

# Effect of Various Handling Methods of Plasma and Serum Samples on Pregnancy Associated Glycoprotein Concentrations

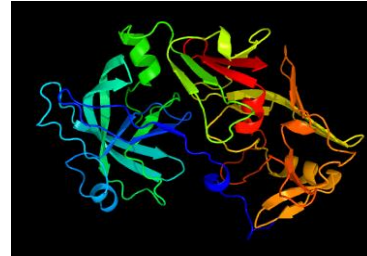
G.M. Wesson, L.F. Montero, R.K. Poole, G.A. Franco, S.T. Reese, and K.G. Pohler  
Department of Animal Science, Texas A&M University, College Station, TX



## INTRODUCTION

Pregnancy-associated glycoproteins (PAG) are produced by bovine binucleate trophoblast cells beginning at approximately three weeks of pregnancy (Wallace et al., 2015). PAG concentration, determined by Enzyme Linked Immunosorbent Assay (ELISA), is a biomarker of pregnancy in ruminants, so understanding PAG is key to improving assisted reproductive techniques.

**Figure 1:** A model of bovine PAG 1, depicting its bilobed shape (Kelley et al., 2015)



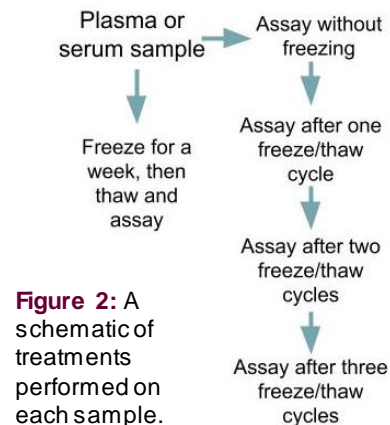
## OBJECTIVE & HYPOTHESIS

**Objective:** To determine if plasma and serum PAG concentrations are altered when centrifugation occurs at different times post-collection, when samples are subjected to repeated freezing and thawing, and when monoclonal antibodies are kept in frequently or infrequently opened containers.

**Hypothesis:** Handling of monoclonal antibodies, day of centrifugation, and repeated freeze/thaw cycles change the measurement of PAG concentrations in plasma and serum.

## MATERIALS & METHODS

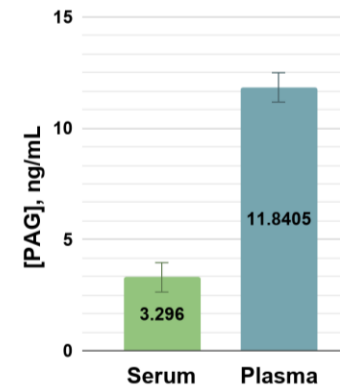
Plasma (n=4) and serum (n=4) samples were collected from two open and two pregnant cows 28 days after artificial insemination. Pregnancy status was determined via transrectal ultrasonography. Plasma and serum samples were evenly divided and either centrifuged on the day of collection or refrigerated at 4°C and centrifuged the next day. The samples that were centrifuged and assayed on the day of collection were evenly divided and assayed using monoclonal antibodies kept in either frequently or infrequently opened containers. An in-house PAG ELISA was performed on all samples before freezing, after being frozen for one week, after one freeze/thaw cycle, two freeze/thaw cycles, and three freeze/thaw cycles. Data were analyzed using one-way ANOVA (GLM procedure, SAS 9.4).



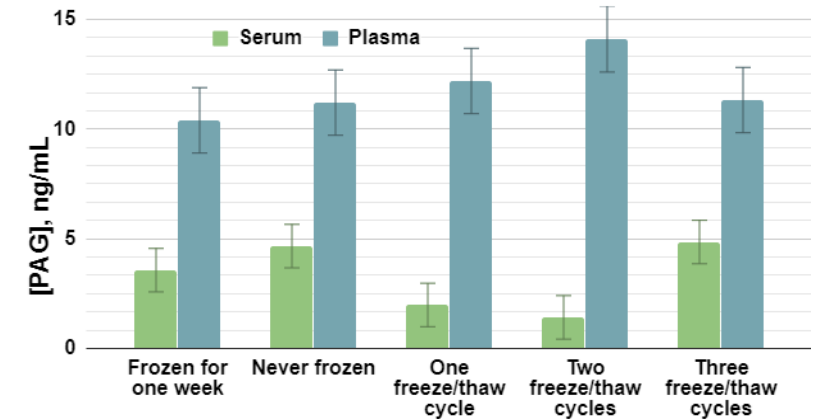
**Figure 2:** A schematic of treatments performed on each sample.

## RESULTS

Frequent exposure of monoclonal antibodies to air through repeatedly opening containers did not affect the detected concentration of PAG (P=0.90). The time of centrifugation of both plasma and serum also had no significant effect on PAG concentration (P=0.50 and P=0.60, respectively). All samples from open cows were below the baseline of the assay.



**Figure 3:** In pregnant cows, average PAG concentrations in plasma samples were less than in serum samples (11.84 vs 3.30 ± 0.66 ng/mL, respectively, P<0.05).



**Figure 4:** Freezing and thawing did not impact PAG concentrations in plasma samples (P=0.19), but did alter serum concentrations (P=0.01). Serum PAG concentrations after the first and second freeze-thaw cycles (1.98 ng/mL and 1.42 ng/mL) were lower compared to serum samples that were never frozen, serum samples that endured three freeze thaw cycles, and samples that were frozen for a week, then thawed (4.66, 4.85, and 3.57 ng/mL, respectively)

## CONCLUSIONS

The PAG ELISA is most reliable in plasma, and detected PAG concentrations in serum are significantly affected by freezing and thawing. While none of these changes were sufficient to misdiagnose pregnancy, freeze/thaw cycles could impact results when using PAG concentrations to predict pregnancy loss (Pohler et al., 2013) or number of progeny for small ruminants. More research is needed to determine the extent of and factors underlying the observed PAG concentration changes.

## References

Wallace et al. 2015. *Reprod.* 149: 115-126  
Pohler et al. 2013. *J. Anim. Sci.* 91:4158-4167  
Kelley et al. 2015. *Nature Protocols* 10, 845-858.