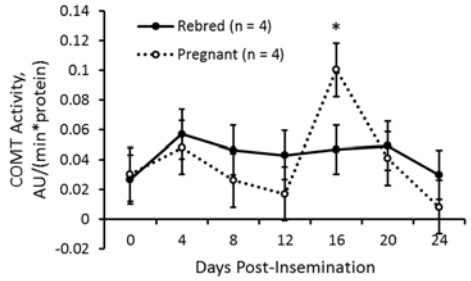



Figure 4.) Catechol-O-methyltransferase (COMT) activity in dairy cows classified as pregnant or rebred. Asterisk (*) represents a significant differences between pregnant and rebred cattle ($P < 0.05$).




During this undergraduate research experience, I gained not only academic credit, but also valuable knowledge that will be applicable throughout my lifetime. I am an aspiring veterinarian, and desire to own a mixed animal practice entailing my sibling as my fellow colleague. However, my interests within the veterinary field lie primarily with large animals, specifically cattle and horses. Therefore, not only will gained knowledge relating to the COMT enzyme function assist me with my future career, but also common practice techniques that I acquired during animal handlings and blood sampling periods set in both lab and field environments. For example, I gained tremendous experience with acquiring blood samples from cattle by method of vena puncture of the jugular vein and also coccygeal vein, with and without assistance of a cattle chute. I also gained experience with valuable laboratory techniques and safety protocols. For example, I learned how to centrifuge blood samples, operate absorbance-

level detecting blood assay machinery, safety protocols accommodated with operating such lab machinery, and I also became familiar with running statistical analysis on samples.

While being enrolled in this undergraduate study, I was simultaneously enrolled in both General Chemistry II and Physiology of Reproduction. Unfortunately, I have always had a very weak chemistry background. However, due to my research advisor's, Dr. Lemley, instructions in the lab I became more experienced in the chemistry field. I became more familiar with balancing chemical equations, converting chemical units, and also preparing chemicals and solutions that were implemented in my project. I strongly believe that his guidance during my research not only allowed me to gain knowledge for me to be successful in the my current chemistry classes during my undergraduate career, but also will better prepare me for my future career. Also, I was able to apply knowledge gained in my Physiology of Reproduction class in my research project. I believe that I was able to better comprehend the material obtained in lecture due to Dr. Lemley explaining and relating reproductive physiological processes pertaining to my research that coincided with the course's lecture material.

Not only did my undergraduate research experience allow me to be more prepared for application to Mississippi State University's College of Veterinary Medicine, but it also opened many current career doors. Another fellow researcher, Megan P.T. Coleson, is a current graduate student who is also directing a research project, advised under Dr. Lemley, in order to gain her Ph.D. Therefore, she required experienced employees to assist her with uterine biopsies, blood sampling and centrifuging, and also transportation of cattle. I am currently employed and once again gaining valuable knowledge and experience for my future career.

In retrospect, being enrolled as a full time student, while simultaneously directing a research project was very demanding yet incredibly rewarding. Due to being enrolled in veterinary emphasized classes, it was challenging maintaining my grades along with successfully directing a research project. However, I further established organizational and time managing skills that will be helpful both currently as an undergraduate student and in the future as veterinarian student. Another rewarding factor related to the undergraduate research program was being able to see acquired knowledge from the classroom be implemented and tested in the field. If I do become a veterinarian, my greatest fear is not being able to implement acquired knowledge successfully in the field due to my forgetful tendencies. However, I realized that no one is perfect. I will make mistakes in the future, but the goal is to minimize mistakes and maximize successes. At the beginning of the undergraduate research term, I was skeptical and unconfident that I would perform well. However, Dr. Lemley's guidance and my determination proved me wrong. To this day, I am still astonished of what our research group has achieved, and I look forward to seeing more valuable results obtained in the future. I would strongly recommend this program to all majors in undergraduate school. It allowed me to acquire a strong foundation in the general science field, professionalism, confidence, and also implementation of knowledge. I believe that current society lacks younger generations with qualities for the professional field. I strongly believe that my experience in this program gave me

a strong enough foundation to allow me to build upon in order to achieve my future aspiration of becoming a veterinarian.