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Use of Pregnancy Associated Glycoproteins to Determine Fetal Age Throughout Gestation and Clearance Rate in Postpartum Beef Cattle

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Objective

The objectives of these studies were to determine whether a commercially available blood pregnancy test that measures pregnancy associated glycoproteins (PAGs) concentrations could be modified to detect stage of pregnancy in cattle and to determine factors that impact clearance of PAGs after calving.

Study Description

Previously identified pregnant females from four different herds and postpartum females from one herd were utilized. Blood samples were collected (n = 1,753; study 1) between d 28 and 285 of gestation and (heifers n = 418 and cows n = 657; study 2) once a week for up to 12 weeks after calving. Serum was tested in duplicate using a commercially available blood pregnancy test, IDEXX Alertys Pregnancy Test. In study 1, procedures were modified to allow PAG concentrations to fall within the detectible range of the assay. Data were analyzed using the MIXED procedure of SAS with cow age and gestational age (also divided into four gestational age groups: 1) < 30 d; 2) 30-90 d; 3) 91-180 d; 4) > 180 d) in the model and then analyzed further using the REG procedure in SAS within gestational age group. In study 2, data were analyzed as repeated measure using the MIXED procedure of SAS with cow age, days postpartum (dpp), and cow age by dpp in the model, then data were analyzed further using the REG procedure in SAS. In study 1, there was a significant effect of gestational age and cow age by gestational age interaction ($P < 0.01$) as well as a tendency of cow age ($P = 0.08$). Among heifers and cows, serum PAG concentrations did not differ between gestational age groups 1 and 2 ($P > 0.84$), however, PAG concentrations differed between groups 2 and 3 ($P < 0.0001$) and 3 and 4 ($P < 0.0001$). There was a positive correlation between gestational age and PAG concentrations ($P < 0.01$; $r2 = 0.2604$). In study 2, there was a significant effect of days postpartum (dpp; $P < 0.01$) on PAG concentrations; however, PAG concentrations were not influenced by cow age ($P = 0.73$) or cow age by dpp ($P = 0.55$). Concentrations of PAGs rapidly decreased from d 0 to 50 postpartum and then continued to gradually decrease ($P < 0.01$; $r2 = 0.8083$). Prior to 42 dpp, PAG concentrations were sufficiently elevated which resulted in false positive readings.

Take Home Points

Concentrations of PAGs using this modified pregnancy test would not make a reliable marker for gestational age due to high variability. Additionally, residual PAGs decreased to undetectable concentrations after 42 dpp in most females; thus, minimizing the likelihood of false positives (test results indicating a cow is pregnant, when she is not pregnant).

Introduction

In order to be more profitable and have a complete and successful reproductive management program, pregnancy diagnosis within an operation is not only important, but necessary (Oltenacu et al., 1990). Fertilization occurs greater than 90% of the time following insemination of beef cows that have been detected in estrus, but calving rates to a single insemination are usually only about 55% (Diskin and Sreenan, 1980). Without an accurate pregnancy detection method, an operation can miss out on both monetary and management alternatives. Transrectal ultrasonography is considered the gold-standard for pregnancy detection, but it is costly and requires a skilled technician (Perry and Cushman, 2016). Transrectal palpation of pregnancy requires even greater skill and can result in some pregnancy loss. An alternative method to determine pregnancy is with blood tests utilizing pregnancy-associated glycoproteins (PAGs; Sasser et al., 1986; Humblot et al., 1988; Zoli et al., 1992; Mialon et al., 1993). These glycoproteins can be detected in the maternal bloodstream as early as day 22 to 24 of gestation (Pohler et al., 2013), and are extremely accurate with a 95 to 99% true positive rate and a 1 to 5% false positive rate compared to transrectal ultrasonography (Pohler et al., 2016). Blood pregnancy tests are also increasing in popularity due to ease of use and the unique feature of not requiring costly equipment or special training.

A downside to blood pregnancy tests is they do not provide any additional results such as fetal age. Pregnancy-associated glycoproteins steadily increase throughout gestation, are elevated at time of parturition, and then decrease after parturition (Sasser et al., 1986; Zoli et al., 1992; Green et al., 2005). They also have a long-half life in the blood of postpartum females which ranges from 80 to 100 d before they become undetectable in the maternal blood supply (Zoli et al., 1992; Kiracofe et al., 1993). Because PAGs peak at parturition and have a long half-life, residual concentrations can still exist in cows at the start of the subsequent breeding season. Thus, when trying to use PAG concentrations as a marker for early pregnancy diagnosis, these residual concentrations can impact the results. Therefore, the objectives of these studies were to 1) 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 and 2) determine factors that impact clearance of PAGs in postpartum beef females.

Experimental Procedures

Experimental Design

In study 1, blood samples (n = 1,753) were collected from Angus and Angus-cross beef females previously identified as pregnant via transrectal ultrasonography from four herds in South Dakota.

In study 2, Angus and Angus-cross postpartum females (n = 114; heifers n = 48 and cows n = 66) from one of the previously mentioned herds were utilized. Animals were managed similarly in two groups; however, females were in separate pastures based on sex of calf. Blood samples were collected once a week for up to 12 weeks post calving (range of first and last sample was 1-7 to 84-91 days postpartum; dpp).

Blood Sampling

In study 1, blood samples were only collected in females identified as pregnant by transrectal ultrasonography. In study 2, blood samples were only collected in females who had calved. Blood samples were collected by venipuncture of either the tail or jugular vein into 10-mL Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) and stored at room temperature for approximately two hours until centrifuged. Samples were centrifuged, serum was collected and stored at -20 °C until PAG assays were conducted.

Bovine Pregnancy Tests

Each serum sample was examined in duplicate using a commercially available blood pregnancy test, IDEXX Alertys Ruminant Pregnancy Test (IDEXX, Westbrook, ME).

In study 1, all samples were analyzed according to the manufacturer’s instructions aside from a few adaptions to the protocol. Since all females were pregnant, PAG concentrations exceeded the threshold of detection of the IDEXX Alertys Ruminant Pregnancy Test assay. Thus, to have PAG concentrations fall within the linear detectable range of the assay, the serum sample was decreased from 100 µL to 8 µL and plates were incubated at 37 °C for more consistency. Optical density was measured at Absorbance of 450 nm and 650 nm using a Molecular Devices SpectraMax 190 microtiter plate reader and recorded.

In study 2, all samples were analyzed according to the manufacturer's instructions with 100 µL of sample used.

Statistical Analysis

In study 1, blood samples were grouped by age (heifers and cows) and were also grouped into 4 gestational age groups (group 1: < 30 d, group 2: 30- 90 d, group 3: 91- 180 d, and group 4: >180 d) to further statistically analyze the data. Groups were established where a natural break in gestational age occurred. Serum PAG concentrations were analyzed using the MIXED procedure of SAS with age and gestational age in the model. Correlations between PAG concentrations and gestational age were analyzed using the REG procedure in SAS.

In study 2, samples were grouped by week of collection and PAG concentrations were analyzed as a repeated measure using the MIXED procedure of SAS (9.4) with age, days postpartum (dpp), and age by dpp in the model. Since there was no effect ($P > 0.10$) of age, effect of dpp on PAG concentrations were analyzed using the REG procedure in SAS. Statistical significance was determined at a $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$ for analysis.

Results and Discussion

Study 1

There was a significant impact of gestational age on PAG concentrations among pregnant females. Pregnancy-associated glycoproteins had a linear increase in both heifers and cows as gestational age increased ($P < 0.01$); however, there was a weak correlation ($r2 = 0.3009$; Figure 1). There was an interaction between age and gestational day ($P < 0.05$), so blood samples were divided into gestational age groups by trimester to further analyze the data. Concentrations of PAGs had a linear increase in both heifers and cows as gestational age group increased ($P < 0.01$); however, there was a weak correlation ($r2 = 0.2604$) to gestational age (Figure 2). Among animals, there was no difference in PAG concentrations between groups 1 to 2 ($P = 0.84$), but there was an increase between groups 2 to 3 ($P < 0.0001$) and then a further increase between groups 3 to 4 ($P < 0.0001$; Figure 3.A). A comparison of age by group was made between heifers and cows (Figure 3.B). Among heifers there was a linear increase from group 1 to 2, group 2 to 3, and then further from group 3 to 4 ($P < 0.0001$; Figure 3.B). Among cows there was no difference between group 1 and 3, but there was a decrease, between group 1 to 2, then an increase from group 2 to 3, and a further increase from 3 to 4 ($P < 0.0001$; Figure 3.B). Between heifers and cows there was a tendency between group 1 ($P = 0.06$) and a difference between groups 2, 3, and 4 ($P < 0.0001$; Figure 3.B).

Study 2

There were no post-calving differences in PAG concentrations between 1st calf heifers and cows (age; $P = 0.73$) and age by dpp ($P = 0.55$). There was, however, a significant effect of dpp on PAG concentrations in postpartum females ($P < 0.01$). There was a linear decrease in PAG concentration from day 0 (calving) to approximately 40 postpartum, at which time PAGs were undetectable (Figure 4; $P < 0.01$). Days postpartum accounted for 67.08% of the variation in PAG concentrations. Females were further separated into 7-d interval groups based on dpp for further analysis; dpp were grouped in blocks of 7 days from 0 to 84 dpp (Figure 5). There was a linear decrease from 0 to 50 dpp and then PAG concentrations plateaued from 50 to 84 dpp (Figure 5). There was again a strong correlation between dpp group and PAG concentrations, accounting for 67.48% of the variance (Figure 5). Since PAGs plateaued from 50 dpp onward further statistical analysis was performed to determine the clearance from 0 (calving) to 50 dpp (Figure 6). Analysis of females when separated into 7-d interval groups was also made from 0 to 50 dpp (Figure 7). Elimination of the samples when they begin to plateau allowed for a stronger correlation between PAG concentrations and dpp, accounting for

81.73% and 80.83% of the variance (Figure 6 and 7, respectively; $P < 0.01$). When determining average PAG concentrations of samples broken down into 7 d intervals through day 84, clearance occurred by 42 dpp (od = 0.26 ± 0.036; Figure 8.A). Animals are considered pregnant when optical density (od) were ≥ 0.3. There was no significant difference between cows and heifers determining the clearance of PAGs with the IDEXX Alertys Ruminant Pregnancy Test ($P = 0.55$).

Implications

Concentrations of PAGs increased with gestational age. However, with the variability in concentrations it is difficult to use PAGs as a marker for fetal age. Concentrations of PAGs decreased rapidly for the first 3 weeks after parturition and after 42 dpp concentrations fell below the minimum level of detection. Thus, more confidence can be gained from the results received from a PAG blood pregnancy test when it is performed at least 42 days after parturition.

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Figures

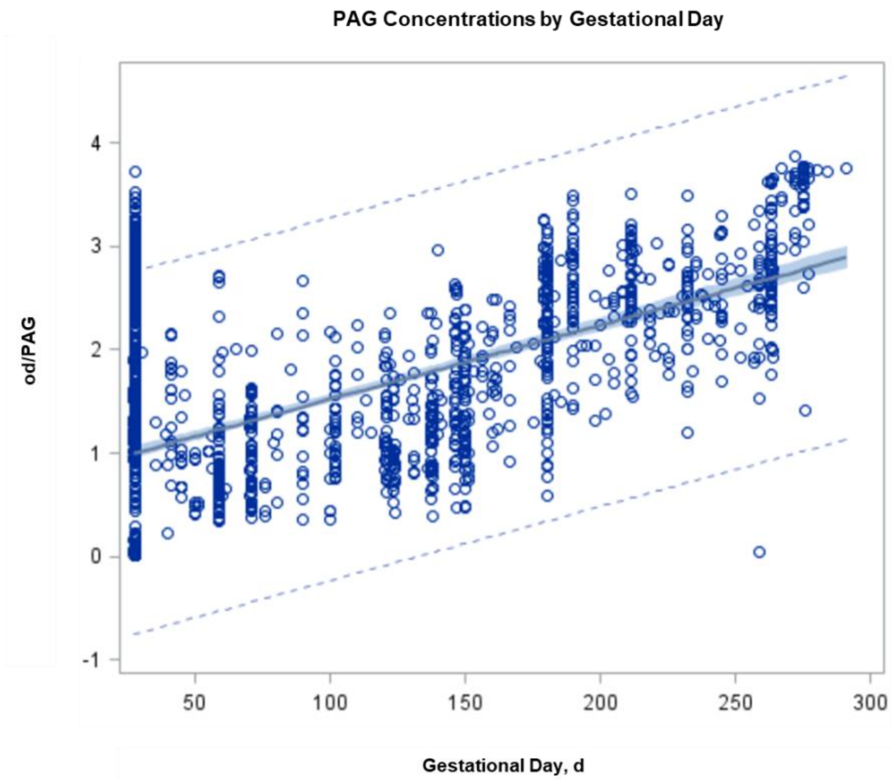

Figure 1. Regression analysis of gestational age on circulating concentrations of pregnancy-associated glycoproteins (PAG) in heifers and cows. Each circle indicates an individual sample. The solid line is the calculated regression line, the blue shaded area is the 95% confidence interval, and the dashed line is the 95% prediction limit. Gestational age 30 and greater ($P < 0.01$; $r^2 = 0.3009$).

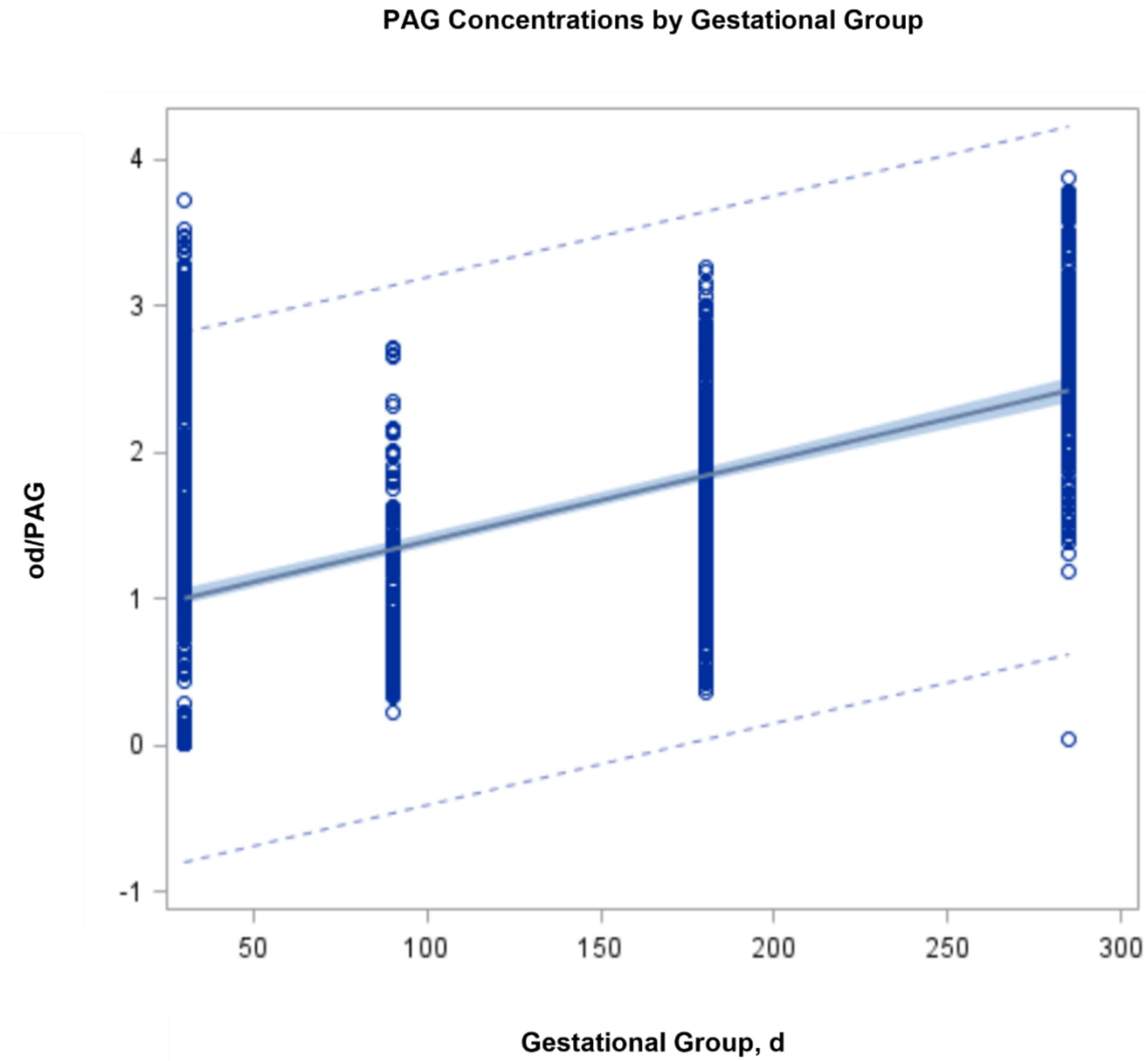

Figure 2. Increase of pregnancy-associated glycoproteins (PAG) concentrations among four different gestational groups (1) < 30 d; 2) 30-90 d; 3) 91-180 d; 4) > 180 d) in heifers and cows. Regression analysis of gestational age group on circulating concentrations of PAG. Each circle indicates an individual sample. The solid line is the calculated regression line, the blue shaded area is the 95% confidence interval, and the dashed line is the 95% prediction limit ($P < 0.01$; $r^2 = 0.2604$).

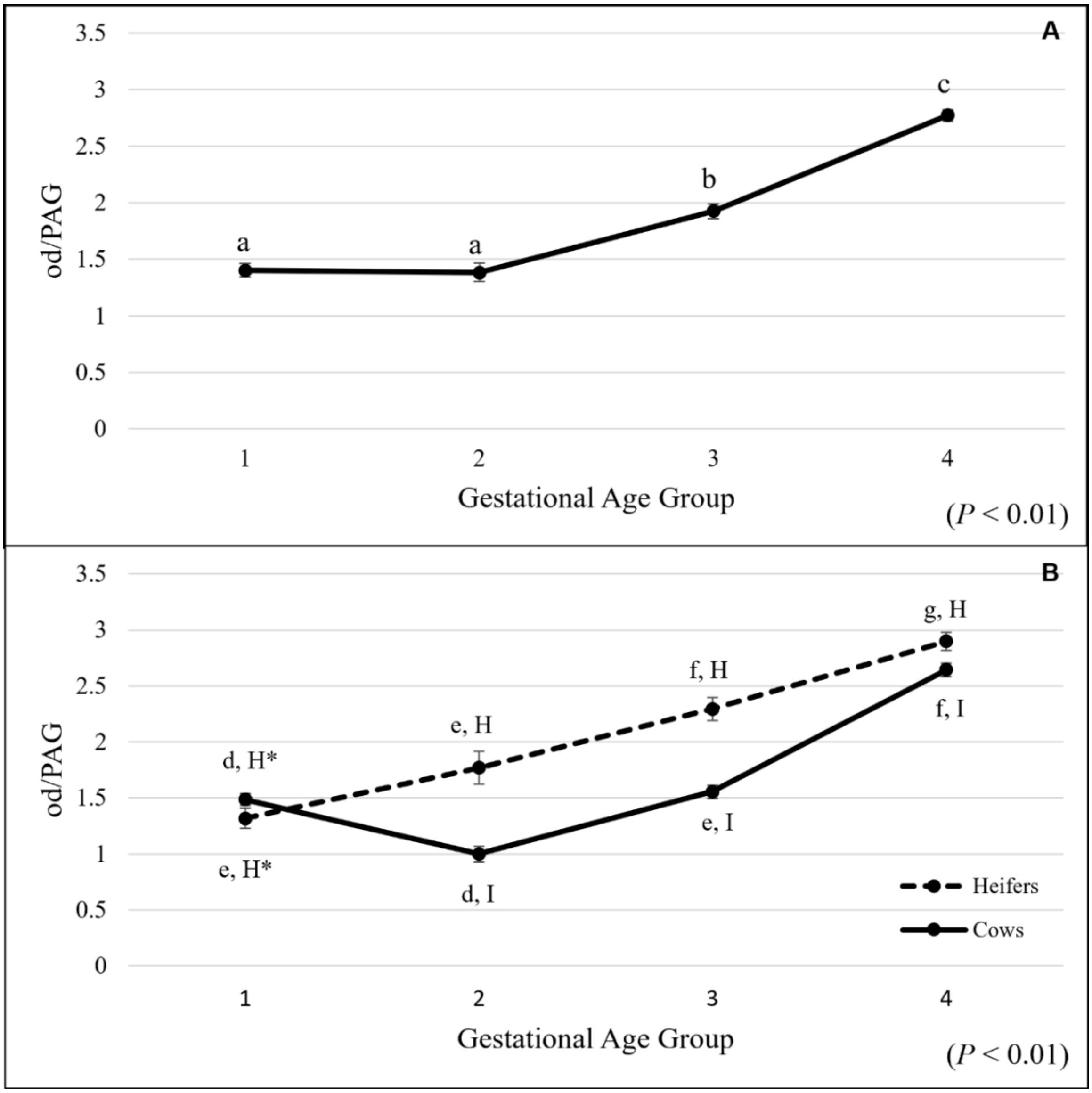

Figure 3. Effect of Group (A) and Age by Group (B) Interaction on PAG Concentrations. Mean (± SEM) serum pregnancy-associated glycoprotein (PAG) among four different gestational groups (1) < 30 d; 2) 30-90 d; 3) 91-180 d; 4) >180 d) of heifers and cows in figure A. Figure B, age represents heifers (dashed line) and cows (solid line). Different superscripts a-c not sharing the same superscripts differ ($P < 0.01$). Different superscripts d-g differ between groups within age not sharing the same superscript ($P \leq 0.01$). Different superscripts H, I represent values between age within group not sharing the same superscript differ ($P \leq 0.01$). Superscript * represents values tended to differ between gestational group ($P = 0.06$).

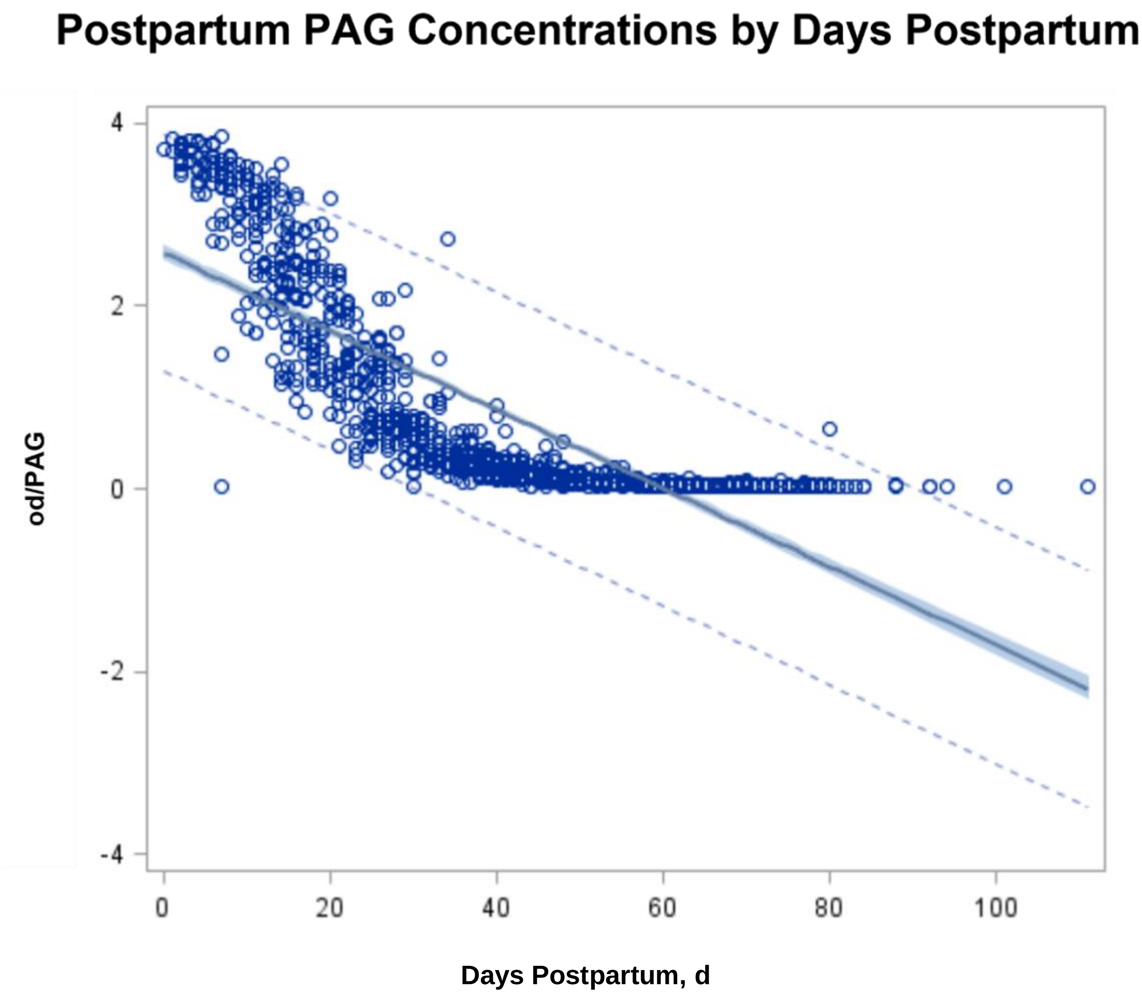

Figure 4. Regression analysis of days postpartum (dpp) on circulating concentrations of pregnancy-associated glycoproteins (PAG) in postpartum beef first-calf heifers and cows. Each circle indicates an individual sample. The solid line is the calculated regression line, the blue shaded area is the 95% confidence interval, and the dashed line is the 95% prediction limit ($P < 0.01$; $r^2 = 0.6708$).

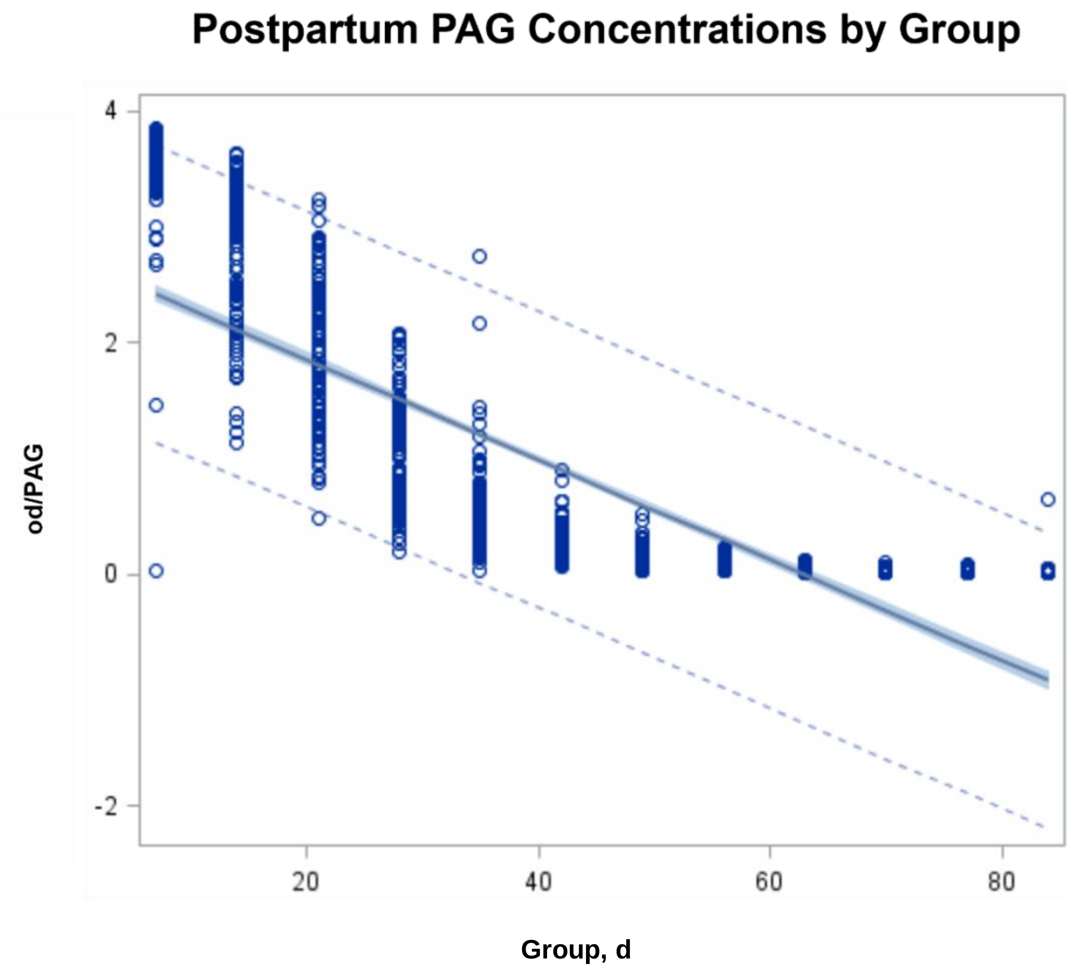

Figure 5. Regression analysis of group (7-day intervals) on circulating concentrations of pregnancy-associated glycoproteins (PAG) in postpartum beef heifers and cows. Each circle indicates an individual sample. The solid line is the calculated regression line, the blue shaded area is the 95% confidence interval, and the dashed line is the 95% prediction limit ($P < 0.01$; $r^2 = 0.6748$).

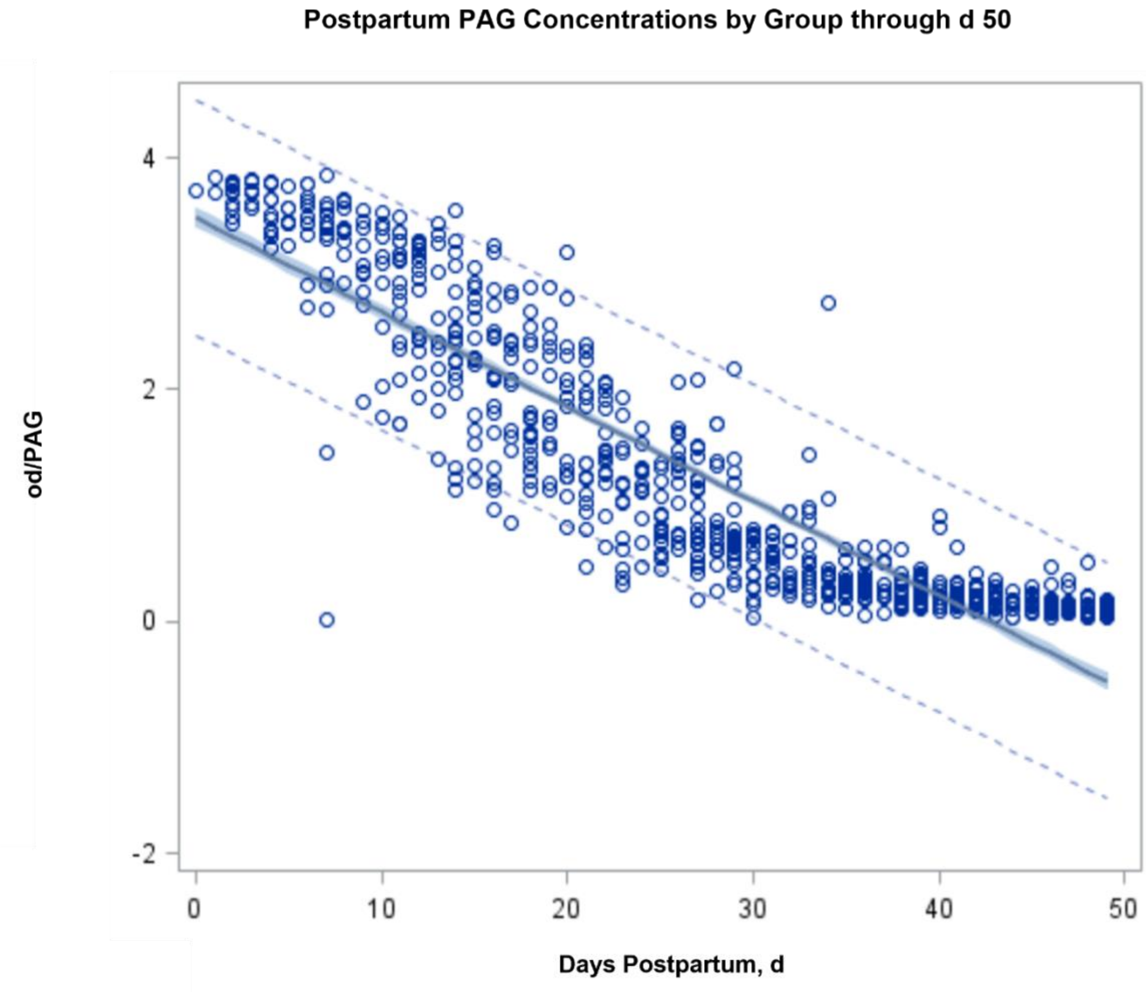

Figure 6. Regression analysis of days postpartum (dpp) on circulating concentrations of pregnancy-associated glycoproteins (PAG) in postpartum beef heifers and cows through d 50. Each circle indicates an individual sample. The solid line is the calculated regression line, the blue shaded area is the 95% confidence interval, and the dashed line is the 95% prediction limit ($P < 0.01$; $r^2 = 0.8173$).

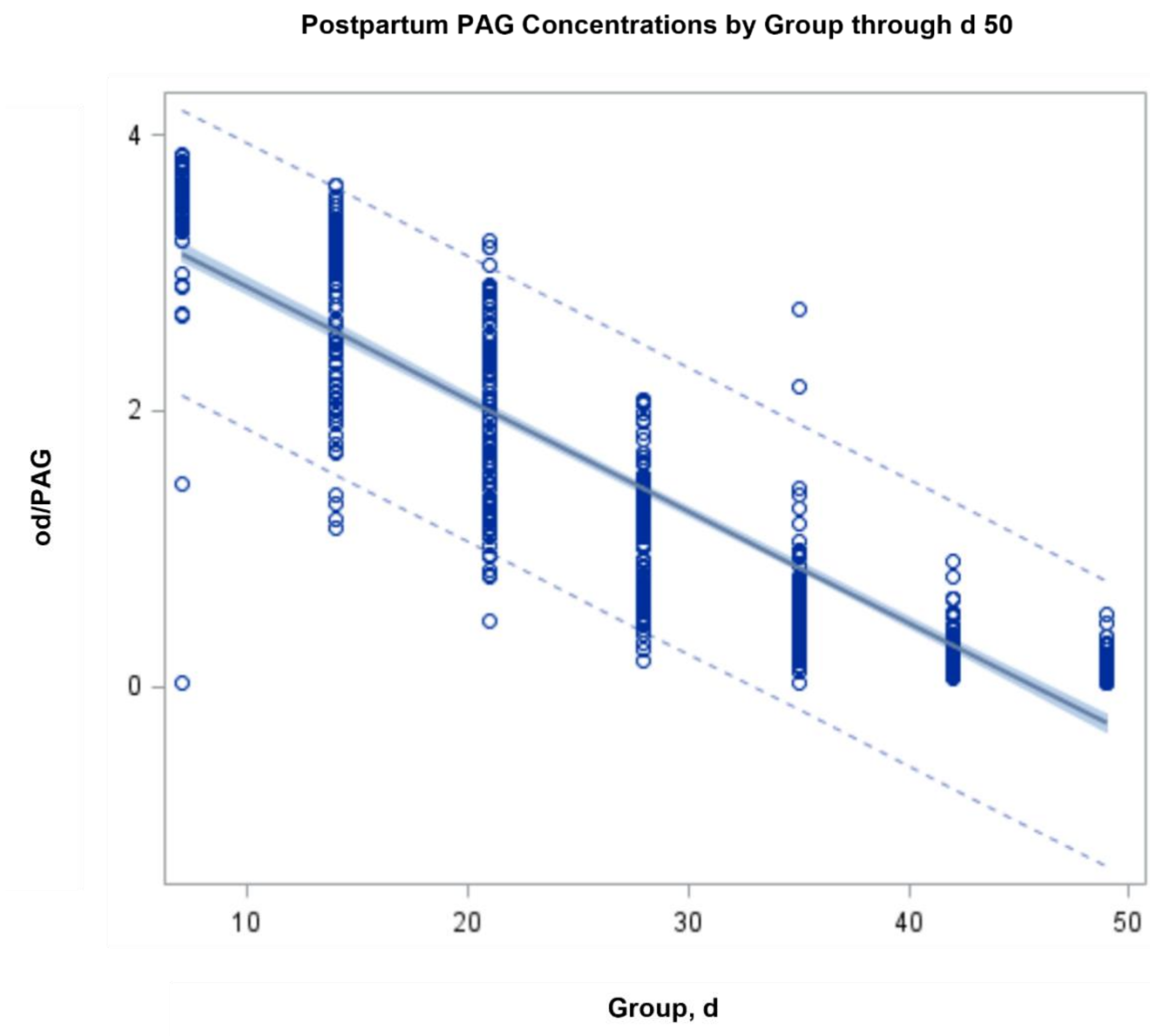

Figure 7. Regression analysis of group (7-day intervals) on circulating concentrations of pregnancy-associated glycoproteins (PAG) in postpartum beef heifers and cows through d 50. Each circle indicates an individual sample. The solid line is the calculated regression line, the blue shaded area is the 95% confidence interval, and the dashed line is the 95% prediction limit ($P < 0.01$; $r^2 = 0.8083$).

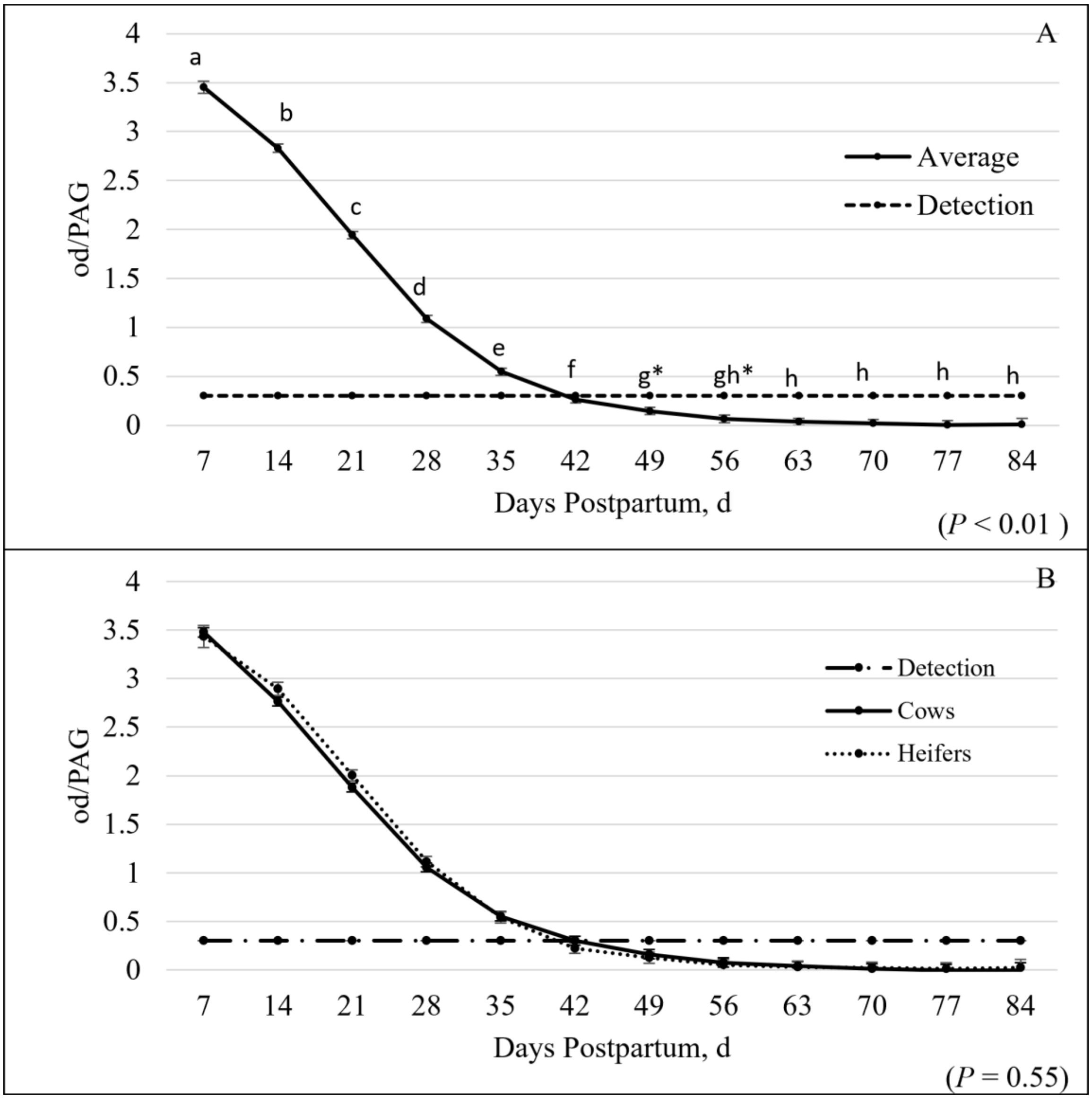

Figure 8. Overall clearance of PAG concentrations in postpartum beef cattle (A) and overall comparison between age (B). Mean (± SEM) serum pregnancy-associated glycoprotein (PAG) concentration levels among postpartum beef females (A). PAG concentration levels fall below the undetectable range by 42 dpp (optical density (od) = 0.2636; A). Different superscripts a-h not sharing the same superscripts differ ($P < 0.01$; A). Superscript * represents values tended to differ ($P = 0.08$; A). In beef cows and heifers there is no significant difference found postpartum ($P = 0.55$; B).