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EVALUATION OF ELISA FOR PREGNANCY ASSOCIATED GLYCOPROTEIN (PAG) DETECTION IN SWAMP BUFFALO

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ABSTRACT

The goal of the present study was to compare the value of PAG concentration in swamp buffalo fluid which was detected by ELISA and RIA technique. The anti-PAG antibodies using as trapping reagents in a 'sandwich' type of ELISA were the same in RIA method. The sera were withdrawn from pregnant buffaloes at different periods of pregnancy. The ELISA was able to detect PAG in all kind of samples, such as fetal serum, buffalo serum, alllantoid fluid and amniotic fluid. The PAG concentration in plasma was not significant different between ELISA and RIA detection method whereas the PAG concentration in amniotic and allantoid fluid detected by RIA methods was higher than those detected by ELISA method ($P<0.05$). Comparison of ELISA with RIA showed a correlation coefficient (r) from 0.90 in amniotic fluid to 0.975 in fetal plasma. In summary, the ELISA method was shown suitable for PAG determination in swamp buffalo.

KEY WORD - ELISA, PAG, Pregnancy diagnosis, RIA, Swamp buffalo

INTRODUCTION

Pregnancy-associated glycoproteins (PAGs) belong to a large family of glycoproteins that are synthesized in the superficial layer of the ruminant placenta according to a spatial and temporal expression pattern [1]. Nowadays, It is become a useful tool for monitoring pregnancy in ruminant species [2]. In 2005, Green *et al*. have developed ELISA method to detect PAG as a method of early pregnancy diagnosis in cattle [3]. The reports have shown that measuring PAGs by RIA, the ELISA was able to detect PAGs unambiguously by 28d after insemination. The comparison the accuracy of determining the pregnancy status of lactating dairy cows 27d after timed AI by detection PAG concentration by ELISA method with transrectal ultrasonography have been done by Silva *et al*. (2007) [4]. The PAG ELISA used for determination of PAG concentration in cows had an accuracy of 93.7 to 96.2% at 27 days after time of AI and is similar to the accuracy of the TU method (93.7 to 97.8%) [4]. These results show that the ELISA method could be able a replacement of conventional RIA methods in determination PAG concentration. Until now, there is no information available on ELISA method developed for swamp buffalo PAG.

The aim of our study was to evaluate the ELISA method for determining PAG concentration on samples withdrawn from swamp buffalo. We also established the correlation between PAG concentrations in different samples of swamp buffalo using ELISA and RIA method detectives.

MATERIALS AND METHODS

Animals and samples collection

This study was conducted in swamp buffalo breeding in Vietnam. The samples containing fetal and maternal plasma, allantoid and amniotic fluid was collected at slaughterhouse.

The blood is commonly extracted into a sterile blood collection syringe (monovette 9ml, sarstedt, Germany) and amniotic and allantoids fluids were collected by aspiration before fetal blood collection by sterile syringe 5ml, which were put into a cool box until centrifugation. The samples were collected by centrifugation at 1500 x g for 20 min, and then separated and stored at -20°C until assayed.

Radioimmunoassay procedure

A PAG radioimmunoassay was performed according to methods of Zoli et al. (1992) slightly adapted to buffalo samples [5]. Briefly, 0.1 ml of PAG standard and 0.05 ml of difference fluid samples was diluted in 0.3 ml of Tris-BSA buffer. An appropriate dilution of antisera (0.1 ml) and 0.1 ml of radiolabelled ^{125}I-PAG was then added. The serum samples and the standard tubes were incubated overnight at room temperature. The following day, 1.0 ml of the second antibody solution was added and the tubes were incubated for 30 min at room temperature. Finally, the tubes were added with 2.0 ml of Tris-BSA buffer and centrifuged (20 min at 1500 g). After centrifugation, the tubes were aspirated and counted using a gamma counter.

Indirect sandwich ELISA

A PAG-ELISA was performed according to methods of Beckers et al. (2011) slightly adapted to buffalo samples. Indirect sandwich ELISA 0.1 ml of either samples from pregnant animals and 0.1 ml serially diluted PAG standards (in buffer solution) was added to duplicate wells previously coated with 0.1 ml 1/64000 antibody solution in bicarbonate buffer pH 9.6 [6]. The standard curve was included as a blank. The plates were incubated at 37^{o}C in 1 hour. Then, the plates were washed three times with 0.3 ml washing solution, and 0.1 ml of IgG coupling Biotin (Pierce Biotechnology, Inc; dilution 1:10.000) added to each well at 37^{0}C for 1 h. The plate was washed, and 0.1 ml of Avidine (Pierce Biotechnology, Inc; dilution 1:8000) was added into the wells (37^{0}C, 20 min). The plate was washed, and 0.15 ml of 1 TMB (Pierce Biotechnology, Inc) was added into each well. The plate was measured the absorbance at 450 nm in the wells.

The PAG standards were adjusted to provide a range from 0.015 to 10 ng.ml^{-1}. A standard curve was included on every ELISA plate and was generated by linear regression of a LOG (ng PAG) versus Absorbance plot by using KCjinor software (Bio-Teck instruments.INC).

Data analysis

The PAG concentrations measured in the samples with three antibodies were calculating correlation by INOVA. The regression and the variance analysis were carried out by excel.

RESULTS

The mean PAG concentration of swamp buffalo were determined by ELISA and RIA method is present in Table 1. In maternal and fetal plasma, concentration PAG measured by two method is not significantly different ($P<0.05$). In allantoids fluid, the mean PAG concentrations determined by ELISA (6.26 ± 10.02 ng/ml). Similar in amniotic fluid, the mean PAG concentration detected by ELISA and RIA were 15.89 ng/ml and 24.03 ng/ml.

The regression between two systems was showed in Table 2. Overall, the PAG concentration determination in the same samples using ELISA and RIA were similar and highly correlated. The correlation coefficient was range from 0.90 in amniotic fluid to 0.98 in fetal plasma.

The correlations between ELISA and RIA systems were showed in Fig. 1 which the value were concentrate in plasma samples and variable in alantoid and amiotic samples.

Table 1. Valuation of Buffalo PAG concentration detection by ELISA and RIA method

Kind of samples	No. sample	ELISA (ng/ml)	RIA (ng/ml)
Fetal plasma	67	20.75 ± 23.61^{a}	19.73 ± 20.02^{a}
Maternal plasma	57	22.13 ± 17.37^{b}	22.44 ± 19.45^{b}
Amniotic fluid	45	6.26 ± 10.02^{c}	12.66 ± 16.56^{d}
Allantoid fluid	62	15.89 ± 32.57^{e}	24.03 ± 48.98^{e}
Total	235		

Table 2. The regression between ELISA and RIA method in determination difference samples

Kind of samples	Linear regression	Coefficient
Fetal plasma	y = 0.8043x + 2.776	0.98
Maternal plasma	y = 1.0225x - 1.1279	0.96
Amniotic fluid	y = 0.9996x + 5.3846	0.90
Allantoid fluid	y = 1.4998x + 3.2684	0.91

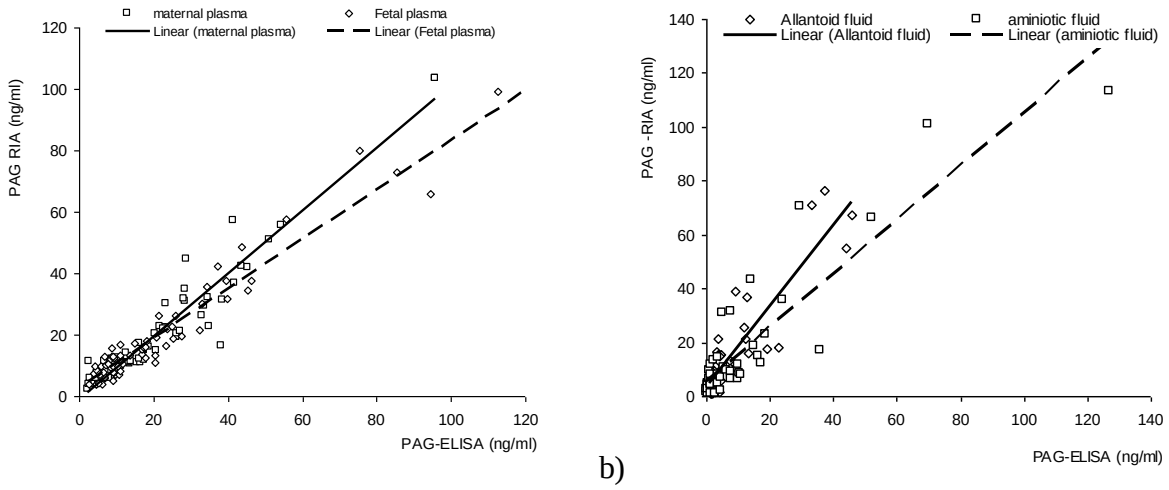

Figure 1. Relationship between PAG concentration detected by ELISA and RIA in fetal and maternal plasma (a) and amniotic and allantoid fluid (b).

DISCUSSIONS

In 2005, Green *et al.*, comparing the profiles of PAG concentration in serum obtainable in the monoclonal-based PAG ELISA with those obtained by others using PAG RIAs, have reported that they were quite similar. The PAG became detectable as early as day 22 in a small percentage of the animals and the concentrations then rise rapidly, peaking temporarily around the fifth week of pregnancy [3]. The result of pregnancy early diagnosis based on plasma PAG concentration by ELISA method was acceptable sensitivity and specificity at 27 d after AI [4]. The used of a new PAG-ELISA for routine pregnancy diagnosis in cattle was reported by Becker *et al*. (2011) [6]. In this research, the doubtful results by RIA, ELISA or both systems were 143(5.2%), 137(4.9%) and 38(1.4%), respectively.

The commercial PAG-ELISA kit was established to use for pregnancy diagnosis and the result used of it were showed in cattle, the sensitivity, specificity, and accuracy of pregnancy detection using for diagnosing pregnancy on dairy farms were conclusion acceptable in both method ELISA and RIA or highly accurate in ELISA in dairy cows on day 28 after AI [9, 12]. However, the milk PAG ELISA was 99.7%, indicating that the expected rate of false negatives with this method of pregnancy determination is very low [8]. But until now, there was no information concerning PAG concentration which was detective by ELISA in other samples such as alantoids or amiotic.

In our result, the PAG concentration in two kinds of sample was variance between ELISA and RIA.

In conclusion, ELISA technique is well suitable to detect of PAG concentration in difference sera of pregnant swamp buffalo. Although the concentration of PAG detected by ELISA had similar accuracy to that by RIA, the ability of using it for early pregnancy diagnosis in swamp buffalo needs to be confirmed.

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