Beef Day 2021

Use of pregnancy associated glycoproteins to determine fetal age throughout gestation in cattle

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Objective

The objective of the current study was to determine if a commercially available blood pregnancy test could be modified to detect differences in pregnancy-associated glycoprotein (PAG) concentrations to indicate stage of pregnancy or fetal age in cattle.

Study Description

Previously identified pregnant females were grouped by age (pre-primiparous or multiparous). Blood samples were collected between day 27 and 190 of pregnancy (n = 176 from pre-primiparous and n = 240 from multiparous) and serum was tested in duplicate using a commercially available blood pregnancy test, IDEXX Alertys Pregnancy Test. Procedures were adapted to allow concentrations to fall within the detectible range of the assay. Animals were grouped by parity (pre-primiparous vs multiparous) into 4 gestational groups (group 1 - < 30 days, group 2 - 30 to 90 days, group 3 - 91 to 178 days, and group 4 - >178 days). Data were analyzed using the MIXED procedure of SAS with parity and gestational age in the model. There was an effect of parity, gestational age, and a parity by gestational age interaction ($P < 0.01$). Pre-primiparous animals had greater concentrations of PAGs compared to multiparous animals. Among pre-primiparous animals, serum PAG concentrations did not differ between gestational age groups 1, 2, or 3 ($P > 0.37$), but group 4 had greater PAG concentrations than all other groups ($P < 0.01$). Among multiparous animals, serum PAG concentrations decreased from group 1 to 2 ($P < 0.01$), and then increased throughout gestation ($P < 0.01$). Data were then analyzed using the REG procedure in SAS within gestational age group. There was a positive correlation between gestational age and PAG concentrations among both pre-primiparous ($P < 0.01$; $R^2 = 0.25$) and multiparous (gestational age 30 and greater $P < 0.01$; $R^2 = 0.64$).

Take Home Points

In conclusion, circulating PAG concentrations among pre-primiparous animals increased with gestational age, but the high variability in concentrations may not make it a reliable marker for gestational age. Among multiparous animals, however, gestational age accounted for 64 % of the variation in concentration of PAGs between day 30 and 190, thus using a modified blood pregnancy test may allow for determining the stage of pregnancy.

Introduction

Pregnancy-associated glycoproteins (PAGs) are a part of the aspartate proteases gene family. These PAGs are synthesized by binucleate giant cells (BNGCs) of the trophectoderm in the ruminant placenta. Binucleate giant cells then migrate to fuse with maternal uterine epithelial cells where the granular content within the BNGCs is released into the maternal circulation. Once the granular content is released in the maternal circulation PAGs can be measured in either milk or blood samples to determine pregnancy status. Pregnancy-associated glycoproteins are detectable in the bloodstream of multiparous and pre-primiparous as early as day 22 to 24 of gestation (Pohler et al., 2013). Pregnancy-associated glycoproteins continue to rise throughout

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gestation and then peak around the time of parturition (Sasser et al., 1986; Zoli et al., 1992; Green et al., 2005). Because PAGs are elevated at parturition, residual levels exist in multiparous cows at the start of the subsequent breeding season that can affect early pregnancy detection.

The PAG family contains more than 20 different proteins which are secreted at different times throughout gestation. The exact purpose of PAGs is still unestablished, but it is estimated that some may play a role in parturition and protection of the fetus from the maternal immune response (Zoli et al., 1992). Blood pregnancy tests are popular due to their ease of use and the unique feature of not requiring costly equipment or special training. Using PAGs for pregnancy determination is extremely accurate with a 95 to 99 percent true positive rate (Pohler et al., 2016). A downside to blood pregnancy tests is they only provide a yes or no answer as to whether an animal is pregnant or open. Currently, fetal aging can only be completed with transrectal palpation or ultrasonography. Therefore, the objective of this study was to determine if a commercially available blood pregnancy test could be modified to detect differences in PAG concentrations to indicate stage of pregnancy or fetal age. It was hypothesized that fetal age would be correlated to increasing concentrations of circulating PAGs.

Experimental Procedures

Experimental Design

Females previously identified as pregnant via transrectal ultrasonography from four different herds in South Dakota were utilized in this study. Animals were grouped by parity (pre-primiparous and multiparous). Blood was collected once a month between day 27 and 190 of pregnancy from either the tail or jugular vein into 10-mL EDTA Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) and stored at room temperature for approximately one to three hours until centrifuged. The whole blood samples were centrifuged at 2,000 x g for 30 minutes for serum collection and stored at -20ºC until testing. Serum was tested in duplicate using a commercially available blood pregnancy test, IDEXX Alertys Ruminant Pregnancy Test (IDEXX, Westbrook, ME). Blood samples were grouped into 4 gestational age groups (group 1 - < 30 days, group 2 - 30 to 90 days, group 3 - 91 to 178 days, and group 4 - >178 days).

Procedures for the commercial PAG assay were adapted to allow concentrations to fall within the linear detectable range of the assay. The IDEXX Alertys Ruminant Pregnancy Test currently detects whether a female is pregnant or open. Since females in this study were all pregnant, their PAG concentrations surpassed the minimum threshold of the assay. Adjustments were made from 100 µL to 8 µL of serum used in the assay in order fit within the detectable range of the assay. Plates were then washed and treated with reagents according to the manufacturer's instructions. The Molecular Devices SpectraMax 190 microtiter plate reader (San Jose, California) determined the PAG concentration for each sample.

Animals were not physically separated for the duration of this study but were separated into 4 different gestational groups (group 1- < 30 days, group 2- 30 to 90 days, group 3- 91 to 178 days, and group 4- >178 days) to statistically analyze the data. Different groups were established where a natural break in gestational age was found. Pre-primiparous and multiparous serum samples were initially run together, but a statistical difference of parity was evident, therefore they were analyzed separately.

Statistical Analysis

Serum PAG concentrations were analyzed using the MIXED procedure of SAS with parity and gestational age in the model. Correlation of PAG concentrations with gestational age were analyzed using the REG procedure in SAS within gestational age group. Significance was determined at a $P \leq 0.05$ and a tendency at $0.10 \geq P > 0.05$.

Results and Discussion

There was a significant impact of gestational age on PAG concentrations. Pregnancy Associated Glycoproteins increased in both pre-primiparous (Figure 1) and multiparous (Figure 4) animals as gestational age increased ($P < 0.01$). Concentrations of PAGs also increased in both pre-primiparous and multiparous animals (Figure 2

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and 5) as gestational age increased ($P < 0.01$). Among pre-primiparous animals, there was no difference in PAG concentrations between groups 1, 2, and 3, but PAGs increased in group 4 (Figure 3). Among multiparous animals, PAG concentrations increased from group 2 to group 3 and further increased to group 4 (Figure 6). We hypothesize that the difference in PAG profiles is due to an effect of parity (pre-primiparous vs multiparous) on PAG concentrations during gestation. Pre-primiparous animals had greater PAG concentrations compared to multiparous animals (Figure 7) early in gestation. We hypothesize this is because pre-primiparous animals have not calved yet and are at smaller body weights, so the PAGs are not diluted as much as they would be in a multiparous animal.

Implications

The present study verified circulating PAG concentrations increase with gestational day. Among pre-primiparous animals, PAG concentrations did increase with gestational age, but with high variability in concentrations, may not be a reliable marker for gestational age. Among multiparous animals, gestational age accounted for 64 percent of the variation in PAG concentrations between d 30 and 190, thus using a modified blood pregnancy test may allow for determining stage of pregnancy. To be enticing for producers to implement into their program, the ideal pregnancy detection method should accurately identify both pregnant and non-pregnant females in the herd (Romano and Larson, 2010). Using PAGs to identify pregnant females within a producer's operation allows for a detection method at a lower cost, requiring less skill, and less strain on their body. With further research, PAG blood pregnancy test may also allow for determining stage of pregnancy.

Acknowledgements

This project was possible through collaboration between Department of Animal Science, South Dakota State University, Brookings SD, U.S.A. and Jim R. Rhoades at IDEXX, Westbrook ME.

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Figures

PAG CONCENTRATIONS BY GESTATION DAY

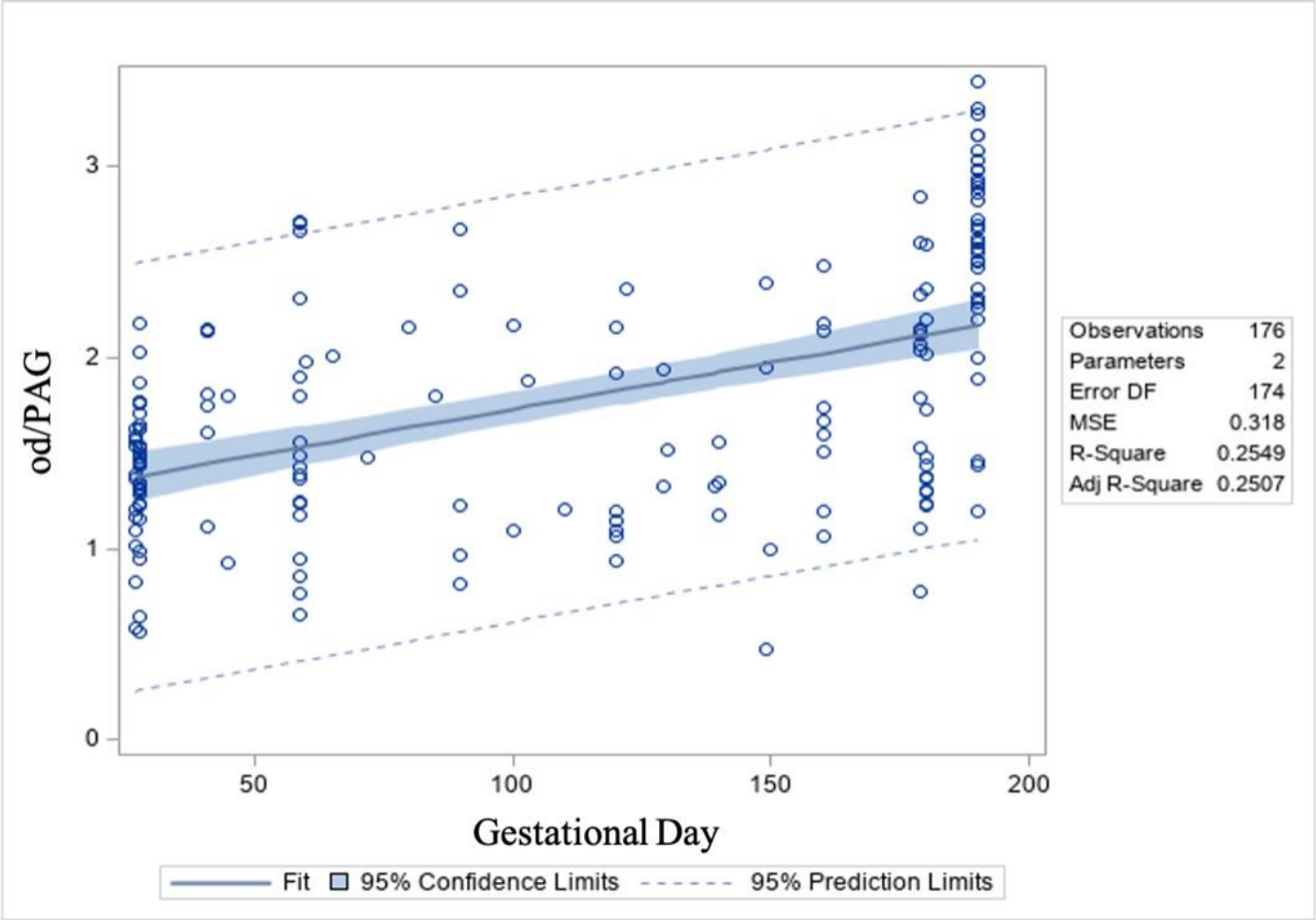

Figure 1. Regression analysis of gestational age on circulating concentrations of pregnancy-associated glycoproteins in primiparous animals. Each circle indicates an individual sample. The solid line is the calculated regression line, with the blue shaded area being the 95% confidence interval. Gestational age 30 and greater (P <0.01; R^2 =0.25).

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PAG CONCENTRATIONS BY GESTATION AGE GROUP

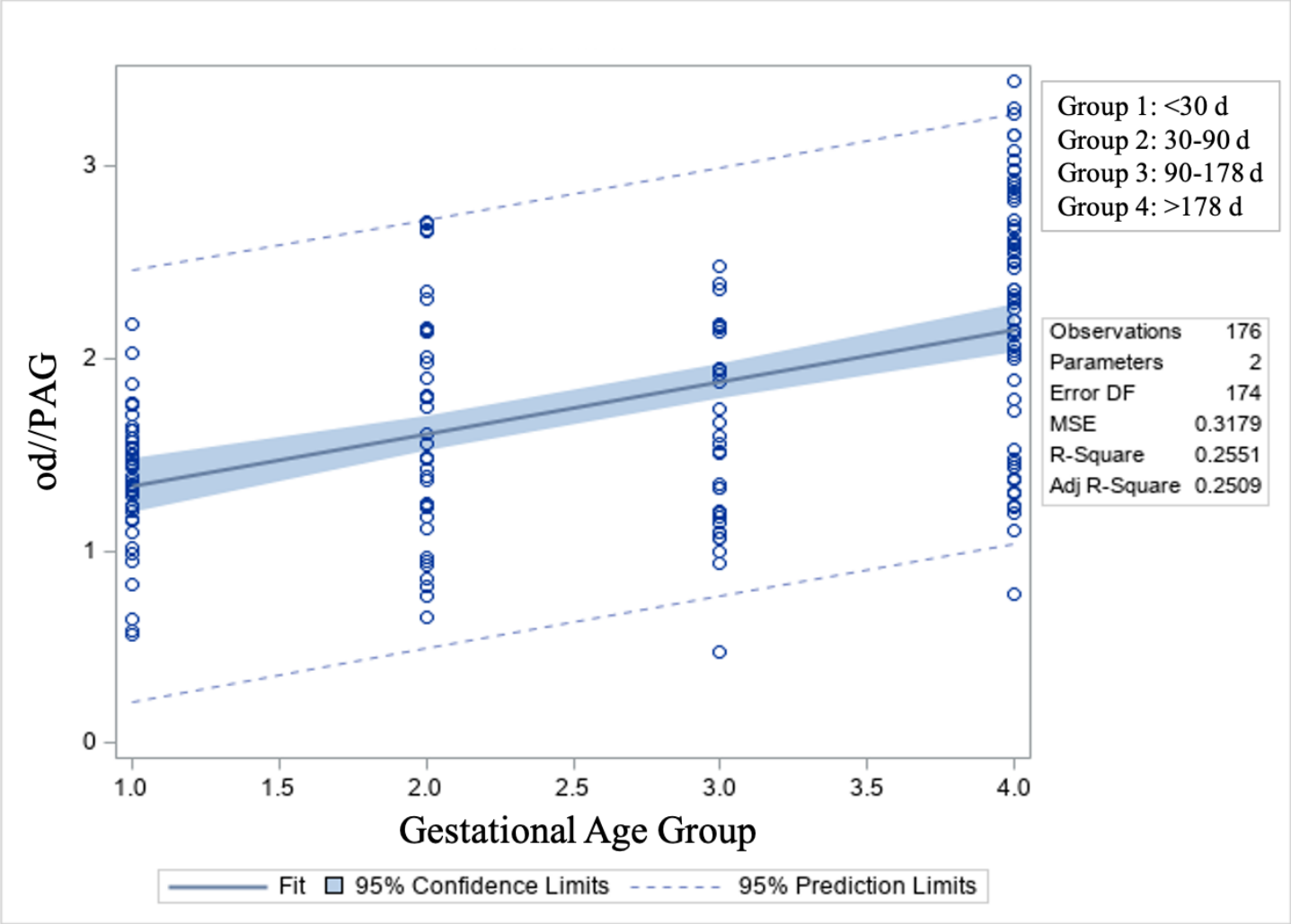

Figure 2. Increase of PAG concentrations among four different gestational groups in primiparous animals. Regression analysis of gestational age group on circulating concentrations of pregnancy-associated glycoproteins. Each circle indicates an individual sample. The solid line is the calculated regression line, with the blue shaded area being the 95% confidence interval (P <0.01; R^2 =0.25).

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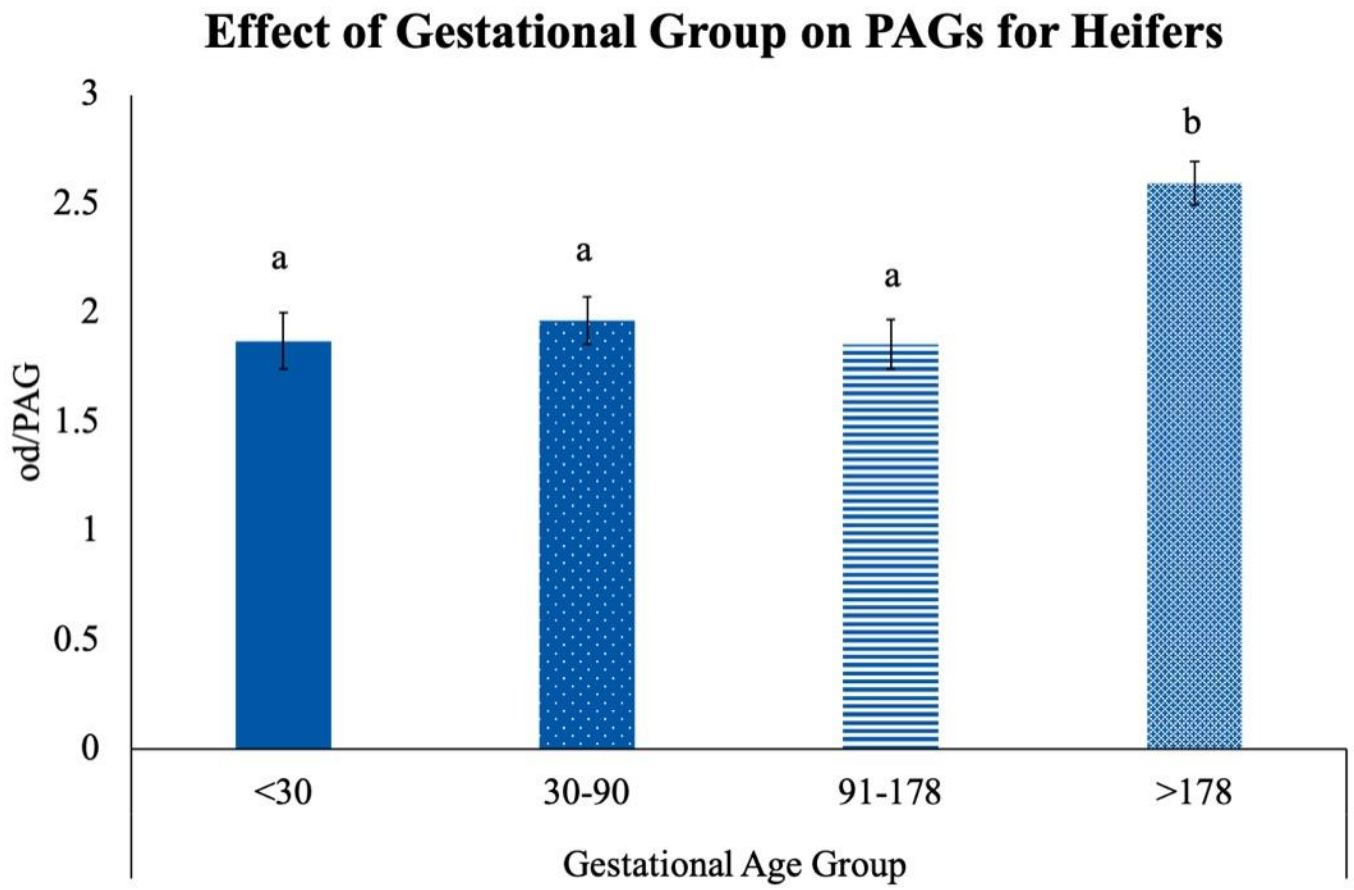

Figure 3. Mean (± SEM) serum PAG concentrations among four different gestational groups (days) of heifers. Different superscripts [a,b] (*P* <0.01).

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PAG CONCENTRATIONS BY GESTATION DAY

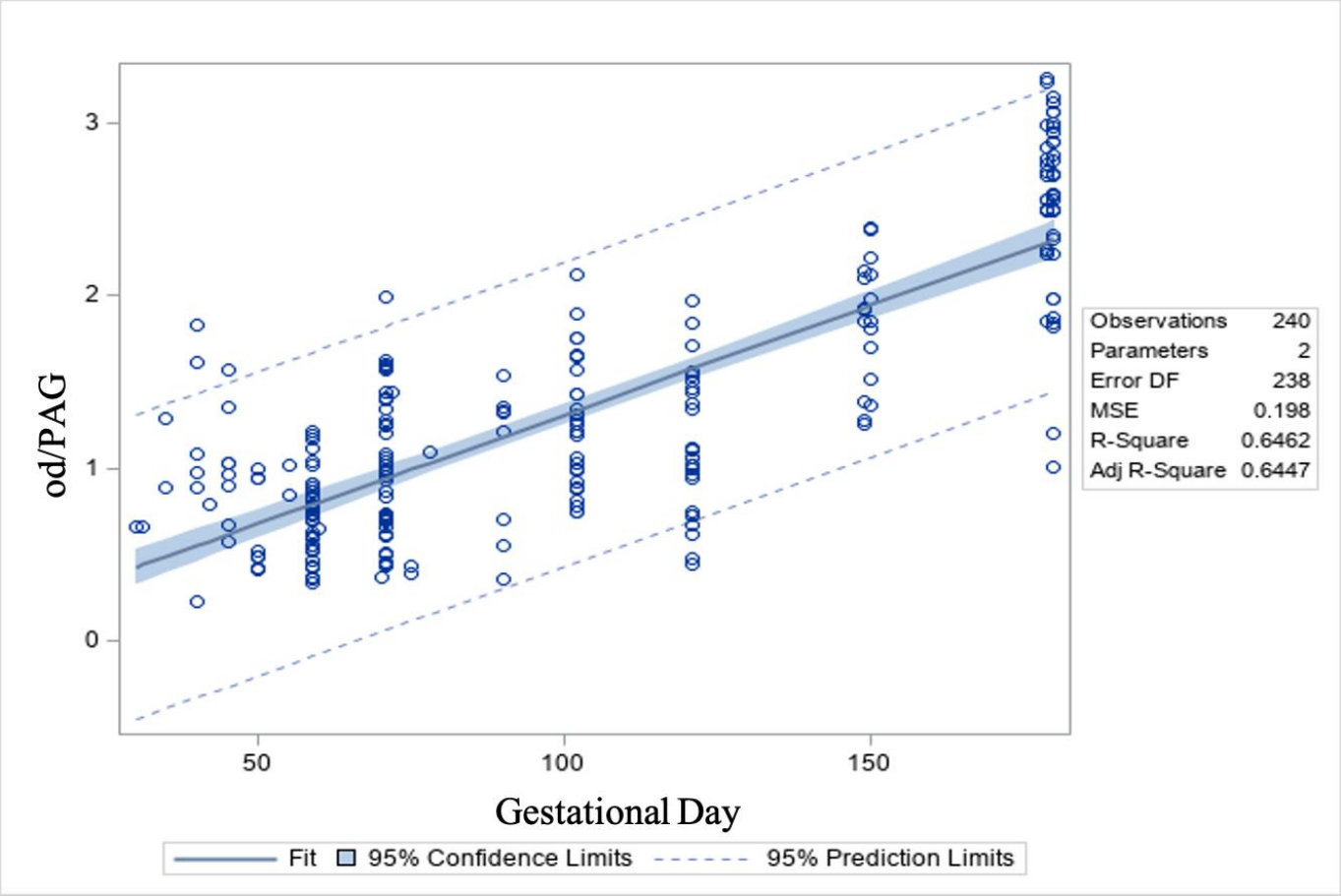

Figure 4. Regression analysis of gestational age on circulating concentrations of pregnancy-associated glycoproteins in multiparous cows. Each circle indicates an individual sample. The solid line is the calculated regression line, with the blue shaded area being the 95% confidence interval. Gestational age 30 and greater (P <0.01; R^2 =0.64).

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PAG CONCENTRATIONS BY GESTATION AGE GROUP

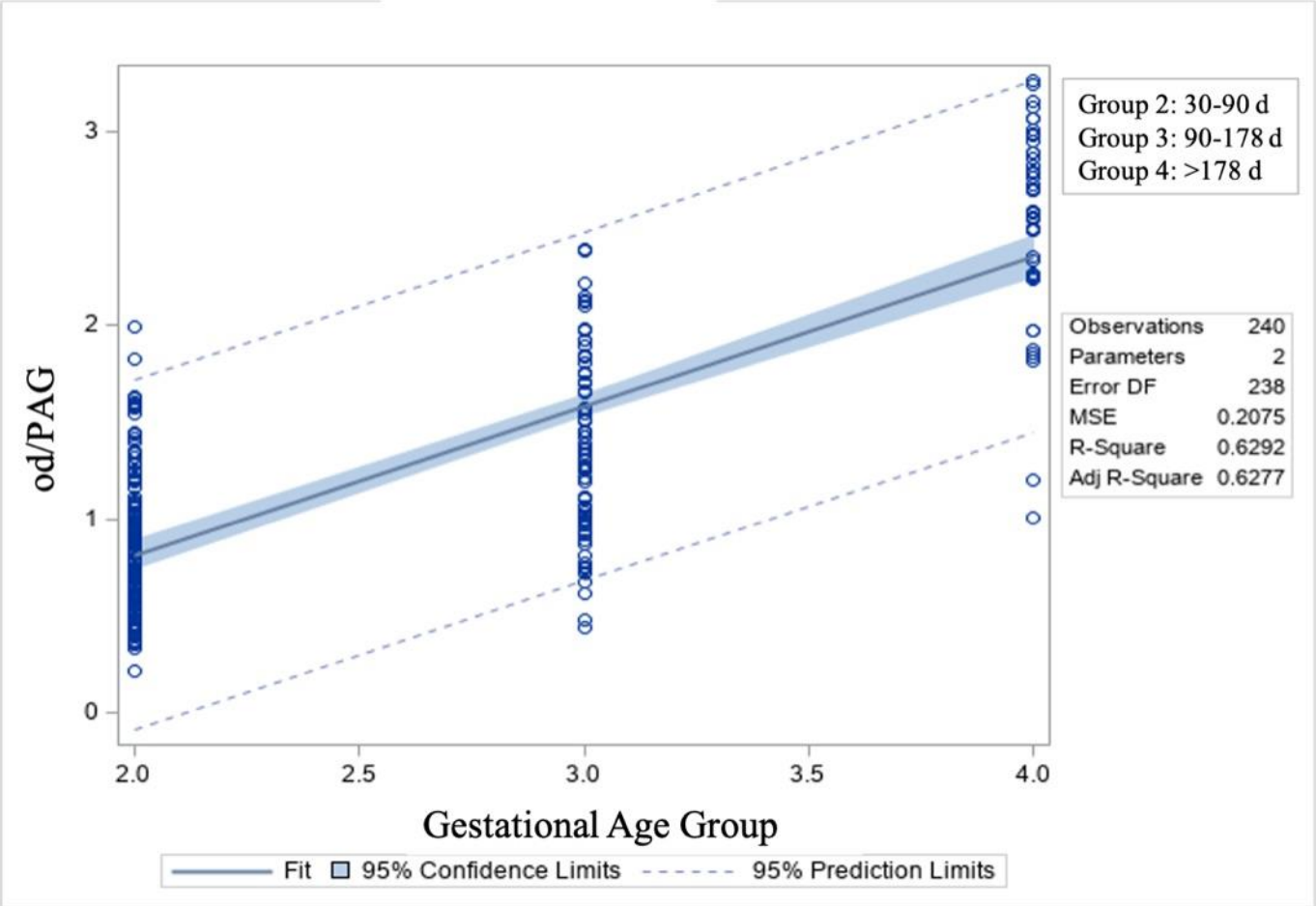

Figure 5. Increase of PAG concentrations among three different gestational groups in multiparous cows. Regression analysis of gestational age group on circulating concentrations of pregnancy-associated glycoproteins. Each circle indicates an individual sample. The solid line is the calculated regression line, with the blue shaded area being the 95% confidence interval (P <0.01; R^2 =0.63).

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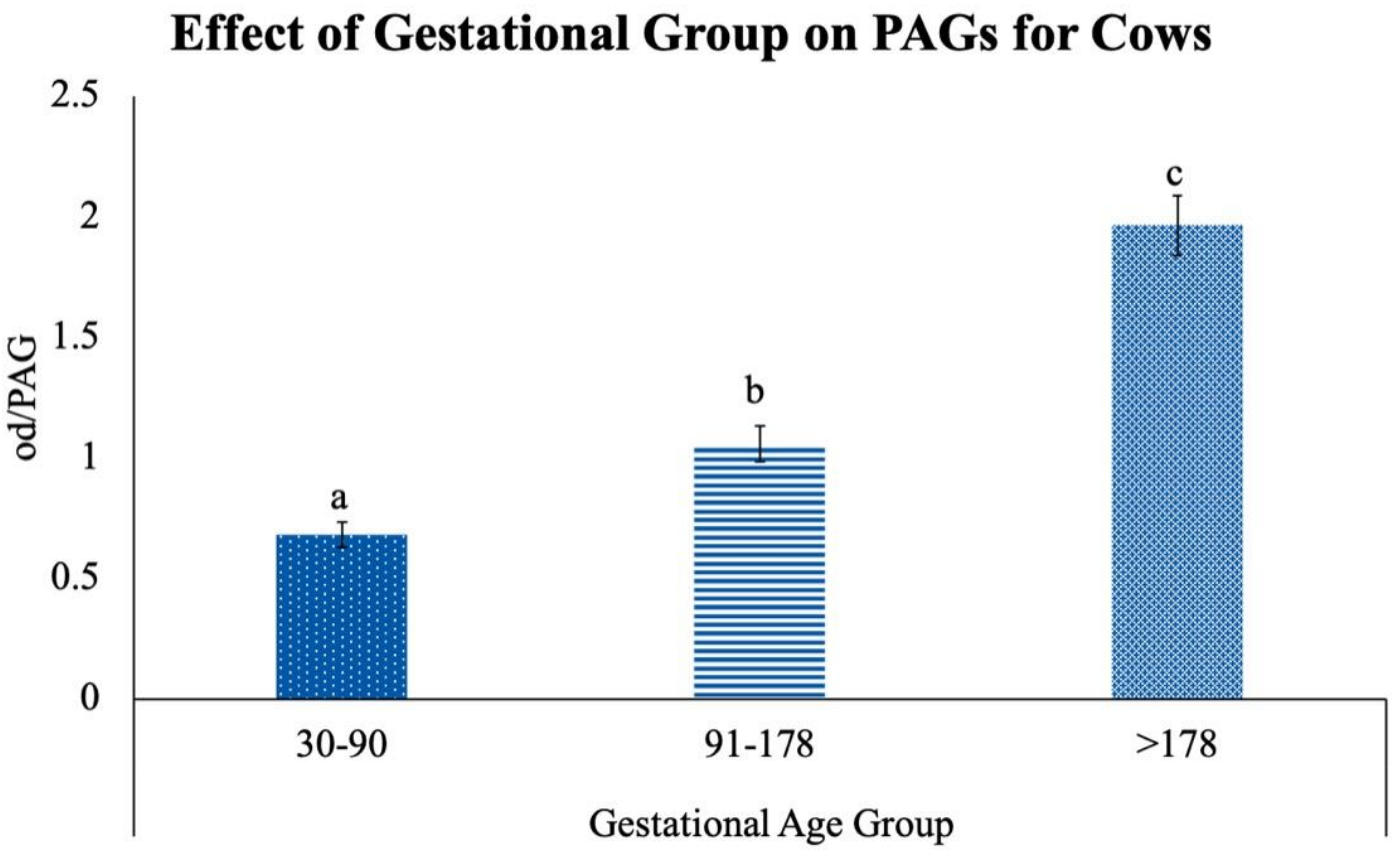

Figure 6. Mean (± SEM) serum PAG concentration levels among three different gestational groups of multiparous cows. Different superscripts [a,b,c] ($P < 0.01$).

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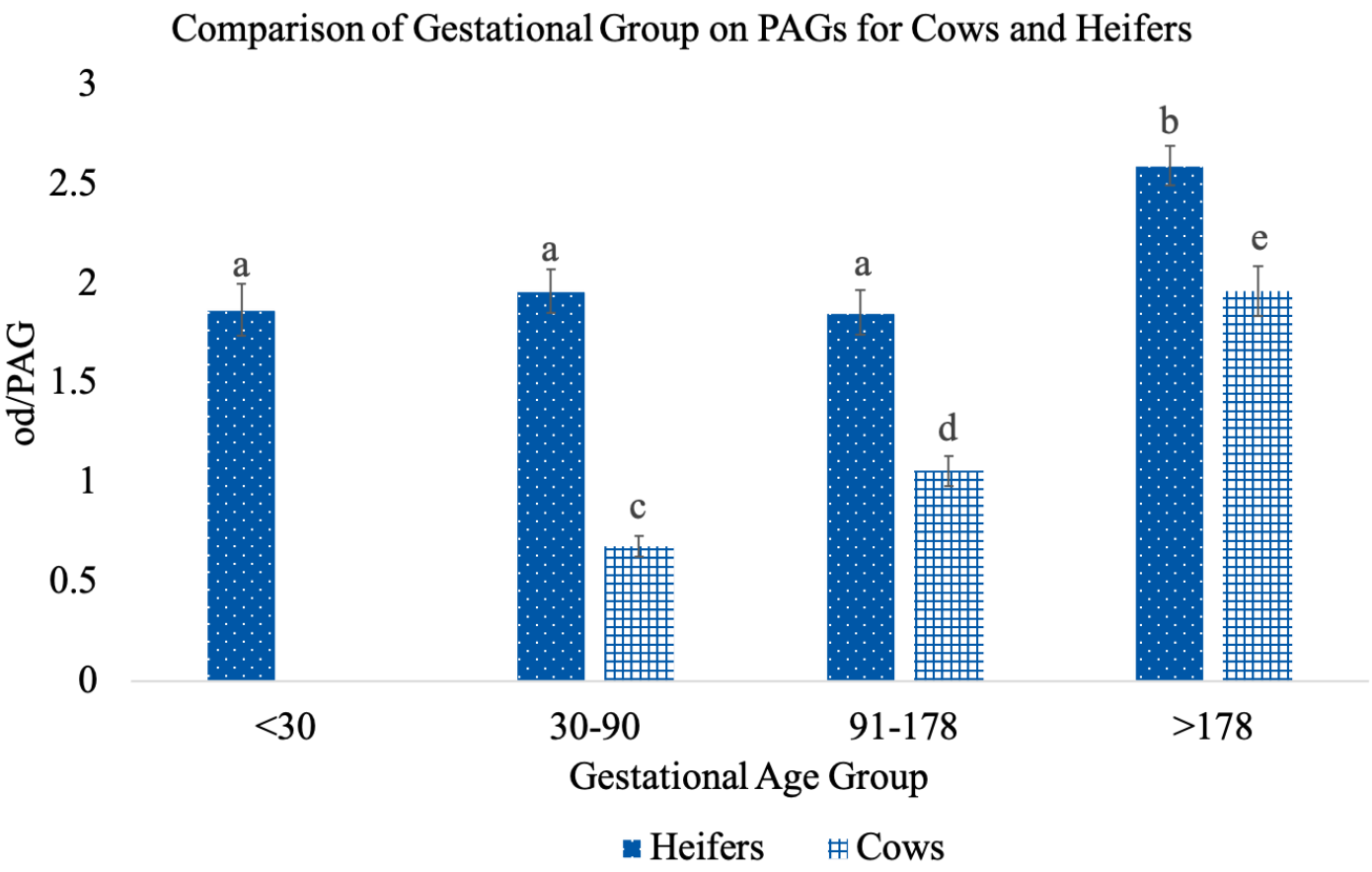

Figure 7. Comparison of PAG concentrations between primiparous and multiparous cows. Different superscripts ^{a,b,c,d,e} ($P < 0.01$).

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