The Detection of Pregnancy Associated Glycoproteins (PAG) in Routine Milk Recording Samples as an Indicator of Pregnancy in Dairy Cattle

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Abstract

Accurate and timely detection of pregnancy in dairy cows is an essential component of any bovine reproductive management programme. Good reproductive performance has multiple financial benefits. Increased profits result from many areas, including decreased average days in milk in the lactating herd - additional milk yield, less culling of good cows due to reproductive failure, more calves per year as replacements, improved genetic advancement, more reliable management and less variation in lengths of lactation and dry period. Veterinarians and farmers use detection of non-pregnant (open) cows to make decisions regarding re-breeding of cattle and to manage calving intervals, thereby maximizing milk production and revenue for the farm. Traditionally, pregnancy is determined through rectal palpation of the cow or by transrectal ultrasound (TU).

An enzyme-linked Immunoassay (ELISA) for the detection of pregnancy-associated glycoproteins (PAGs) in bovine milk samples and has been developed to provide a laboratory-based method for the accurate detection of pregnancy which could offer veterinarians and dairy farmers an important tool for the identification of open cows in the dairy herd.

This paper presents the results of trial work examining the use of a milk based pregnancy test as part of routine herd recording and discuss the concept of pregnancy testing through milk recording laboratories to provide additional information to customers, thereby maximizing the use of the milk sample as a herd management resource.

Keywords: bovine, pregnancy associated glycoproteins, milk, ELISA

Introduction

Reproductive performance and efficiency in dairy herds depends greatly on the accurate and timely diagnosis of pregnancy (Oltenacu et al., 1990; De Vries 2006). Pregnancy diagnosis in dairy cattle is traditionally conducted by rectal palpation or transrectal ultrasonography. Recently, several immunological detection assays for the quantification of pregnancy associated glycoproteins (PAG) in blood serum have been developed as accurate, cost-effective and convenient alternatives (Sasser et al., 1986; Sousa et al., 2006; Whitlock & Maxwell, 2008, Silva et al., 2009). Additional efficiencies would be realized if these assays could be applied with similar accuracy and performance to milk samples collected during routine animal recording test dates. However, data from previous studies have indicated a significant reduction in performance when existing serum-based assays were applied to milk samples (Friedrich & Holtz, 2008; Gajewski et al., 2008).

The IDEXX Bovine Pregnancy test for use on serum or EDTA plasma is based on the target antigen, PAG. They are expressed specifically in the maternal and embryonic regions of the placenta and are a sub-group of the aspartic protease family. More than 22 bovine

transcribed genes have been identified and these are expressed temporally at varying levels throughout gestation. IDEXX has developed a lab-based immunological detection assay (ELISA) which can reliably and accurately detect PAG in bovine milk samples in less than 4 hours.

Materials and methods

Diagnostic method

Serum samples were run on the IDEXX Bovine Pregnancy Test and milk samples on the IDEXX Milk Pregnancy Test at IDEXX Inc., (One IDEXX Drive, Westbrook, Maine). The IDEXX ELISA uses monoclonal antibodies directed against PAG, which are set on a microplate that will capture the PAG present in the sample. Secondary anti-PAG antibodies, coupled to a signal amplification system, are used as the detection reagent. Enzyme substrate (TMB) is used as a coloured indicator to reveal the PAG contained in the sample. After stopping the reaction the optical density of each well is read at a wavelength of 450 nm. Results are calculated and expressed as sample – negative (S-N). For serum samples, if the result is ≥0.3 samples are classed as positive (pregnant), and below 0.30 classed as negative (not pregnant). For milk samples, if the result is ≥0.15 the samples are classed as positive (pregnant), and below 0.10 classed as negative (not pregnant). Results falling between 0.10 and 0.15 are classified as re-check in the milk assay.

Temporal study

Milk samples (n=291) were collected from thirteen cows at regular intervals during lactation to assess changes in milk PAG levels throughout gestation.

Post-calving decline in milk PAG levels

Milk samples were obtained from 134 cows at varying days post-calving, but prior to service to analyze speed of decline in milk PAG levels after parturition and to determine the day post-calving at which PAGs are no longer detected.

Test performance evaluation

From Herd A, 192 paired milk and serum samples were collected from individual lactating Holstein cattle greater than 60 days post-partum. Samples were collected for cows throughout gestation from 40 days post breeding up to a maximum of 227 days post breeding.

From Herd B, 120 paired milk and serum samples were collected from lactating Holstein cows greater than 60 days post-partum. Samples were collected for cows throughout gestation from 40 days post breeding up to a maximum of 243 days post breeding.

In addition to milk and serum samples, all cows were tested by rectal palpation or ultrasound to confirm pregnancy status at the time of sample collection.

In order to determine diagnostic specificity of the test, 290 paired milk and serum samples were collected from five geographically diverse herds of lactating Holstein cattle. Cows greater than 60 days post-partum but not bred or not pregnant where included in the sample

set. In addition to milk and serum samples, all cows were tested by rectal palpation or ultrasound to confirm pregnancy status at the time of sample collection.

Results

Temporal study

Results from the temporal study show the presence of PAG in milk throughout gestation (Figure 1). Whilst a high degree of variability in PAG levels was observed for different cows, PAGs are detectable as early as 24 days of pregnancy and remain elevated throughout gestation. Assay response declines slightly from day 40 through day 90 of gestation, but PAG levels steadily increase during mid and late gestation.

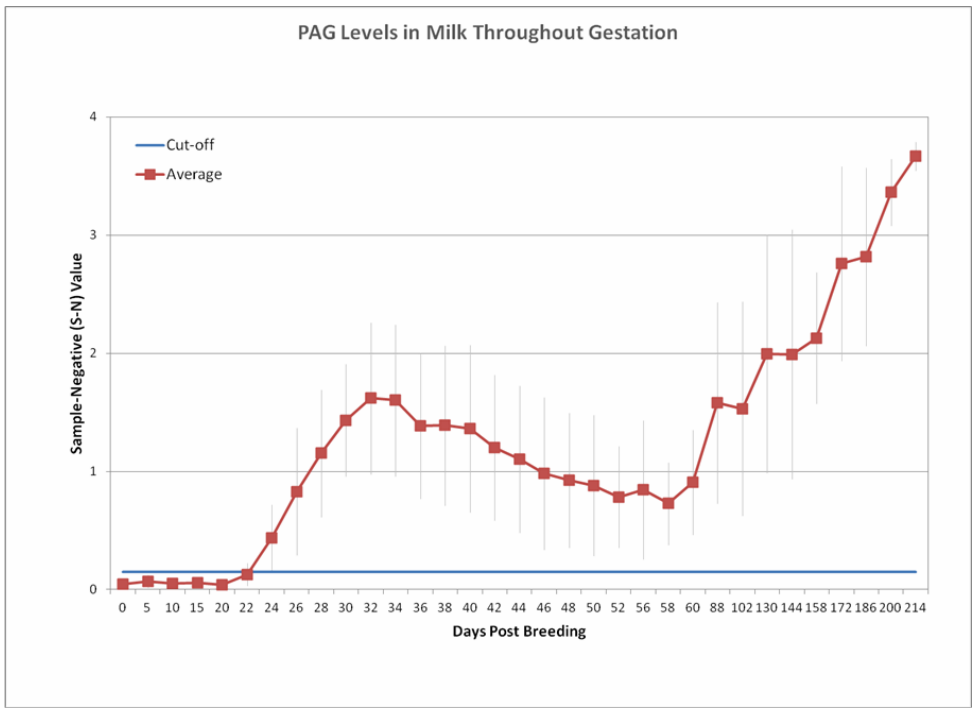

Figure 1. *PAG levels in milk throughout gestation in Holstein cows*

Post-calving decline in milk PAG levels

For the 134 cows sampled post-calving, but prior to breeding, milk PAG levels were below the cut-off for all cows by 60 days post-calving (Figure 2). The results show a rapid decline in PAGs after parturition with an observed specificity of 100% at 60 days post-calving but prior to breeding.

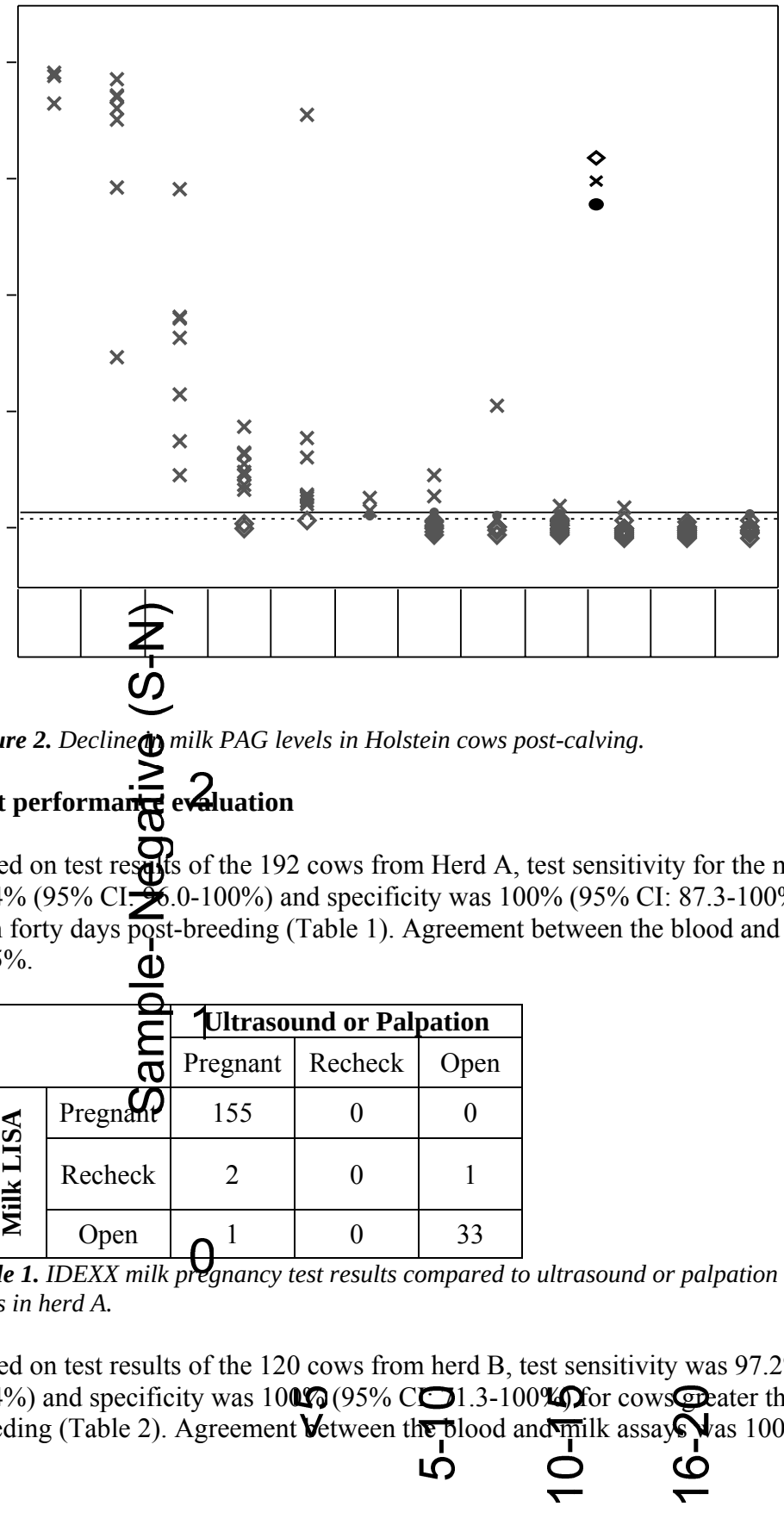

Figure 2. *Decline in milk PAG levels in Holstein cows post-calving.*

Test performance evaluation

Based on test results of the 192 cows from Herd A, test sensitivity for the milk ELISA was 99.4% (95% CI: 96.0-100%) and specificity was 100% (95% CI: 87.3-100%) for cows greater than forty days post-breeding (Table 1). Agreement between the blood and milk assays was 94.5%.

		Ultrasound or Palpation		
		Pregnant	Recheck	Open
IDEXX Milk LISA	Pregnant	155	0	0
	Recheck	2	0	1
	Open	1	0	33

Table 1. *IDEXX milk pregnancy test results compared to ultrasound or palpation results in Holstein cows in herd A.*

Based on test results of the 120 cows from herd B, test sensitivity was 97.2% (95% CI: 91.6-99.4%) and specificity was 100% (95% CI: 91.3-100%) for cows greater than forty days post-breeding (Table 2). Agreement between the blood and milk assays was 100%.

		Ultrasound or Palpation		
		Pregnant	Recheck	Open
IDEXX Milk ELISA	Pregnant	104	0	0
	Recheck	0	0	1
	Open	3	0	12

Table 2. *IDEXX milk pregnancy test results compared to ultrasound or palpation results in Holstein cows in herd B.*

Figure 3 shows the results of the milk PAG ELISA on 290 open cows (not bred or confirmed open by ultrasound or palpation) from five geographically diverse herds. Overall specificity was 96.7% (95% CI: 94.9-98.9%. Fewer open samples are available in late gestation, but specificity after day 90 of gestation was 100%.

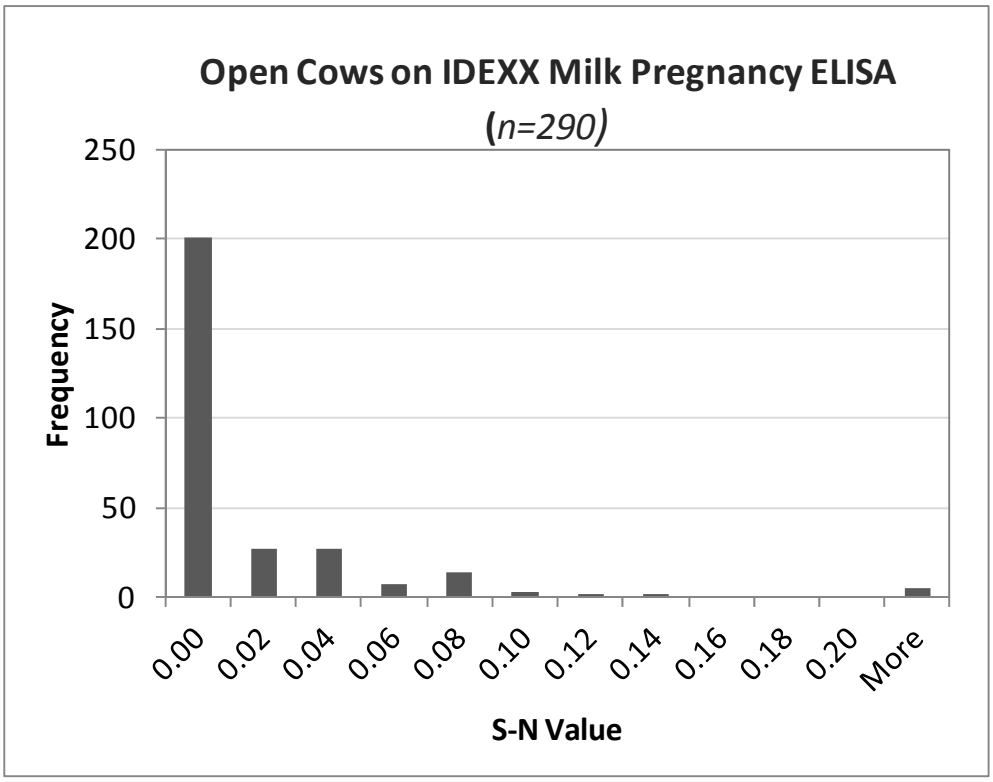

Figure 3. *Frequency of IDEXX milk pregnancy test values in non-pregnant Holstein cows (not bred or confirmed by ultrasound or palpation) from diverse geographical regions.*

Discussion

Data from the current study show that the IDEXX Milk Pregnancy Test is able to accurately detect pregnancy status using milk samples from dairy cows throughout gestation. Compared to tests used for milk PAG analysis in previous studies (Friedrich & Holtz, 2008; Gajewski et al., 2008), the current test was able to consistently detect the presence of PAG in milk from pregnant cows much earlier in gestation (24 days vs 150 days). Although milk PAG levels

varied markedly between individual animals throughout gestation, sensitivity and specificity of the current milk PAG ELISA was similar to that reported for commercially available serum PAG ELISA (Paré et al., 2008; Breed et al., 2009).

Use of serum PAG ELISA is typically restricted until 60 to 90 days after parturition to allow clearance of PAG from previous pregnancy to improve specificity. As with serum PAG levels, the current data show that milk PAG levels are highest near parturition and it is important to ensure that PAG from the previous pregnancy do not interfere with the current pregnancy diagnosis. Data from 134 cows tested between 1 and 60 days after parturition in the temporal study show that milk PAG levels decline rapidly and by day 60 are below the detection limit of the assay. Since the typical voluntary waiting period for breeding dairy cows is greater than 50 days, the milk PAG ELISA could be applied for early pregnancy diagnosis (≥28 days) post breeding.

The performance of the IDEXX Milk Pregnancy Test was evaluated on a total of 602 cows (265 pregnant and 337 open) from 7 geographically diverse herds. Using the cut-off value of 0.15 and a recheck classification between 0.10 and 0.15 for data interpretation, the milk PAG ELISA detected 261 of 265 pregnant cows for an overall sensitivity of 98.5%. Of the 337 open cows, the milk PAG ELISA correctly identified 326 cows as open for an overall specificity of 96.7%. Lower specificity in early compared to late gestation (100% after day 90) is likely due to higher rates of pregnancy wastage in early gestation and the slow clearance of circulating PAG (Whitlock & Maxwell 2008).

Conclusion

Using the IDEXX Milk Pregnancy Test, the presence of PAG in milk can be detected with suitable analytical accuracy for pregnancy detection in dairy herds. The detection of PAG in milk throughout gestation offers great scope for the application of the test through herd recording schemes.

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