

## **Increased Plasma Progesterone Levels During the Estrous Cycle and Altered Follicular Fluid IL-13 May Determine if High A4 Cows Ovulate or Develop Persistent Follicles.**

**Manjula P.S. Magamage**<sup>1,2</sup>; Mariah Hart<sup>1</sup>; Ligia D. Prezotto<sup>1</sup>; Mohamed A. Abedal-Majed<sup>3</sup>; Renata Spuri Gomes<sup>1</sup>; Caleb O. Lemley<sup>4</sup>; Carol Casey<sup>5</sup>; Scott G. Kurz<sup>1</sup>; John S. Davis<sup>6,7</sup>; Jennifer R. Wood<sup>1</sup>; Andrea S. Cupp<sup>1</sup>

<sup>1</sup>University of Nebraska–Lincoln; Department of Animal Science, 3940 Fair Street, Lincoln, NE 68583-0908, USA

<sup>2</sup> Department of Livestock Production, Sabaragamuwa University of Sri Lanka, Belihuloya, RN 70140, Sri Lanka

<sup>3</sup>Department of Animal Production, School of Agriculture, The University of Jordan, Amman 11942

<sup>4</sup>Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS, 39762, USA

<sup>5</sup>University of Nebraska Medical Center, Department of Internal Medicine, Section of Gastroenterology, 98200 Nebraska Medical Center, Omaha, NE 68198-2000, USA

<sup>6</sup>University of Nebraska Medical Center, Olson Center for Women’s Health; 983255 Nebraska Medical Center, Omaha, NE 68198-3255, USA

<sup>7</sup>VA Nebraska-Western Iowa Health Care System, Omaha, NE 68105, USA

Our research identified a subpopulation of cows with naturally elevated androgen levels (specifically androstenedione, or A4; High A4 cows;  $A4 \geq 40$ ng/ml) in follicular fluid compared to controls ( $A4 \leq 20$ ng/ml). Interestingly, these High A4 cows have molecular characteristics similar to those observed in women diagnosed with polycystic ovary syndrome (PCOS). High A4 cows also are sub-fertile with a 17% reduction in calving rate, increased ovarian fibrosis, and oxidative stress. This study aimed to investigate whether elevated ovarian cortex A4 levels in cows with ovulatory (HA4O) or irregular (HA4I) cycles cause hormonal, metabolic, and immune changes compared to controls. We hypothesized that excess androgen alters hepatic liver enzymes, and endocrine hormones to allow for ovulation to occur in HA4O compared to anovulatory HA4I cows. Interestingly, HA4O cows had the highest A4 levels in ovarian cortex media, followed by HA4I and then control cows. The HA4O cows had greater circulating concentrations of progesterone (P4) and reduced estradiol (E2) during the estrous cycle when compared to the HA4I and control groups. Circulating Leptin secretion was greater in control animals compared to HA4O and HA4I groups indicating there may be differences in metabolism in High A4 cows. There were no differences in circulating non-esterified fatty acids (NEFA’s) or triglycerides between the HA4O and HA4I groups. Hepatic tissue had increased cytochrome 2C (CYP2C;  $p < 0.05$ ) and a tendency ( $p < 0.06$ ) for increased aldo-keto reductase 1C (AKR1C) activity in High A4 groups compared to controls suggesting that steroid hormones would be metabolized at a faster rate in excess androgen females. Interleukin-13 (IL-13) concentrations were increased in follicular fluid of dominant follicles of HA4I ( $p < 0.03$ ) compared to HA4O and Control cows. Elevated IL-13 has been demonstrated to cause inflammation, suggesting that increased inflammation may be a factor contributing to anovulation in the HA4I cows. In summary, while both High A4 groups had increased liver enzyme activity and reduced circulating Leptin, HA4O had increased circulating P4, reduced E2, and increased dominant follicle follicular fluid IL-13. The cytokine, IL-13, is an anti-inflammatory cytokine that causes inflammation and may contribute to development of persistent follicles resulting in anovulation in HA4I cows. Furthermore, elevated plasma progesterone with reduced follicular fluid inflammation may allow for HA4O cows to ovulate despite the high levels of A4 in the ovarian microenvironment.