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COMPARATIVE EVALUATION OF IDEXX RAPID VISUAL PAG ELISA TEST AND ULTRASONOGRAPHY FOR EARLY PREGNANCY DIAGNOSIS IN BUFFALOES

Prasanth Kumar Manigaradi[1,*], Murugavel Kailasam[1], Hemalatha Harikrishnan[1], Vivek Srinivas Mouttou[2] and Kantharaj Sammantham[1]

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ABSTRACT

The aim of this study was to evaluate the efficacy of IDEXX rapid visual PAG ELISA kit for early diagnosis of pregnancy in Buffaloes. The study was carried out with 148 Murrah graded buffaloes in a private dairy farm in Nalgonda district, Telangana state. Artificial Insemination was done for all the buffaloes with good quality frozen thawed semen and breeding records were maintained. Pregnancy diagnosis was done on day 30 post insemination in all animals by means of ultrasonography device with a 5-Mhz Trans rectal probe, Blood samples were collected from all the animals to study the presence of bovine pregnancy-associated glycoproteins (bPAGs) with IDEXX rapid visual kit. The results were determined based on bPAGs levels and it is compared with USG findings. In present study, 54 animals were confirmed pregnant by ultrasonography. In IDEXX rapid visual kit method, 49 buffaloes out of 54 animals were shown positive for pregnancy, while 5 of them were found to show false negative. However, all the animals that were declared non-pregnant based on ultrasonographical findings, showed negative result with the kit. The accuracy rate for pregnancy diagnosis using IDEXX rapid visual PAG ELISA test was 96% in case of pregnancy and 100% in case of non-pregnant status. It can be concluded that the IDEXX rapid visual kit may be utilised as a next option for early pregnancy in buffaloes under field conditions where ultrasound facilities are not available, as the accuracy rate is 100% especially in identifying non pregnant animals.

Keywords: *Bubalus bubalis*, buffaloes, pregnancy-associated glycoproteins, ultrasonography

INTRODUCTION

Early and accurate pregnancy detection is essential for effective reproductive management in the dairy industry, as it allows for proper care of pregnant females and the timely resynchronization and re insemination of non-pregnant females. Detecting pregnancy shortly after breeding

[1]Department of Veterinary Gynaecology and Obstetrics, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry, India, *E-mail:prashanthshetty274@gmail.com
[2]Department of Veterinary Microbiology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry, India

maximizes reproductive efficiency, enhances overall herd productivity, and reduces the time between calving cycles (Gradela *et al*., 2009; Green *et al*., 2011). Pregnancy diagnosis methods must be practical, accurate, easy to apply, and affordable (Fricke *et al.,* 2016). Different methods include per-rectal palpation, Transrectal real-time B-mode ultrasonography, serum progesterone assay and pregnancy associated glycoprotein (PAG). Per- rectal method involves palpation of the amniotic vesicle (Wisnicky and Cassida, 1948) and the slipping of the chorioallantoic membranes (Zemjanis, 1970). Progesterone assays measure hormone levels that increase up to 14 days post-estrus and continue rising until day 21 after successful fertilization, with a 98% specificity (Nebel *et al*., 1987; Waldman, 1993). PAG detection using IDEXX rapid ELISA Kit is a chemical method for diagnosing early pregnancy (Bulut and Taşdemir 2021). Trans rectal real-time B-mode ultrasonography is regarded as the "gold standard" for pregnancy diagnosis in diary animals. Despite the availability of various pregnancy detection methods for dairy animals, trans rectal ultrasonography has remained a dominant technique since 1980s (Reeves *et al*., 1984) due to its early detection capability and accuracy in assessing fetal heartbeat and embryo vitality.

During pregnancy in both humans and many animal species, specific proteins and hormones either emerge for the first time or show a marked increase in the maternal bloodstream. This shift is largely influenced by the presence of the fetus and placenta, rather than the dam itself (Chard, 1986). Once a connection between the dam and the calf is established, pregnancy-associated glycoproteins (PAGs) are secreted by cotyledons and trophoblastic cells (both mononucleate and binucleate) and enter the maternal blood circulation. Pregnancy-associated glycoproteins (PAGs) are considered to be pregnancy specific markers (Touzard *et al*., 2013). Sasser *et al*. (1989) first identified specific proteins in the blood of pregnant cows. They named this protein as "pregnancy-specific protein B" (PSPB), which belongs to the PAG family produced by the placenta. Biochemically, PSPB is equivalent to boPAG-1 (Green *et al*., 2005). PAGs share some similarities with human chorionic gonadotropin (hCG), the hormone used in human pregnancy test, as both are placental glycoproteins with long half-lifes in the blood. However, despite these similarities, PAGs and hCG have distinct molecular structures and functions. While both can be detected in blood, PAGs, unlike hCG, are not known to be detectable in urine. Pregnancy-associated glycoproteins (PAGs) have been described as a good marker of pregnancy and fetal viability, with increasing concentrations in maternal blood from the 22nd day until the last week of gestation (Abdulkareem *et al*., 2012). However, variations in PAG levels can occur based on species, breed, and even the number of fetuses during pregnancy. Recent research has concentrated on using PAG levels in maternal blood to detect pregnancies, with highly promising results. As per literature available, no work has been done to test the efficacy of PAG levels and ultrasonography method for early detection of pregancy in buffaloes. Hence, the present study was planned to evaluate IDEXX Rapid Visual PAG ELISA kit for early pregnancy diagnosis in buffaloes.

MATERIALS AND METHODS

The study was carried out in a private

dairy farm in nalgonda district Telangana state. A total of 148 animals were utilized for the study. All the 148 buffaloes were inseminated with frozen semen straws, and the breeding history were recorded. On day 30 post insemination, all the buffaloes were subjected to Trans rectal real-time B-mode ultrasonography. The presence of anechoic (allantoic) fluid, embryo proper, slipping of membranes the embryo's heartbeat was considered as positive sign indications for pregnancy. At the time of rectal examination, blood samples were collected from the jugular vein from all the animals. The collected blood samples were centrifuged at 3000 rpm for 15 minutes then the serum was stored at - 40°C for PAG analysis. Plasma PAG levels were determined by a commercial ELISA test (Figure 3)-IDEXX bovine pregnancy test, IDEXX Laboratories, Switzerland, AG) at The Centre for Translational Research and Centre Instrumentation facility (Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry). The samples and controls were loaded according to the manufacturer assay protocol. (Figure 1).

The results were measured by optical density at 450 nm using a microplate reader (Figure 3 Thermo Scientific, Multiskan FC, USA). The PAG levels of the samples were determined by calculating from the optical density (OD) at 450 nm of the sample (S) minus the OD of negative control (N).

By comparing the colour changes in the wells representing each animal to the positive and negative control wells, the pregnancy status of the animals was assessed. (Figure 4) Colour changes from light blue to dark blue as in the positive control group in the wells were accepted as positive pregnancy. No colour change as in the negative control group was accepted as negative for pregnancy.

RESULTS

Out of 148 buffaloes, 54 animals were confirmed pregnant. Based on USG examination, on day 30 post insemination. Out of 54 pregnant animals only 49 animals were found to be pregnant, based on IDEXX rapid visual PAG ELISA kit. For the remaining 5 pregnant animals PAG ELISA kit showed false negative result. All the 96 non-pregnant animals showed, absence of (PAG) level while using IDEXX kit.

DISCUSSION

While ultrasound has certain advantages over the PAG blood test, such as providing immediate pregnancy results and allowing for the assessment of uterine and ovarian health in non-pregnant animals, it can also help detect nonviable pregnancies and dead embryos. However, these benefits should be considered alongside the expense of ultrasound equipment, the technical expertise required to perform the procedure, and the availability of skilled personnel to conduct early pregnancy diagnoses using ultrasound (Fricke, 2002). In PAG method, 5% of animals diagnosed non pregnant. The false-negative results in the present study were probably due to the consequence of very low serum bPAG concentrations at the time of blood sample collection due to variations in bPAG production among other. The PAGs assay might have lower sensitivity at certain stages of pregnancy, particularly during very early or late stages. In 5 cases, the PAGs assay may show non-pregnancy due to cross-reactivity, even if the animal is pregnant. (Zoli *et al*., 1992)

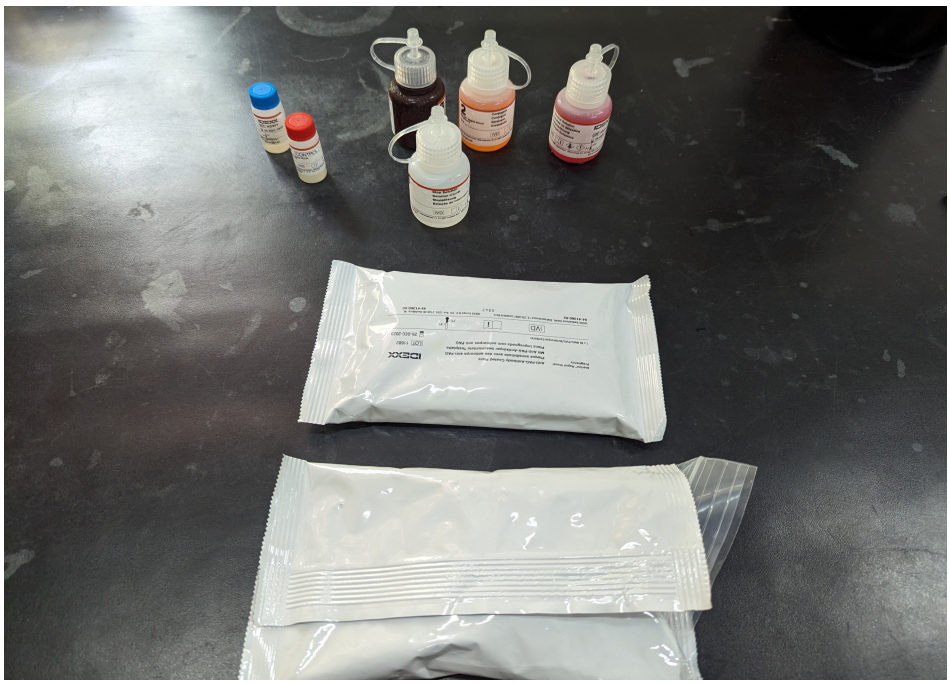

Figure 1. Composition of the IDEXX rapid visual PAG ELISA kit: (a); positive control (b)negative control (c) detector solution(d); Conjugate (e); TMB substrate (f); Stop solution.

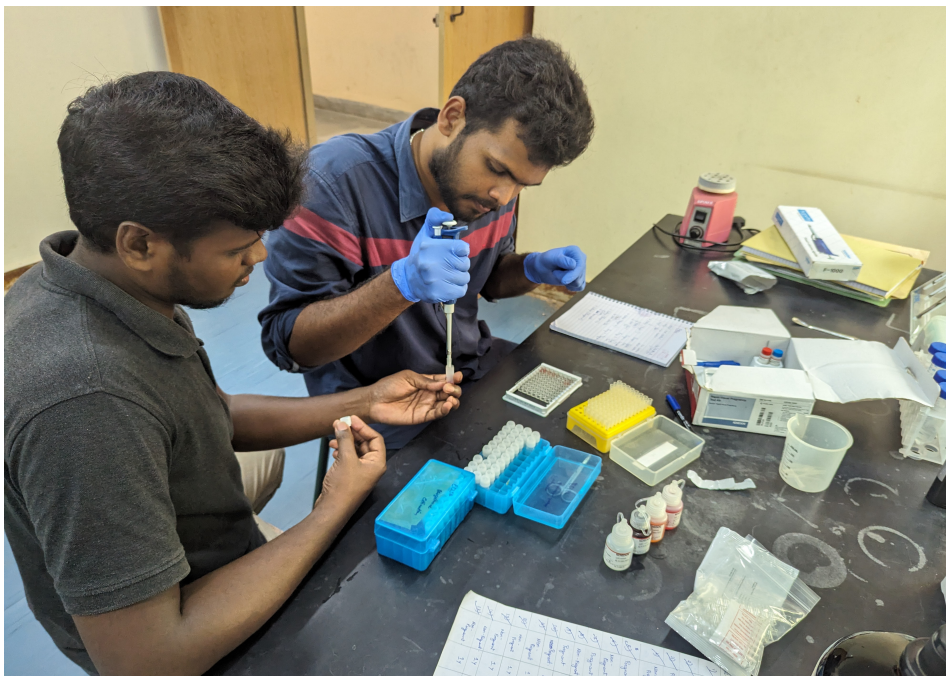

Figure 2. Filling of the serum samples to the microtiter plate.

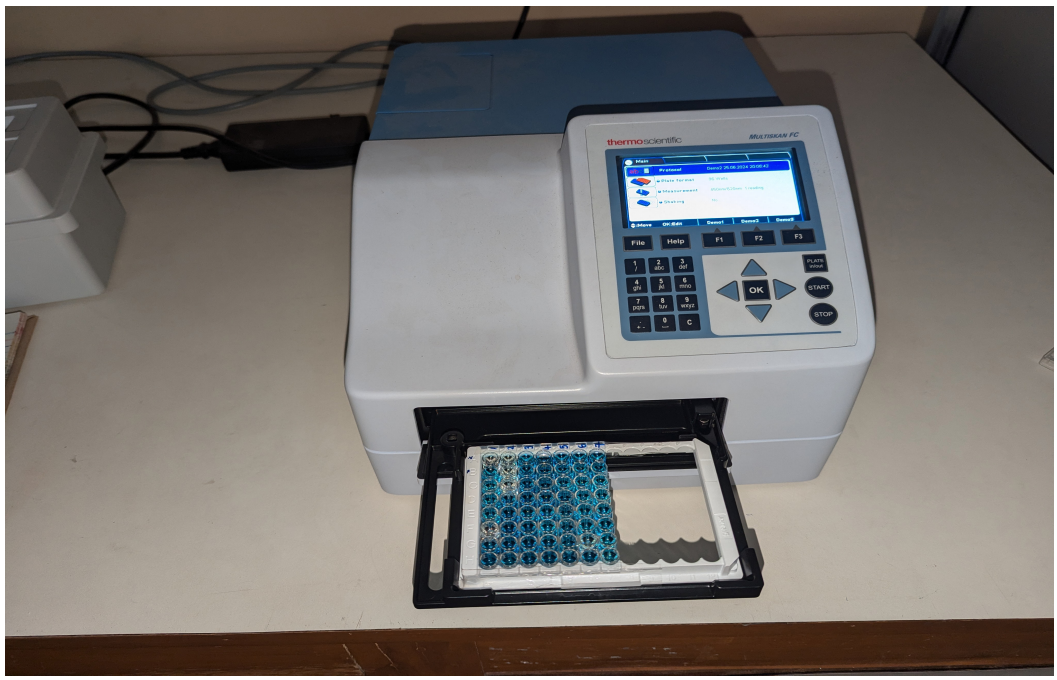

Figure 3. Microplate reader (Thermo Scientific, Multiskan FC, USA).

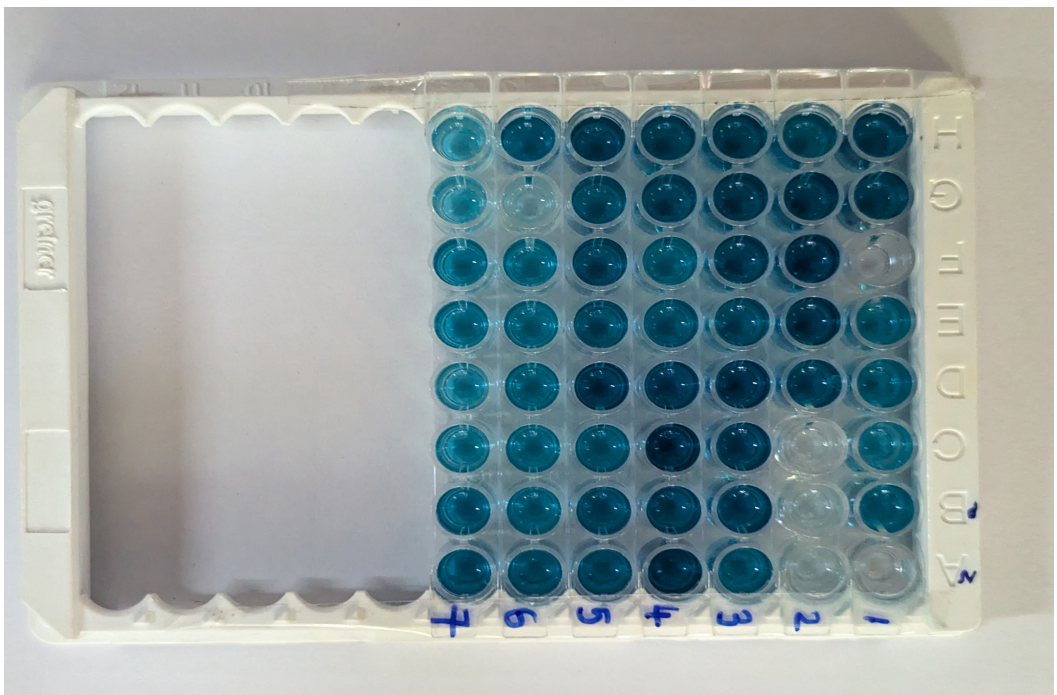

Figure 4. Colour changes of samples in the microtiter plate.

Table 1. Relative efficacy of early pregnancy diagnostic methods in cows (n=148).

Method of early pregnancy diagnosis	Accuracy (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
USG	100%	100%	100%	100%	100%
PAG	96%	90%	100%	100%	94%

CONCLUSION

From the above findings, it can be concluded that five animals showed false negative whereas, there is no false positive result while using IDEXX rapid visual PAG method, when compared to USG examination. The development of reliable and cost-effective PAG assays for pregnancy testing in dairy animals offers the possibility of early pregnancy detection, typically 30 days post-insemination (Fricke, 2002). The comparative study of PAG method with ultrasonography method has revealed the liability and precocity of the biochemical methods. The PAG's are good markers of pregnancy diagnosis in dairy cows. Performed on blood serum, this method is totally non-invasive and viable since day 30 post insemination. From present study, it can be concluded that rapid test can be done in field conditions, However, sensitivity is 90 % in detecting pregnant animals. It can be used as a real breakthrough in pregnancy diagnosis and for the detection of embryonic losses and early abortions.

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