

## Negative Prediction Value of Pregnancy-associated Glycoprotein Contributes to Reduce the Days During Which Nonpregnant Holstein Cows are Subjected to Diverse Strategies of Hormonal Synchronization

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**Abstract:** The objective of the present study was to determine the accuracy and the negative prediction value of Pregnancy-Associated Glycoprotein (PAG) at 29 d post artificial insemination by ELISA. Blood serums of multiparous Holstein cows were used (n = 275). Pregnancy diagnosis was carried out by rectal palpation at 40 d and it was considered as reference test. Pregnancy results based on the PAG-ELISA and the pregnancy diagnosis at 40 d showed high concordance with a kappa value of 0.7. The accuracy test based on PAG-ELISA at 29 d post artificial insemination compared with pregnancy diagnosis by rectal palpation was 88%. The values of sensibility, specificity, positive prediction value, negative prediction value and positive and negative likelihood ratio were 96, 78, 83 and 95%, 4.5 and 0.0442, respectively. It is concluded that pregnancy diagnosis of PAG-ELISA is reliable and accurate at 29 d post artificial insemination. The PAG-ELISA's negative prediction value diminishes the number of days in subjecting cows diagnosed as nonpregnant to diverse strategies of hormonal synchronization protocols.

**Keywords:** Dairy cattle, pregnancy diagnosis, pregnancy-associated glycoprotein, rectal palpation

### INTRODUCTION

To reach acceptable productive and reproductive efficiency in a dairy herd, diverse factors play pivotal role like the level of milk production, fertility, days in milk, interval between births, timely detection of estrous, open days, early pregnancy diagnosis of both pregnant and nonpregnant cows (Löf *et al.*, 2007; Silva *et al.*, 2007). Cows that were first diagnosed as pregnant but later on turned out not to be, have a deep and negative influence on the herd's reproductive efficiency parameter. These pre-natal losses are probably the factor that more affects productivity of dairy cattle herds (López-Gatius *et al.*, 2007). In daily routine and practice, pregnancy diagnosis is carried out usually by two techniques: transrectal ultrasonography and rectal palpation (Zemjanis, 1984; Frike, 2002; Romano *et al.*, 2006, 2007; Bartolome *et al.*, 2012). However, controversies exist for the advantages and disadvantages of these two techniques. For example, transrectal ultrasonography when pregnancy diagnosis in cows is carried out between 24 and 30 d post-service,

taking the moment of estrous as day 0 (Romano *et al.*, 2006). In fact, rectal palpation continues being a useful tool in pregnancy diagnosis for a considerable number of dairy herds, since the veterinarian does not always count with an ultrasound device for pregnancy diagnosis. Another methodology is related to placental glycoproteins, denominated pregnancy specific B proteins; these proteins are isolated from the fetal membranes (Butler *et al.*, 1982; Sasser *et al.*, 1989; Zoli *et al.*, 1992). Placental glycoproteins are expressed in the mononuclear cells or binuclear cells of trofoectoderm and they are secreted to the peripheral blood, thus it is possible to detect them in serum of pregnant cows from 15 d of conception (Sasser *et al.*, 1986; Wooding *et al.*, 2005). The concentration of Pregnancy-Associated Glycoprotein (PAG) is gradually increased from 15 to 35 d of pregnancy, reaching its pick before the birth and remaining in the blood circulation for several weeks post-partum (Zoli *et al.*, 1992; Green *et al.*, 2005; Haugejorden *et al.*, 2006). The objective of the present study was to determine the accuracy and negative prediction value of pregnancy-

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associated glycoprotein at 29 d post-service in Holstein cows as compared with pregnancy diagnosis on d 40 by rectal palpation.

## MATERIALS AND METHODS

**Animals and herd management:** The study was carried out in April, in a commercial dairy herd located in northern Mexico Laguna Region. The cows used in the study did not present neither reproductive disorders nor clinical illness. Cows were in open pens and were milked twice times daily, they were fed according to a total mixed ration of rolled corn, sorghum silage, alfalfa hay and soya flour, with free access to mineral salts and water. Semen was obtained from bulls of proven fertility. Two hundred seventy-five multiparous Holstein cows were synchronized and inseminated. They had an average of 146 days in milk.

**Blood samples:** Blood samples were obtained from cows at 29 d after having been subjected to a program of estrous synchronization for Artificial Insemination (AI). Vacutainer tubes without anticoagulant were used (BD Vacutainer, Franklin Lakes, NJ) and blood was obtained by caudal venopunction. Immediately the samples were transported to a laboratory, where were centrifuged at 3500×g for 29 min and blood serums were frozen at -20°C until their analysis.

**Detection of PAG-ELISA:** The ELISA was used to detect and to quantify specific antibodies. Protocol was carried out according to the commercial kit bovine pregnancy test instructions (IDEXX Laboratories, Inc. Westbrook, Maine, USA). Kit of bovine pregnancy detection is an enzymatic immunoassay that detects the early presence of serum PAG which acts as markers of pregnancy. The reader BioTek ELx800 was used to measure the absorbance. The results, calculations and the interpretation of each sample were carried out by the software provided with the kit.

**Pregnancy diagnosis by means rectal palpation:** Pregnancy diagnosis by rectal palpation was carried out at 40 d post artificial insemination by a veterinarian with wide experience in animal reproduction. These results were considered as the reference test (true value).

**Statistical analysis:** In order to evaluate the capacity of PAG-ELISA test and to correctly classify a cow as pregnant or nonpregnant, the approach of validity of the coefficient of agreement denominated statistical kappa was used, which indicates the concordance grade among the two tests. The value kappa of 0.4 to 0.5

indicates moderate concordance, 0.5 to 0.6 means good and >0.6 indicates high concordance (Carletta, 1996; Silva *et al.*, 2007). The accuracy of the assay is represented by the percentage of cows diagnosed correctly [(number of true positive results + number of true negative results) / (number of observations)]. The sensibility of the assay was expressed as the percentage of pregnant cows with a positive result of PAG-ELISA [number of true positive results / (number of true positive results + number of negative false results)]. By contrast, the specificity of the assay was calculated as the proportion of nonpregnant cows with a negative result in the test [number of true negative results / (number of true negatives + number of positive false results)]. The Prediction Positive Value (PPV) was calculated as the proportion of pregnant cows [number of pregnant cows with true positive results / (number of true positive results + number of positive false results)], while the Prediction Negative Value (PNV) is the percentage of cows with negative pregnancy results according to PAG-ELISA which turned out as nonpregnant by rectal palpation [true negative results / (number of cows with true negative results + number of cows with false negative results)]. To determine the certainty of the test, two additional calculations were carried out that include the positive and negative likelihood ratio. The positive likelihood ratio is the probability of obtaining a pregnant cow result, when this cow is nonpregnant, this proportion is related to the test of specificity [(sensibility / (1 - specificity)]. While negative likelihood ratio is the probability of obtaining a negative pregnant result in a pregnant cow (1 - sensibility / specificity), this proportion is related to the sensibility test (Henneken and Buring, 1987; Argimón-Pallás and Jiménez-Villa, 2004; Silva *et al.*, 2007).

## RESULTS

**Prediction negative value and prediction positive value:** The prediction negative value of the assay was 95% (103/108) which is the percentage of nonpregnant cows at 40 d post-AI that were negative by PAG-ELISA. While the prediction positive value was 83% (139/167) which is the percentage of pregnant cows at 40 d post-AI that was positive by PAG-ELISA (Table 1).

**Accuracy of the test and likelihood ratio:** The accuracy of the test was 88% which is the percentage of cows diagnosed correctly (Table 1). The obtained kappa value was 0.7±0.04. Positive likelihood ratio was 4.5 which mean that a cow diagnosed as pregnant by PAG-ELISA is 4.5 times more likely to be pregnant. While negative likelihood ratio was 0.0442, is the probability that a pregnant cow was diagnosed as nonpregnant.

Table 1: Contingency table to evaluate sensibility<sup>1</sup>, specificity<sup>2</sup>, prediction positive value<sup>3</sup>, prediction negative value<sup>4</sup> and accuracy<sup>5</sup> in detection of Pregnancy-Associated Glycoprotein (PAG-ELISA) and pregnancy diagnosis on 40 days post-artificial insemination in Holstein cows <sup>1</sup>[a/ (a + b)] × 100, <sup>2</sup>[d/ (d + c)] × 100, <sup>3</sup>[a/ (a + c)] × 100, <sup>4</sup>[d/ (b + d)] × 100, <sup>5</sup>(a + d) /n] × 100

PAG-ELISA	Rectal palpation 40 days post-AI		
	Pregnant	Nonpregnant	Total
Pregnant	139 <sup>b</sup>	28 <sup>c</sup>	167
Nonpregnant	5 <sup>b</sup>	103 <sup>d</sup>	108
Total (n)	144	131	275

n: Number of cases

**Sensibility and specificity:** The sensibility of the assay was 96% (139/144) which is the percentage of pregnant cows with positive result of PAG-ELISA. The specificity of the assay was 78% (103/131) which is the percentage of nonpregnant cows with negative result of PAG-ELISA (Table 1).

## DISCUSSION

The results obtained in this study indicate a high concordance between PAG-ELISA and pregnancy diagnosis by rectal palpation, since the accuracy of the assay was 88% and the statistical kappa was 0.7 (Henneken and Buring, 1987; Argimón-Pallás and Jiménez-Villa, 2004; Silva *et al.*, 2007). Also our results show that the prediction negative value was 95%, which means that there was a high the rate of nonpregnant cows diagnosed by PAG-ELISA. Indeed when the pregnancy diagnosis by rectal palpation was carried out at 40 d post-AI, those cows usually were nonpregnant. This finding is similar to those reported by Silva *et al.* (2007) where the prediction negative value was 97.1% at 27 d post-AI between the pregnancy diagnosis of PAG-ELISA and transrectal ultrasonography. The accuracy of our assay was 88%, which differs with 94.7% found by Zoli *et al.* (1992). It is probably because they carried out the pregnancy diagnosis at 35 d post-AI, in cows transferred with embryos, whereas in our study it was made at 29 d post-AI. In spite of it, the accuracy value was high and was confirmed by the values obtained with positive and negative likelihood ratio of this assay. The accuracy and the prediction negative value in pregnancy diagnosis by PAG-ELISA compared with pregnancy diagnosis on 40 d by rectal palpation have not been previously reported. The 96% sensibility found in our study coincides with other reports, where they found 93.5% when carrying out the pregnancy diagnosis by transrectal ultrasonography between 24 and 30 d post-AI in cows subjected to estrous synchronization (Romano *et al.*, 2006). In another study, a 96.8% sensibility is reported between the PAG-ELISA and the

pregnancy diagnosis by transrectal ultrasonography carried out at 27 after timed artificial insemination (Silva *et al.*, 2007). On the contrary, low sensibility values (75 and 81.2%) have also been reported in cows diagnosed as pregnant before 29 d post-service and diagnosed by PAG-RIA (Szenci *et al.*, 1998). In this study it was found that the assay specificity was 78% which differs with the 96.8% reported by Silva *et al.* (2007), which suggest that probably more cows are diagnosed as negative at 27 d post-AI on fixed time than among 29 post-AI subjected to an estrous synchronization protocol. Actually the pregnancy diagnosis is mostly performed by transrectal ultrasonography, a technique broadly used in dairy herds with technologies of vanguard (Romano *et al.*, 2006; López-Gatius *et al.*, 2007; Silva *et al.*, 2007; Bartolome *et al.*, 2012). However, there are frequent errors in pregnancy diagnosis before 30 d post-service; since the use of this technique in those days reduces the benefit of having an early pregnancy diagnosis (Badtram *et al.*, 1991; Silva *et al.*, 2007). Indeed, rectal palpation continues being a frequent and accurate practice (Vaillancourt *et al.*, 1979; Romano *et al.*, 2007, 2011). The 83% prediction positive value in our study was similar to 87.3% reported by Silva *et al.* (2007); these data indicate that a high percentage of cows are diagnosed as pregnant by the PAG-ELISA on 27 d post-AI, confirming this diagnosis by transrectal ultrasonography and by rectal palpation. The high prediction negative value of 95% obtained in this study suggests that few cows (4.6%) did suffer an embryonic loss (Silva *et al.*, 2007). Also in this test, the risk of causing microabortions is low, if the cows were subjected to a treatment with prostaglandins for estrous or ovulation synchronization (Frike *et al.*, 2003; Romano *et al.*, 2006). From the practical point of view, the prediction negative value PAG-ELISA's test result at 29 d post-AI contributes to the fact that cows do not have to suffer a delay of 11 d, to be diagnosed by rectal palpation at 40 d gestation period, which is common in dairy herds in this region of northern Mexico. Then, once cows are diagnosed as nonpregnant, they could immediately be subjected to programs of hormonal synchronization used in diverse reproductive strategies in dairy herds. It would be advisable to carry out this technique in all dairy herds, especially during months of heat stress, that are very intense in this region (Cruz-Velázquez *et al.*, 2009). This could contribute substantially to detect the nonpregnant cows in advance.

## CONCLUSION

Early pregnancy diagnosis by PAG-ELISA is an accurate technique. The prediction negative value of

pregnancy-associated glycoprotein contributes to reduce the days during which nonpregnant cows are subjected to diverse hormonal synchronization protocols.

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