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REGULAR ARTICLES

# Efficacy of a commercial test kit to determine early pregnancy in cows using whole blood and blood serum

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## Abstract

The objective of this study was to determine the efficacy of the Fassisi®BoviPreg visual test kit (Fassisi®BoviPreg) in determining early pregnancies in cows by measuring the pregnancy-associated glycoprotein (PAG) in whole blood and blood serum. The study was conducted on 50 cows, the artificial insemination (AI) dates of which were designated as day 0. Pregnancy diagnosis was performed with transrectal ultrasonography (USG), and serum samples were simultaneously collected and used with Fassisi®BoviPreg to determine pregnancies on 30 days after AI. The results of the Fassisi®BoviPreg test on serum and whole blood samples, respectively, on 30 days after AI were as follows: sensitivity, 61.54% and 50.0%; specificity, 79.17% and 75%; accuracy, 70.0% and 62.0%; positive predictive values, 76.2% and 68.4%; negative predictive values, 65.5% and 58.1%; false-positive diagnoses, 23.8% and 31.6%; and false-negative diagnoses, 34.5% and 41.9%. On day 50 after AI, sensitivities were 63.64% and 50.0%, specificities were 100.0% and 100.0%, and accuracies were 75.0% and 65.62% in serum and whole blood, respectively. Higher pregnancy rates were obtained using Fassisi®BoviPreg in cows with ≥ 5 ng/mL P4 ($P < 0.001$). In conclusion, the results from the Fassisi®BoviPreg tests on cows on 30 and 50 days after AI showed that the use of blood serum is more accurate and suitable than that of whole blood. The results also showed a higher confidence level in specificity 50 days after AI.

**Keywords** Cow · Early pregnancy · PAG · Progesterone

## Introduction

Pregnancy detection in the early stages after artificial insemination (AI) is important for the future economy of cattle breeders. The determination of open (non-pregnant) cows, which can reveal problems such as disease and bad nutrition, can help provide suitable treatments and is a highly economical practice in dairy herds. There are many methods for diagnosis of pregnancy in cows. Estrus observation, rectal palpation, and ultrasonography (USG) are generally used as examination methods by which to determine pregnancies in cows. Some of the molecules related to pregnancy in cows are interferon-stimulated gene (ISG), progesterone (P4), and pregnancy-associated glycoproteins (PAGs) (Lucy et al. 2011). Laboratory tests such as for milk/blood PAGs, plasma/serum P4 levels, early pregnancy factors, and pregnancy-specific protein B (PSPB) are the methods by which to clinically diagnose pregnancy (Balhara et al. 2013, Fricke et al. 2016).

PAGs are inactive aspartic proteases produced and secreted by placental binuclear cells and detected in maternal circulation on days 24–25 of gestation (Zoli et al. 1992, Telugu et al. 2009). The biological function of PAGs is not completely clear; however, they are used to determine pregnancies in cows, ewes, and other ruminants because of placental-specific secretions (Gabor et al. 2007). Bovine PAGs are more localized in trophoblast binuclear cells in caruncular epithelial cells (Zoli et al. 1992). PAG and PAG-like proteins are produced from cow placenta on day 25 of gestation. Zoli et al. (1991) have suggested that these proteins can be used to

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determine pregnancy and embryonic loss. PAG concentrations were determined as 1.5, 1.9, and 2.2 ng/mL on days 33, 34, and 44 after AI, respectively (Piechotta et al. 2011). In cows for which a miscarriage (embryo loss) had been determined, PAG concentration was < 2.5 ng/mL (Piechotta et al. 2011).

This study for the first time was aimed at determining the efficacy of a visual commercial test kit (Fassisi®BoviPreg) using PAG in whole blood and blood serum to determine pregnancy at 30 and 50 days after AI in cows under field conditions in Turkey.

## Material and methods

The present study was conducted with approval from Kafkas University Ethical Committee, Kars, Turkey (KAU-HAYDEK 2015-110).

## Animals

Fifty clinically healthy primiparous cows (18 Simmental and 32 Brown Swiss) 3–5 years old were used in this study. The body condition score (BCS) of each cow was assessed according to a 5-scale system developed by Edmonson et al. (1989). The mean milk yield per day was 15–20 L in Simmental cows and 10–15 L in Brown Swiss cows.

The cows were fed twice a day just before milking with a total mixed ration (TMR) that was prepared using feed-mixing machines. TMR comprised a concentrated feed with 18% crude protein providing 2750 kcal/kg metabolic energy and also hay, trefoil, and corn silage as crude feed. Salt (0.5%) was added to TMR and water was provided ad libitum to all animals during the study.

## Procedures

Blood samples from the AI cows were collected from the *vena coccygea* into tubes with or without heparin 30 and 50 days after AI. USG using the SonoSite Titan® ultrasound machine (SonoSite, Inc., Bothell, WA, USA) was used on 30 and 50 days after AI to monitor the cows' pregnancy. The pregnancies were also simultaneously assessed using Fassisi®BoviPreg (Fassisi, Gesellschaft für Veterinardiagnostik und Umweltanalysen mbH, Göttingen, Germany) from whole blood according to the manufacturer's instructions under field conditions (15–30 °C) just after USG examination.

The blood samples in the empty tubes were centrifuged at 3000 rpm for 10 min to obtain the serum. The serum samples were transferred into microcentrifuge tubes and stored at − 20 °C until use in P4 analysis. Simultaneously, the pregnancy status of the cows was immediately determined from serum samples using Fassisi®BoviPreg to compare the results from those using whole blood samples. The pregnancy status was determined as either pregnant or open from the serum and whole blood samples. In addition, miscarriage was determined using USG between 30 and 50 days after AI.

Progesterone levels from the serum samples were determined by electrochemiluminescence immunoassay (ECLIA) using the Test Roche E170 as described by Kaya et al. (2017). The lower detection limit of progesterone was 0.03 ng/mL. The intra-assay and interassay coefficients of variation of progesterone were 0.7% and 1.8%, respectively.

## Statistical analyses

Statistical analyses of the data were conducted using SPSS v. 18.0 (SPSS Inc., Chicago, IL, USA). The results of the hormone analyses were determined as the mean ± standard deviation. Serum P4 levels in the cows were compared using Student's *t* test. Pregnancy rates were compared using the chi-squared test. Results of $P < 0.05$ were considered statistically significant.

The results of current study were formulated according to the literature as follows (Sinedino et al. 2014, Karen et al. 2015, Commun et al. 2016):

*Sensitivity* = number of correct pregnancies using Fassisi®BoviPreg / number of truly pregnant cows (determined using USG) x 100.

*Specificity* = number of correct open cows determined using Fassisi®BoviPreg / number of truly nonpregnant cows number (determined using USG).

*Positive predictive value (PPV)* = number of pregnancies correctly determined using Fassisi®BoviPreg / all pregnancies (USG + Fassisi®BoviPreg) x 100.

*Negative predictive value (NPV)* = number of open cows correctly determined using Fassisi®BoviPreg / all open cows (USG + Fassisi Kit) x 100.

*Accuracy* = all true results using Fassisi®BoviPreg (true pregnancies + open) / all determinations x 100.

## Results

A 52% (26/50) pregnancy rate was determined during the transrectal USG examination 30 days after AI. The sensitivity percentages from serum and whole blood were 61.54% and 50.00%, respectively, 30 days after AI. The specificity rate from serum was 79.17%; that from whole blood was 75.00% (Table 1). In addition, the sensitivity rates from serum and whole blood 50 days after AI were 63.64% and 50.00%, respectively. The specificity rate from serum and whole blood was 100.00% for each. The accuracy rates from serum and whole blood 30 and 50 days after AI are provided in Table 1. Table 2 also lists the PPV, NPV, false-negative, and false-positive rates for the serum tests 30 days after AI.

The mean P4 level in pregnant cows was 8.3 ± 3.2 ng/mL; that in non-pregnant cows was 2.5 ± 3.1 ng/mL ($P < 0.001$). P4 levels in the pregnant Simmental and Brown Swiss cows were 7.8 ± 1.8 and 10.3 ± 4.3 ng/mL, respectively ($P > 0.05$).

Fassisi®BoviPreg used to determine P4 levels resulted in a high pregnancy rate in cows with ≥ 5 ng/mL P4 30 days after AI ($P < 0.001$) but a lower rate in cows with < 5 ng/mL P4 (Table 3).

The sensitivity and specificity results from the Fassisi®BoviPreg tests on whole blood and serum comparing P4 levels 30 days after AI are provided in Table 4.

The differences in the sensitivity results between the Simmental and Brown Swiss cows are shown in Fig. 1. The sensitivity rate from serum was 93.33% in the Simmental cows and 27.27% in the Brown Swiss cows. The sensitivity rate from tests on whole blood was 66.67% in the Simmental cows and 27.27% in the Brown Swiss cows.

The specificity rates from tests on serum and whole blood 30 days after AI was 66.67% in the Simmental cows and 80.95% in the Brown Swiss cows.

The sensitivity rate from the serum of cows with BCS ≥ 3 was 83.33% but was 12.50% for cows with BCS < 3 using the same parameters. The specificity rates for cows with BCS ≥ 3 or BCS < 3 were 100.00% and 75.00%, respectively. The accuracies of the tests were 86.36% and 57.14% for cows with BCS ≥ 3 or < 3, respectively (Fig. 2).

It was determined that whole blood sensitivity 30 days after AI was 56.56% in cows with BCS ≥ 3 and 37.50% in cows with BCS < 3. Using the same parameters, the specificities were 75.00% in both groups. The accuracy rates for cows with BCS ≥ 3 or < 3 were 59.09% and 64.29%, respectively (Fig. 3).

USG conducted 50 days after AI determined that four embryos (two from each breed) were lost (15.4%) in the 26 cows for which USG had determined a pregnancy 30 days after AI.

**Table 1** Sensitivity and specificity rates in cows evaluated 30 days after artificial insemination

| Parameters | Day of pregnancy | Serum | Whole blood |
|---|---|---|---|
| Sensitivity (%) | 30 | 61.54 | 50.0 |
| Specificity (%) | | 79.17 | 75.0 |
| Accuracy (%) | | 70.0 | 62.0 |
| Sensitivity (%) | 50 | 63.64 | 50.0 |
| Specificity (%) | | 100 | 100 |
| Accuracy (%) | | 75.0 | 65.62 |

**Table 2** Serum and whole blood positive identification, negative identification, false-positive, and false-negative rates 30 days after artificial insemination in cows

| Parameters | Day 30 | |
|---|---|---|
| | Serum (%) | Whole blood (%) |
| Positive predictive value | 76.2 | 68.4 |
| Negative predictive value | 65.5 | 58.1 |
| False positive | 23.8 | 31.6 |
| False negative | 34.5 | 41.9 |

## Discussion

Because cattle breeding is an economical issue, the sensitivity for detecting open cows is more important (a true determination of pregnant cows) than the specificity (a true determination of non-pregnant cows) (Giordano et al. 2013). Test kits used to determine open cows within an early period should have sensitivity rates of 94.00% on day 24 and 96.00% on day 31 after AI to predict an economical value in the dairy industry (Giordano et al. 2013). It is well known that 32 days after AI in cows is the best time to determine pregnancies when considering PAG levels (Ricci et al. 2015). Ricci et al. (2015) have found that sensitivity and specificity were 100.00% and 87.00%, respectively, in PAG-positive cows 32 days after AI. The accuracy rate was 92.00% in this same study. LeBlanc (2013) has pointed out a 99.20% sensitivity and 95.50% specificity in the study conducted based on PAG levels in milk. In cows whose PAG levels were determined with ELISA between 31 and 35 days after AI, sensitivity, specificity, PPV, NPV, and accuracy rates were 98.70%, 88.10%, 83.70%, 99.10%, and 92.20%, respectively. In the present study, the sensitivity and specificity rates obtained using the visual Fassisi®BoviPreg were relatively low compared with those in previous studies.

Serum PAG concentrations can be affected by breed differences. PAG concentrations in Boran and Boran x Holstein-Friesian cows crossbred during pregnancy are different (Lobago et al. 2009). In pregnant cows, the mean concentration of blood PAG was 9.50 ± 5.33 ng/mL on day 31 of gestation. Pohler et al. (2016a) have also indicated that serum

**Table 3** Comparison of serum progesterone (P4) levels and pregnancy rates with the Fassisi®BoviPreg test kit in sera 30 days after artificial insemination in cows

| P4 (ng/mL) | Pregnancy rate (%) | *P* value |
|---|---|---|
| 1–5 | 25.0 | < 0.001 |
| > 5 | 76.9 | |

*P4* progesterone

**Table 4** Comparison of serum progesterone (P4) levels with parameters determined by the Fassisi®BoviPreg test kit in sera 30 days after artificial insemination in cows

| Parameters | P4 (ng/mL) | | | |
|---|---|---|---|---|
| | Serum | | Whole blood | |
| | 0–5 | > 5 | 0–5 | > 5 |
| Sensitivity rate (%) | 66.66 | 60.0 | 50.0 | 55.0 |
| Specificity rate (%) | 77.77 | 83.33 | 70.0 | 83.33 |
| Accuracy rate (%) | 75.0 | 65.38 | 66.66 | 61.54 |

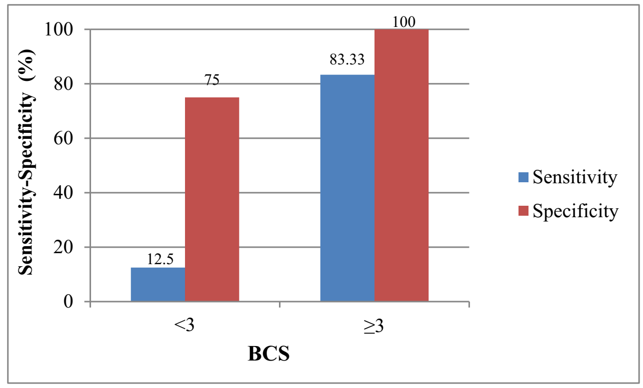


**Fig. 2** Sensitivity and specificity rates in sera according to body condition score 30 days after artificial insemination (%)

bPAG concentration was 15.11 ± 9.92 ng/mL on day 28 in Nelore cows. In the present study, using Fassisi®BoviPreg, P4 levels 30 days after AI were higher in the pregnant cows, and there was a statistical difference in these levels between pregnant and non-pregnant cows. In addition, the P4 levels in the pregnant Brown Swiss cows were higher than those in the pregnant Simmental cows; this difference was also statistically significant. These findings are consistent with those of other studies on the effect of breeds on PAG concentration (Pohler et al. 2016b). Similar accuracy rates have been determined with analyses of bovine PSPB and bovine PAG in pregnant cows 33 and 34 days after AI; however, the determination of non-pregnant cows was more difficult than that of pregnant cows (Szenci et al. 1998).

Pohler et al. (2016b) have not determined any relationship between body weight and PAG concentration in cows. They have assessed a 12.00% embryonic loss between days 31 and 59 of pregnancies using USG. PAG concentration in pregnant cows from days 31 to 59 after AI was 9.58 ± 0.31 ng/mL on day 31, but PAG concentration in cows that had miscarried during that same period was only 4.15 ± 0.33 ng/mL. The embryonic loss increased to day 59 of gestation when the PAG levels on day 31 of gestation were low; however, these same findings were not confirmed from day 59 of gestation to parturition. Although the mean PAG concentration on day 24 of gestation was 3.06 ± 0.49 ng/mL, that in open cows was 0.94 ± 0.16 ng/mL; the difference was statistically significant. In pregnant cows, PAG concentration on day 31 of gestation was 9.06 ± 4.48 ng/mL and P4 concentration was 8.01 ± 4.61 ng/mL. In a study conducted on dairy cows, PAG levels were related to P4 concentrations as long as gestation continued (Barbato et al. 2013). In the present study, the high percentage of pregnancy detection using visual PAG kits in cows, which had higher P4 concentration during gestation, might be associated with resuming their embryo viability and increasing of PAG secretion.

Breukelman et al. (2012) have not found a relationship between late embryonic loss and P4 concentration but have shown a positive correlation between the health of the embryo and PAG concentration in the blood. On the contrary, Starbuck et al. (2004) have determined a relationship between the P4 concentration at week 5 of gestation and the health of the embryo in dairy cows. Embryonic loss that was detected in the present study was believed to be related to PAG concentration, which peaked on 30 days after AI and suddenly decreased at day 50 of gestation when a miscarriage had occurred.

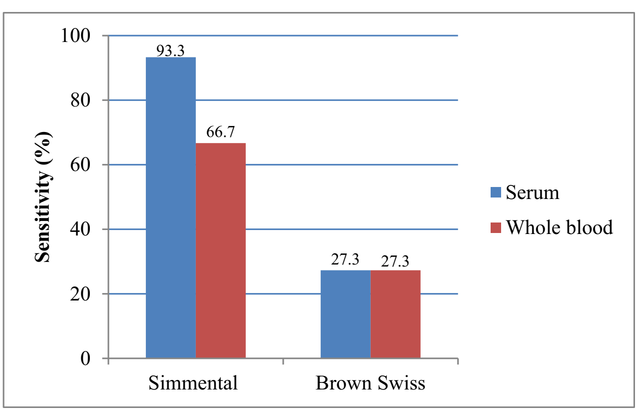


**Fig. 1** Sensitivity rates (%) of serum and whole blood according to breed differentiation in Simmental and Brown Swiss cows 30 days after artificial insemination

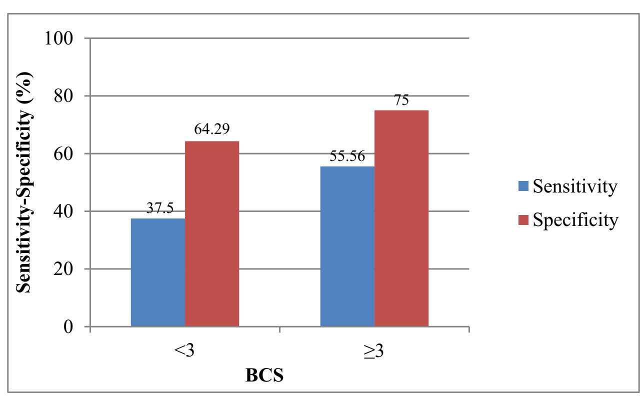


**Fig. 3** Whole blood sensitivity and specificity ratio (%) according to body condition score (< 3 and ≥ 3) 30 days after artificial insemination

It was determined that there was no relationship between PAGs and BCS in beef cows (Kiracofe et al. 1993). Moreover, a negative correlation between PAG and BCS concentration was detected in dairy cows; PAG concentration decreased while BCS increased (Ricci et al. 2015, Stratman et al. 2016). In another study, it was pointed out that there were no changes in statistical significance in the PAG concentrations in primiparous or multiparous cows among high, normal, and low BCS (Mercadante et al. 2016). In the present study, the rates of sensitivity, specificity, and accuracy in serum was higher in cows with BCS ≥ 3, while the sensitivity rate in whole blood was higher in cows with BCS ≥ 3. The specificity and accuracy rates in whole blood were not changed by the BCS values. According to the results from the present study, we believe that the use of visual PAG test kits, such as Fassisi®BoviPreg, have some advantages for determining pregnant and non-pregnant cows from serum samples, especially for those with higher BCS values.

## Conclusion

We believe that the use of blood serum might be more efficient than that of whole blood in a visual PAG kit, such as Fassisi®BoviPreg, and that there is a higher confidence level for specificity values obtained on day 50 of gestation in either serum or whole blood using Fassisi®BoviPreg to determine pregnancies on day 30 or 50 after AI. In addition, we suggest that the test results might be different in different cattle breeds, given that the sensitivity rate in the blood of Simmental cows was higher than that in Brown Swiss cows. Additional more detailed studies should be conducted on Fassisi®BoviPreg when used to determine early pregnancies in cows.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that there are no conflicts of interest.

**Statement of animal rights** All institutional and national guidelines for the care and use of laboratory animals were followed.

## References

Balhara, A.K., Gupta, M., Singh, S., Mohanty, A.K. and Singh, I., 2013. Early pregnancy diagnosis in bovines, current status and future directions. Scientific World J., 958540.

Barbato, O., Merlo, M., Celi, P., Sousa, N.M., Guarneri, L., Beckers, J.F. and Gabai, G., 2013. Relationship between plasma progesterone and pregnancy-associated glycoprotein concentrations during early pregnancy in dairy cows. Vet. J., 195, 385-387.

Breukelman, S.P., Perenyi, Z., Taverne, M.A., Jonker, H., Van der Weijden, G.C., Vos, P.L., de Ruigh, L., Dieleman, S.J., Beckers, J.F. and Szenci, O., 2012. Characterisation of pregnancy losses after embryo transfer by measuring plasma progesterone and bovine pregnancy associated glycoprotein-1 concentrations. Vet. J., 194, 71-76.

Commun, L., Velek, K., Barbry, J.B., Pun, S., Rice, A., Mestek, A., Egli, C. and Leterme, S., 2016. Detection of pregnancy-associated glycoproteins in milk and blood as a test for early pregnancy in dairy cows. J. Vet. Diagn. Invest., 28, 207-213.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T. and Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci., 72, 68-78.

Fricke, P.M., Ricci, A., Giordano, J.O. and Carvalho, P.D., 2016. Methods for and implementation of pregnancy diagnosis in dairy cows. Veterinary Clinics of North America-Food Animal Practice, 32, 165–180.

Gabor, G., Toth, F., Ozsvari, L., Abonyi-Toth, Z. and Sasser, R.G., 2007. Early detection of pregnancy and embryonic loss in dairy cattle by ELISA tests. Reprod. Domest. Anim., 42, 633-636.

Giordano, J.O., Fricke, P.M. and Cabrera, V.E., 2013. Economics of resynchronization strategies including chemical tests to identify nonpregnant cows. J. Dairy Sci., 96, 949-961.

Karen, A., De Sousa, N.M., Beckers, J.F., Bajcsy, Á.C., Tibold, J., Mádl, I. and Szenci, O., 2015. Comparison of a commercial bovine pregnancy-associatedglycoprotein ELISA test and a pregnancy-associatedglycoprotein radiomimmunoassay test for early pregnancy diagnosis in dairy cattle. Anim. Reprod. Sci., 159, 31-37.

Kaya, S., Kaçar, C., Polat, B., Çolak, A., Kaya, D., Gürcan, I.S., Bollwein, H. and Aslan, S., 2017. Association of luteal blood flow with follicular size, serum estrogen and progesterone concentrations, and the inducibility of luteolysis by PGF2α in dairy cows. Theriogenology, 87, 167-172.

Kiracofe, G.H., Wright, J.M., Schalles, R.R., Ruder, C.A., Parish, S. and Sasser, R.G., 1993. Pregnancy-specific protein B in serum of postpartum beef cows. J. Anim. Sci., 71, 2199-2205.

LeBlanc, S.J., 2013. Field evaluation of a pregnancy confirmation test using milk samples in dairy cows. J. Dairy Sci., 96, 2345-2348.

Lobago, F., Bekana, M., Gustafsson, H., Beckers, J.F., Yohannes, G., Aster, Y. and Kindahl, H., 2009. Serum profiles of pregnancy-associated glycoprotein, oestrone sulphate and progesterone during gestation and some factors influencing the profiles in Ethiopian Borana and crossbred cattle. Reprod. Domest. Anim., 44, 685-692.

Lucy, M., Green, J. and Poock S., 2011. Pregnancy determination in cattle: A review of available alternatives. In: Proceedings, Applied Reproductive Strategies in Beef Cattle. 31 August-1 September, Joplin, MO, pp. 367-376.

Mercadante, P.M., Ribeiro, E.S., Risco, C. and Ealy, A.D., 2016. Associations between pregnancy-associated glycoproteins and pregnancy outcomes, milk yield, parity, and clinical diseases in high-producing dairy cows. J. Dairy Sci., 99, 3031-3040.

Piechotta, M., Bollwein, J., Friedrich, M., Heilkenbrinker, T., Passavant, C., Branen, J., Sasser, G., Hoedemaker, M. and Bollwein, H., 2011. Comparison of commercial ELISA blood tests for early pregnancy detection in dairy cows. J. Reprod. Dev., 57, 72-75.

Pohler, K.G., Pereira, M.H.C., Lopes, F.R., Lawrence, J.C., Keisler, D.H., Smith, M.F., Vasconcelos, J.L.M. and Green, J.Z., 2016a. Circulating concentrations of bovine pregnancy-associated glycoproteins and late embryonic mortality in lactating dairy herds. J. Dairy Sci., 99, 1584-1594.

Pohler, K.G., Peres, R.F.G., Green, J.A., Graff, H., Martins, T., Vasconceles, C.L.M. and Smith, M.F., 2016b. Use of bovine pregnancy-associated glycoprotein to predict late embryonic mortality in postpartum Nelore beef cows. Theriogenology, 85, 1652-1659.

Ricci, A., Carvalho, P.D., Amundson, M.C., Fourdraine, R.H., Vincenti, L. and Fricke, P.M., 2015. Factors associated with pregnancyassociated glycoprotein (PAG) levels in plasma and milk of Holstein cows during early pregnancy and their effect on the accuracy of pregnancy diagnosis. J. Dairy Sci., 98, 2502-2514.

Sinedino, L.D.P., Lima, F.S., Bisinotto, R.S., Cerri, R.L.A. and Santos, J.E.P., 2014. Effect of early or late resynchronization based on different methods of pregnancy diagnosis on reproductive performance of dairy cows. J. Dairy Sci., 97, 4932-4941.

Starbuck, M.J., Dailey, R.A. and Inskeep, E.K., 2004. Factors affecting retention of early pregnancy in dairy cattle. Anim. Reprod. Sci., 84, 27-39.

Stratman, T.J., Moore, S.G., Lamberson, W.R., Keisler, D.H., Poock, S.E. and Lucy, M.C., 2016. Growth of the conceptus from day 33 to 45 of pregnancy is minimally associated with concurrent hormonal or metabolic status in postpartum dairy cows. Anim. Reprod. Sci., 168, 10-18.

Szenci, O., Beckers, J.F., Humblot, P., Sulon, J., Sasser, G., Taverne, M.A., Varga, J., Baltusen, R. and Schekk, G., 1998. Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology, 50, 77-88.

Telugu, B.P., Walker, A.M. and Green, J.A., 2009. Characterization of the bovine pregnancy-associated glycoprotein gene family-analysis of gene sequences, regulatory regions within the promoter and expression of selected genes. BMC Genomics, 185, 1-17.

Zoli, A.P., Beckers, J.F., Ballman, P.W., Closset, J., Falmagne, P. and Ectors, F., 1991. Purification and characterization of a bovine pregnancy-associated glycoprotein. Biol. Reprod., 45, 1-10.

Zoli, A.P., Guilbault, L.A., Delahaut, P., Ortiz, W.B. and Beckers, J.F., 1992. Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: Its application for pregnancy diagnosis. Biol. Reprod., 46, 83-92.

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