



Full Length Article

Early Detection of Pregnancy in Non-Descript Goats (*Capra hircus*) in the Philippines using Pregnancy-Associated Glycoproteins (PAGs) as Biomarkers through Enzyme-Linked Immunosorbent Assay (ELISA)

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Abstract

Early and accurate method of pregnancy diagnosis is crucial in goat production to shorten the kidding interval that eventually will result to the increase in production efficiency of the animals. Recent development in pregnancy diagnosis involves the use of Pregnancy – associated glycoproteins (PAGs) as biomarkers that can be detected in the maternal circulation in the definitive time of placental attachment. The present study aims to detect early pregnancy in naturally-bred goats using PAG as biomarkers using PAG-Enzyme-Linked Immunosorbent Assay test kit and compare the levels of PAG between pregnant and non-pregnant goats. A commercial PAG-ELISA (BioPRYN Flex[®]) was used to detect early pregnancy in goats. Thirty (n = 30) non-descript goats were subjected to natural breeding followed by blood collection post-breeding. Of 30 naturally-bred goats, 23 (76.67%) were categorized as pregnant based on PAG level at day 23 post-breeding. The pregnancy status of the 23 pregnant goats was confirmed using transrectal ultrasonography at days 30–35 post-breeding. Additionally, significant differences between the PAG level of pregnant and non-pregnant goats were observed on days 20, 23, 25, 28 and 30 post-breeding. The mean PAG level of non-pregnant goats remained low on days 15 to 30 post-breeding. The present study demonstrated and appeared to be the first in the Philippines to use PAG as a biomarker for early detection of pregnancy in naturally-bred goats through plasma PAG-ELISA. Lastly, the use of PAG-ELISA is a reliable tool and can be used as an alternative to detect early pregnancy in goats.

Keywords: Goats; Pregnancy diagnosis; Pregnancy-associated glycoproteins; ELISA

Introduction

In the Philippines, goats are raised in most of the households due to their low production inputs and minimal management needed. Most Filipinos consumed goat meat or “chevon” prepared in different types of delicacies and served during special occasions (Soriano *et al.* 2021). A large portion of the goat population in the country is accounted for backyard farming; however, production efficiency in this type of farming is low due to poor reproductive management (Barcelo *et al.* 2016) and little or no practice of pregnancy diagnosis being applied that result in a long kidding interval. One key factor to shorten the kidding interval is through early detection of pregnancy.

Early detection of pregnancy is such a crucial tool in

animal reproductive management as it will allow proper feeding management between non-pregnant and pregnant animals. It also provides appropriate nutritional management for pregnant animals especially during the late stage of pregnancy to avoid pregnancy toxemia, and other factors affecting production losses. Detection of early pregnancy can be used for selection of animals to identify infertility and to address pregnancy problems. It would be also possible for re-breeding or culling of non-pregnant animals (González *et al.* 2004; Amiri *et al.* 2015; Al-Hassan and Al-Samawi 2017; Alkan *et al.* 2020). Several methods of pregnancy diagnosis have been applied in goats including abdominal palpation, radiography, and ultrasonography. The simplest method of pregnancy diagnosis in goats is through observing the signs of estrus

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such as mounting and bleating at 21 days post-breeding. However, this method is inaccurate due to several factors. Some non-pregnant goats fail to return to estrus at the expected time, and changes in the estrous cycle of female goats may be attributed to issues such as luteal cysts or prolonged luteal phases (Al-Hassan and Al-Samawi 2017). The use of ultrasonography for pregnancy diagnosis in small ruminants is such a reliable tool and can detect pregnancy at day 30 post-breeding. Conversely, this method requires an expensive machine and technical expertise to interpret the sonogram images. The method of pregnancy diagnosis in goats will depend upon the availability of equipment, known number of days post-breeding, and accuracy (González *et al.* 2004). Advanced methods of pregnancy diagnosis in livestock animals utilizing hormones and biomarkers are being employed to detect early pregnancy (Tadeo *et al.* 2021). One of the biomarkers that is used for pregnancy detection is the Pregnancy-associated glycoproteins (PAGs).

PAGs belong to the aspartic proteinases secreted by the mono- and binucleate cells in the trophoblast (Xie *et al.* 1991; Barbato *et al.* 2022), which migrate from the embryo to the uterine tissue that fuses to the maternal tissue, and can be detected in the maternal blood at the time of placentation (Al-Hassan and Al-Samawi 2017; Barbato *et al.* 2022). PAGs have become a valuable tool in diagnosing pregnancy in ruminants and can be used as an indicator for embryo viability (Sharma *et al.* 2020). Utilization of PAGs has existed for many years but not on a large extent (Rigos *et al.* 2022).

In the Philippines, Balbin *et al.* (2020) reported that barium chloride (BaCl_2) added with urine can be used in detecting pregnancy in goats with 62% accuracy in classifying pregnant and non-pregnant, with specificity and precision of 49 and 48%, respectively. In terms of the use of PAGs, there are reports that it is used for the early detection of pregnancy in water buffaloes using milk (Tadeo *et al.* 2021), and blood (Rigos *et al.* 2022); however, there are no reported studies that PAG is being applied in detection of pregnancy in goats. Hence, the study aims to detect early pregnancy in naturally-bred goats using PAG as biomarkers determined through the PAG-ELISA test kit. The study also compares the PAG levels between pregnant and non-pregnant goats.

Materials and Methods

Selection of animals

A total of thirty non-descript female goats were used in this study, with a body condition score (BCS) of not less than three (≥ 3), regardless of age and parity were selected and underwent transrectal ultrasonography for further evaluation. Those diagnosed as non-pregnant were placed in a pen with the bucks. Goats were closely monitored for signs of estrus and successful mountings were considered as

day 0 of breeding. Subsequently, blood samples were collected from animals that exhibited successful mounting.

Blood plasma samples

Blood samples (5 mL) were collected from the jugular vein on days 15, 20, 23, 25, 28 and 30 post-breeding. Blood samples were placed in an iced box and brought to the laboratory for centrifugation. Plasma was separated by centrifugation at 15,000 rpm for 20 min and then placed in a 1.5 mL-capacity Eppendorf tube and stored at -20°C until used for analysis.

Pregnancy diagnosis through trans-rectal ultrasonography

All animals used in the study were subjected to trans-rectal ultrasonography to confirm pregnancy on day 30–35 post-breeding. Trans-rectal ultrasound scanner (Honda, HS-1600 V, Japan) equipped with 7.5 MHz linear array transducer designed for intra-rectal placement (Mehrajuddin *et al.* 2013) was used to conduct examinations. The rectal probe was attached to a PVC pipe cut into halves in length wise and serves as the hand to manipulate that probe inside the rectum. The presence of amniotic fluid and an embryo with a beating heart were the indicators of positive pregnancy.

PAG/PSPB-ELISA assay (BioPRYN® Flex)

The PAG-ELISA analysis in blood plasma was performed using commercial ELISA Test kit (BioPRYN® Flex, Bovine and Small Ruminant Pregnancy ELISA, Bio Tracking, Inc., 1150 Alturas Drive, Suite 105, Moscow, ID, USA). PAG-ELISA analysis was conducted according to the manufacturer's instructions by trained technicians.

The 96-well of a polystyrene microtiter plate is coated with antibody against the PSPB/PAG. Briefly, detector solution was loaded in each well followed by PSPB/PAG standards and plasma samples and were incubated for 2 hours at 24°C with continuous shaking. Following incubation, unbound conjugate was washed away, the binding of the labeled antibody conjugate was detected by the addition of enhancer solution followed by 3, 3', 5, 5'-tetramethylbenzidine (TMB) and quantified by the subsequent color development which corresponds to the amount of PAG in the samples. Strong color indicates binding and substrate reactivity, while weak color development indicates little or no binding of the labeled antibody conjugate.

Readings were then exported as an excel file to generate and interpret results. Pregnancy results were validated and interpreted based on the protocol provided by the PAG-ELISA manufacturer. The average Optical Density (OD) value of plasma samples of 0.847 and above was interpreted as Pregnant, and the average OD value of plasma samples below 0.504 as non-pregnant.

Table 1: Comparison of PAG levels (OD value) between pregnant and non-pregnant goats during early pregnancy

Groups	Pregnancy-Associated Glycoproteins (mean $\pm$ SEM)					
	Day 15	Day 20	Day 23	Day 25	Day 28	Day 30
Pregnant (n = 23)	0.18 $\pm$ 0.022 ^a	1.26 $\pm$ 0.149 ^a	1.85 $\pm$ 0.077 ^a	2.10 $\pm$ 0.074 ^a	2.33 $\pm$ 0.034 ^a	2.36 $\pm$ 0.047 ^a
Not Pregnant (n = 7)	0.16 $\pm$ 0.021 ^a	0.13 $\pm$ 0.004 ^b	0.14 $\pm$ 0.004 ^b	0.13 $\pm$ 0.006 ^b	0.14 $\pm$ 0.009 ^b	0.14 $\pm$ 0.008 ^b

^{a,b} means with different superscripts within column are significantly different at a 5% significance level

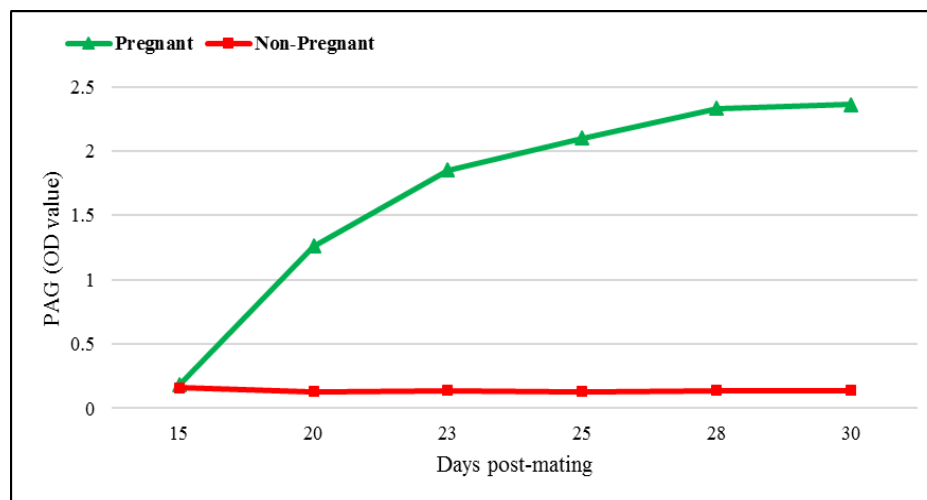


Fig. 1: Average PAG levels (OD value) in blood plasma days after natural mating in non-descript goats (Pregnant and Non-pregnant). The PAG levels in blood plasma were validated and interpreted from the average optical density (OD value) of the PSPB/PAG standards that served as the control (Mean Hi = 0.847; Mean Lo = 0.504)

Statistical analysis

Data were presented as the mean $\pm$ standard error of the mean (SEM). An independent samples t-test ($P < 0.05$) was used to compare the average PAG levels (OD value) between pregnant and non-pregnant goats.

Results

The findings of the study showed an overall conception rate of 76.67% at days 23, 25, 28 and 30 post-breeding. Pregnancy of the goats (n = 23) was confirmed using transrectal ultrasonography as gold standard at day 30–35 post-breeding. Table 1 depicts the results of PAG assay in blood plasma of pregnant and non-pregnant goats for the early detection of pregnancy during days 15, 20, 23, 25, 28 and 30 post-breeding.

The results of plasma PAG-ELISA done during the early pregnancy in goats showed a significant difference ($P < 0.05$) in the mean levels of PAG (OD value) between the pregnant and non-pregnant goats at days 20, 23, 25, 28 and 30, except for the mean plasma PAGs on day 15 ($P > 0.05$). An increase in the plasma PAG level was observed from 0.18 $\pm$ 0.02 on day 15 to 1.26 $\pm$ 0.15 on day 20 to 1.85 $\pm$ 0.08, 2.10 $\pm$ 0.07, 2.33 $\pm$ 0.03 and 2.36 $\pm$ 0.05 on days 23, 25, 28 and 30, respectively. However, the mean plasma PAG level for non-pregnant goat can be observed at a constant low level from 0.16 $\pm$ 0.02 on day 15 to 0.13 $\pm$ 0.004 on days 20, 23, 25, 28 and 30, respectively.

0.00, 0.14 $\pm$ 0.00, 0.13 $\pm$ 0.01, 0.14 $\pm$ 0.01 and 0.14 $\pm$ 0.01 on days 20, 23, 25, 28 and 30, respectively (Fig. 1).

Results revealed that 30 blood samples were negative at day 15 post-breeding. On day 20, PAGs were detected in the 50.00% (15/30) of animals and were categorized as pregnant. On day 23, PAGs were detected in an additional 8 animals resulting in an overall conception rate of 76.67% (23/30). The detection of PAG continued on days 25, 28, and 30 post-breeding in the same animals.

Discussion

Early detection of pregnancy is an important tool in goat production for rebreeding of non-pregnant, and for selection for culling of unproductive does. This can be used to establish breeding programs for the herd and implement improved nutritional management practices (González *et al.* 2004). Pregnancy diagnosis is usually done through rectal palpation at day 60 post-breeding in large ruminants; however, this method is impossible for small ruminants due to their body size.

The present study appears to be the first in the Philippines to use PAG as biomarker for early detection of pregnancy in naturally-bred goats. The pregnancy-associated glycoproteins (PAGs) belong to aspartic proteinase family that is secreted by the binucleate and mononucleate cells and can be detected in the maternal circulation at the time of implantation during early stage of

gestation and throughout pregnancy period (Karen *et al.* 2003; Rovani *et al.* 2015; Commun *et al.* 2016).

The PAG-ELISA test kit (BioPRYN® Flex) used in the present study revealed significant results to detect early pregnancy in naturally-bred goats. The findings revealed that PAG can be detected in maternal circulation and categorized as pregnant from day 20 post-breeding. The majority of the goats were identified as pregnant and could be distinguished from non-pregnant counterparts as early as day 23 post-breeding. These results align with the findings of Al-Hassan and Al-Samawi (2017) in detecting early pregnancy in Aardi goats. Various studies have reported that PAG can be utilized to detect pregnancy as early as day 21 post-AI in Northern Moroccan goats (Chentouf *et al.* 2008), day 24 post-AI in Moxoto and Caninde goats (Sousa *et al.* 1999), and day 21 post-mating in Alpine goats (Batalha *et al.* 2001). The variations in detecting PAG in the blood plasma for early detection of pregnancy may be due to the differences in the breeds, age (Meshref *et al.* 2022), and physiological differences among goat breeds (Sousa *et al.* 1999). The number of fetuses (singleton or twin-pregnancy) may also affect the expression of PAG in the maternal circulation. Moreover, the increase in the level of PAG as gestation progresses could be influenced by the increase in the number of placentome (Rigos *et al.* 2022) and large surface contact of fetal-maternal as the size of the litter increases (Chentouf *et al.* 2008). Spencer and Bazer (2004) stated that the binucleate cells start to migrate and fuse in the endometrial LE to form syncytia between days 14-16 in sheep. Furthermore, there are PAG that are expressed at different times during gestation (Tadeo *et al.* 2021). Some PAG are expressed earlier during the gestation period, while others are expressed later (Barbato *et al.* 2017; Rigos *et al.* 2022). This explains that there are goats in the present study detected pregnant as early as day 20 post-breeding. However, the correlation between the number of fetuses and the level of detectable PAGs in maternal circulation for early pregnancy detection is not addressed in the present study. In addition, the discrepancy in the results of detecting early pregnancy could be attributed to the test kits used, as the present study used ELISA, while previous studies employed RIA.

On the other hand, the constant low level of PAG in non-pregnant goats from days 15 to 30 post-breeding is in agreement with all other detected non-pregnant goats previously studied. This indicates that PAG can be used to detect early pregnancy in goats and distinguish between pregnant and non-pregnant. PAG is not solely secreted by the placenta; they are also released from various organs and tissues (Rigos *et al.* 2022), implying the presence of detectable low level PAG in non-pregnant goats.

Conclusion

An early and accurate tool for early detection of pregnancy is vital for effective reproductive management of a herd. By

identifying and subsequently rebreeding of non-pregnant goats, the kidding interval can be reduced, leading to enhanced production efficiency. The present study showcased the use of PAGs as biomarkers for early detection of pregnancy in naturally-bred goats through PAG-ELISA. Notably, PAGs can be detected in the blood plasma as early as day 20 post-breeding, and the majority of the animals diagnosed as pregnant can be distinguished from non-pregnant goats as early as 23 days post-breeding. PAG-ELISA proves to be a reliable tool for early pregnancy detection in goats and can serve as an alternative to ultrasonography.

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Author Contributions

JOCL and ECA planned the experiments, LCO and EPA interpreted the results, VDV, LMR and JOCL did the write up, JOCL and ECA statistically analyzed the data and made illustrations.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

All procedures that involve the use of animals for research purposes were approved by the Ethics Committee of the Philippine Carabao Center, National Headquarters and Genepool.

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