University of Thi-Qar Journal of agricultural research
ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 12, Issue 1 (2023) PP 201-210
https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v12i1.252*

Evaluate the effectiveness of Pregnancy-Associated Glycoprotein and Progesterone in predicting the gestational status in Goats

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Abstract

The objective of this study was to evaluate the PAG and P4 profiles in pregnant and non-pregnant does using Enzyme Immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA), respectively. Additionally, the study aimed to compare the sensitivity (Se), specificity (Sp), and accuracy (Acc) of pregnancy diagnosis using PAG and P4 detection in serum samples. Twenty does were synchronized using P4 sponge+eCG for 12 days, followed by breeding after estrus. Blood samples were collected at different experimental periods (22, 30, 40, and 60 days post-mating) and analyzed using EIA and ELISA. The lambing and Ultrasonographic examination on day 60 were used as a golden standard for pregnancy evaluation. The results demonstrated that both PAG and P4 mean concentrations significantly increased in pregnant does compared to non-pregnant does in all experimental periods. When comparing the efficiency of each method for pregnancy prediction, the PAG assay method showed a sensitivity (Se) similar to P4 (85%) at day 22 post-mating, but reached 100% at day 30 for PAG and day 40 for P4. Additionally, the accuracy (Acc) of the PAG analysis method was higher than P4 at day 22 (80% vs. 75%) and day 30 (95% vs. 85%), and reached 100% on day 40. In contrast, the Acc of the P4 assay for pregnancy diagnosis was 95% at days 40 and 60. The specificity (Sp) was lower in both methods, but the diagnosis using the PAG assay was better than P4 on all days, reaching its optimal value on day 40, while reach to 83% for P4 assay method on day 40 and 60 PM. The present study concluded that both PAG and P4 analyses were specific and reliable methods for pregnancy determination in does starting from day 22 onward, but the PAG assay was more accurate than the P4 assay.

Keywords: Pregnancy-Associated Glycoprotein, Progesterone, pregnancy diagnosis, Does.

I. INTRODUCTION

The local goat breed is a significant component of ruminant animals primarily utilized for meat and dairy production, they are known for their high milk yield and a high twinning rate (Ishwar et al., 1995). Goats, in general, are considered an emerging domesticated ruminant worldwide due to their

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remarkable ability to adapt to changing environmental circumstances and dietary requirements (Haibel, 1990). In Iraq, it can improve the reproductive performance of local goats through hormonal treatment with a very high response (Bamerny et al., 2022). Generally, early pregnancy diagnosis is a very important practice that can improve reproductive performance and minimized economic losses (Singh et al., 2004; Sharma et al., 2020).

The major prevalent tools of determine pregnancy in small ruminants include; the managemental method; abdominal ballottement and non-return-to-heat, clinical; ULR and hormonal; progesterone assay and PAG with variable diagnostic accuracies (González et al., 2004; Bello et al., 2023; Younis and Hatif, 2023).

Pregnancy-associated glycoprotein is a more appropriate distinct indicator for goat pregnancy (pregnancy-specific glycoprotein) because it is exclusively produced by the placenta in ruminants (El Amiri et al., 2015). It belongs to a major family that shares the same function (inactive aspartic proteinase), which serves a wide range of functions and is exclusively expressed by both placental Mono- and Binucleate cells (BNCs) (Green et al., 1998).

Pregnancy-associated glycoprotein become detectable in the peripheral blood of pregnant doe around the time of attachment of the conceptus extraembryonic membranes with endometrium, this occurs when trophoblastic BNCs begin to migrate and merge with endometrial cells, to form embryo-maternal syncytium (Wooding, 1984). Consequently, PAG serves as a reliable marker for both pregnancy as well as embryo-placental function (Garbayo et al., 2008). Additionally, the evaluation of PAG plasma concentration in does serve as an indicator for early pregnancy detection (Shahin et al., 2013) and also in ewes (El Amiri et al., 2015). It allowed for accurate prediction of pregnancy starting from 21 days after breeding and beyond (Green et al., 2005), and also to estimate the embryo/fetal count in dairy goats (Chentouf et al., 2008). It can be measured by radioimmunoassay (RIA) (Zoli et al., 1992) and ELISA (Green et al., 2005).

Progesterone analysis is a practical, cost-effective, and efficient method used for early pregnancy prediction in small ruminants with discriminatory levels to differentiate between gravid/non-gravid does (Boscos et al., 2003). Progesterone level assay is a very useful tool to estimate embryos/fetal number and fetometry in pregnant does (Capezzuto et al., 2008; Yazici et al., 2018).

According to a recent study, 22-59% of the slaughtered doe were pregnant, and about 66% of pregnant doe carrying twins, which caused an intensive economical loss in Nigeria (Okorie-Kanu et al., 2018), also more than 7- 23% of slaughtered small ruminant (ewes and goat) were pregnant in Switzerland and Nigeria (Chama et al., 2019; Pagamici and Stephan, 2022). Therefore, pregnancy diagnosis is very crucial to minimize these losses. The current study aimed to compare the Accuracies of pregnancy detection in the goat's use of PAG and P4 tests in different pregnancy periods.

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II. MATERIALS AND METHODS

Experimental animals

Twenty healthy mature, polyparous local does (1 to 3 parity) were utilized in this study, the does were maintained in one flock in Al-Saqlawiyah, Al-Anbar governorate, Iraq. All does were synchronized and mated by three breeding bucks. According to lambing results (Golden standard) and ultrasonography, the animals were subdivided equally into two groups; pregnant and non-pregnant groups. Fourteen does were pregnant and six were not pregnant. Before synchronization, all experimental does were checked with transrectal (6.5-7.5 MHz) and transcutaneous (3.5-4.5 MHz) ultrasonography (Chison ECO2/China), and it was confirmed the physiological condition of non-pregnancy. The does were managed in a semi-intensive program with uniform feeding conditions throughout the period of study. The experiment extends from August 2022 until April 2023.

Estrus Synchronization

The estrus was synchronized according to the manufacturer protocol and According to Kuru et al. (2022); P4 containing an intravaginal sponge was inserted for 12 days, and the PMSG with a dose of 500 IU was intramuscularly injected at the time of sponge withdrawal. The estrus appeared within three days, and all does were mated by the breeding buck. The day of breeding was considered day 0.

Progesterone assay

Blood collection

Five mL of whole blood was collected from all does (n=20) (control group) and from the treatment group (n=20) ewes at days (22, 30, 40, and 60) PM for both P4 and PAG assays. The blood was transported into a gel tube (SABA/Jordon) after centrifuging. Then, the plasma was isolated to another test tube and froze until assayed.

Plasma P4 concentration determination

The peripheral P4 concentrations were detected using the EIA Analyzer (Roche/Switzerland) with a human P4 EIA-kit P4 (Roche/Switzerland).

Pregnancy-Associated Glycoprotein Assays

The serum PAG concentration was measured using two commercially available assays: Sheep Pregnancy-AssociatedGlycoprotein (PAG) ELISA Kit (SunLong Biotec/China), a rabbit antiserum raised against ovine and caprine PAG55. The assay range is 20ng/ml -0.312 ng/mL, and the Se is 0.06 ng/ml. The protocol include several steps according to the manufacturer's instructions; Firstly, the Standard was dissolved and diluted by Standard Diluent into different concentrations (50%, 25%,

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12.5%, 6.2%, 3.1%, 1.55%). Secondly, about 100µL standards and samples were added to each corresponding well and incubated for 1.5 hr at 37℃. Next, 100µL Biotinylated Antibody was added to individual wells and incubated for one hr, and washed two times. About 100µL of HRP-Avidin was added to each well (except blank) and also incubated for one hr, then washed three times. After that, 100µL of the prepared Color Reagent was to each well (including blank) and incubated for half hr. Finally, 100µL Stop Solution was added to each well (including the blank well), mixed well, and read on 450nm within 10 min.

Statistical analysis

The data analysis was achieved via SAS (2018) (v9.6). The Least significant differences (LSD) and one-way ANOVA were done to estimate the significant variations among means.

III. RESULTS AND DISCUSSION

The PAG levels in pregnant and non-pregnant does for a different period

The mean PAG level showed a steady rise in gravid does, while they remained at a basal level for non-gravid does. The PAG level in serum samples of gravid does were significantly higher ($P<0.05$) compared to plasma samples of non-gravid does throughout the entire experimental period. In empty does, the average PAG level remained below the cutoff point in the majority of samples (Table 1).

Table 1: The Progesterone level in pregnant/non-pregnant does for different experimental periods

PAG (Pg/mL) Mean± SD	Day 22	Day 30	Day 40	Day 60
Pregnant	252±19*a	627±16*a	548.5 ±40*a	409±23*a
Non-pregnant	153 ±23b	131 ±13b	147 ±9 b	172.4± 13b

Means with a different letter are significantly different ($P<0.05$)

According to Chentouf et al (2008), the PAG was first detected in 70% of pregnant does at day 20 PM, the level increased gradually after that until day 60 PM. Additionally, Batalha (2001) reported that PAG begin to rise on day 35 PM until day 60-70, then decreased slightly in the 2nd trimester toward kidding. Zamfirescu et al. (2011) declared that it can detect pregnancy by measuring the PAG concentration on days 14-20 PM in does, the PAG level decline in case of embryonic death in periods 21-30 PM.

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The P4 levels in pregnant and non-pregnant does for different periods

The peripheral P4 levels began to increase consistently along with pregnancy progression in the pregnant group. While the level declined significantly in non-pregnant does at day 22, a further drop occurs in days 30, 40, and 60 PM (Table 2).

Table 1: The Progesterone level in pregnant/non pregnant does for different experimental periods

P4 (Pg/mL) Mean± SD	Day 22	Day 30	Day 40	Day 60
Pregnant	2.815±0.56*a	3.3957±0.41*a	6.813±0.84*a	8±0.81*a
Non-pregnant	1.16±0.37b	0.85±1.14b	0.72±1.17b	0.68±1.31b

Means with a different letter are significantly different (P<0.05)

During pregnancy, because of the maternal recognition, the luteolysis was blocked and CL still secrete P4, therefore, the mean peripheral P4 level increased on day 22, while, a decline in most non-pregnants. The findings agreed with Sousa et al (1999), who recorded that the P4 level was higher significantly (P<0.05) in pregnant than non-pregnants on days 18, 20, and 24 PM. The same findings were reported by Gonzalez et al. (2004) in day 22 PM.

Compare the efficiency of pregnancy-associated glycoprotein and Progesterone for pregnancy determination

The PAG level was higher in 12/14 does on day 22 PM (Se 85%) with very low false negative, all pregnant does had PAG concentrations higher than 200 Pg/mL on day 30 PM and above (100%). While 2/6 (Sp 66%) and 1/6 (Sp 83%) non-pregnant does have PAG concentrations higher than 200 Pg/mL on day 22 and 30, respectively (false positive), the Sp reaches optimum value on day 40 without false positive. The Acc of PAG was reliable on day 22 (80%), it was increased on day 30 (95%) and reached optimum on day 40 PM onward (Table 3).

Twelve does out of 14 showed levels of P4 above the discriminatory point (>2.5 ng/ml) on day 22 PM. There were three non-pregnant does that represent 50% with P4 levels similar to pregnant goats, while another three of these goats (50%) showed basal levels of P4 during the same period. On day 30 PM,

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the P4 level declined to basal level and was far away from the cutoff point (> 3) in 66% of goats. On days 40 and 60 PM, the P4 level stayed elevated slightly one non-pregnant doe higher than the discriminatory point (<3) (false positive) (Table 3).

Table 3: The effectiveness of PAG and P4 methods in diagnosing pregnancy in does

Day after mating / Cutoff point	Predicted Value +		Predicted Value -		Senstivity		Specificity		Accuracy	
	PAG	P4	PAG	P4	PAG	P4	PAG	P4	PAG	P4
	< 200	< 2.5	< 200	< 3	< 200	< 3	< 200	< 3	< 200	< 3
Day 22 PI	(12/14) 85%	(12/16) 75%	(4/6) 66%	(3/4) 75%	(12/14) 85%	(12/14) 85%	(4/6) 66%	(3/6) 50%	(16/20) 80%	(15/20) 75%
Day 30 PI	(14/15) 93%	(13/16) 81%	(5/5) 100%	(3/4) 75%	(14/14) 100%	(13/14) 92%	(5/6) 83%	(4/6) 66%	(19/20) 95%	(17/20) 85%
Day 40 PI	(14/14) 100%	(14/15) 93%	(6/6) 100%	(5/5) 100%	(14/14) 100%	(14/14) 100%	(6/6) 100%	(5/6) 83%	(20/20) 100%	(19/20) 95%
Day 60 PI	(14/14) 100%	(14/14) 100%	(6/6) 100%	(5/6) 83%	(14/14) 100%	(14/14) 100%	(6/6) 100%	(5/6) 83%	(20/20) 100%	(20/20) 95%

These outcomes came constant with Karen et al. (2003) dissertation, which find that the Se of the PAG assay method in ewes was high with two cases showing false negative results (level lower than the threshold), it reach 100% on day 29 onward. Additionally, they mentioned that Sp recorded optimum value just one false positive case was reported on day 29. The same findings were reported by Batalha et al. (2001) study, which mentioned that Se and Sp were 100% and 91% on day 35 with no false negative and 9% false positive.

Depending on the P4 test, the Se and Acc were very high on day 22 and reach 100% on day 40, but the Sp was lower than the PAG test. According to these findings, the Se was very high at day 22 PM (85%), the same as the Se of PAG during the same period, it increased steadily along with the progression of pregnancy, reaching 100% at day 40 PM onward. The Acc of P4 analysis was leaser than PAG on day 22, but still reliable, it also raised progressively and reach 95% on days 40 and 60 PM.

The Sp in both P4 and PAG assays was lower, which reflects the ability of methods to detect non-pregnancy in a specific period, but the Sp of the PAG assay was still higher than the P4 method (66% Vs 50%), it reach 83% in days 40 and 60 PM, is much lower than PAG method, whereas, PAG level decline underneath the discriminatory point in 5/6 doe in day 30 (Sp 83%) and all does in day 40 and 60 PM. Additionally, Gonzalez et al. (2004) results showed that the Se of the PAG assay was slightly lower than the P4 test on day 22, while, the Sp and Acc of the PAG test were much higher than the P4 in the same period. Moreover, a recent study showed that Se of PAG was lower than P4 (88% Vs 94%)

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tests and Sp of PAG was higher than P4 (91% Vs 80%) on day 28 PM for does by using bovine PAG kit (Doğan and Köse, 2022). Furthermore, Karen et al (2003) find out that Se of PAG was higher than the P4 assay on days 22, 36, and 50 PM. Karen et al (2003) reported false positives in seven cases depending on the P4 test, which they attributed to the occurrence of irregularity of estrus, embryonic death, and pyometra.

The PAG is a pregnancy-specific hormone that depends on the presence of active syncytial tissue, PAG is a unique glycoprotein secreted particularly from placental Binucleate Jiant cells (Wallace et al., 2015), therefore, the high PAG level than threshold gives a strong indicator on viable syncytial tissue. That reflects very high Se, Sp, and Acc without false results especially from day 40 onward. In contrast, the P4 test is a non-specific method, it depends on the presence of active CL, the rise of the P4 level above the threshold is not necessarily related to pregnancy, and many pathological cases related to persisting CL in goats such as embryonic death, endometritis/pyometra and ovarian cyst.

In does, a higher Embryo/fetal mortality (19%) was detected between periods 20-23 PM and (11%) in periods 47-54 PM (Samir et al., 2016). According to Beena et al. (2015), a high incidence of pathological lesions in the genital tract of does were recorded; endometritis was the major pathological lesion (24%), followed by ovarian cyst (14%). According to Garba et al. (2019), the incidence of endometritis was 11.9%, uterine hemorrhage 3.8%, also 2.8% for each cervicitis, pyometra, and mucometra. These pathological conditions increase the false positive and decrease the Sp of the P4 method to predict pregnancy. Both P4 and PAG tests methods showed that (2/6) does expert embryonic death after day 22, because PAG and P4 were still elevated, the P4 level does not decrease below the cutoff point in two does on day 40 and in one doe in day 60 PM, it may attribute to presence pathological lesion and endometritis/pyometra.

The study concluded that pregnant does exhibited higher levels of PAG and P4 starting from day 22 and beyond, compared to non-pregnant does. Accurate detection of PAG and P4 levels was possible from day 22 onwards, with PAG demonstrating higher Se, Sp, and Acc than P4 across all periods. Furthermore, PAG test reached optimal diagnostic values earlier than the P4 assay method, therefore, it more reliable than P4 assay

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