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Pregnancy-Associated Glycoprotein Level in Early Pregnancy Detection with Prediction of Fetal Number and Gender in Iraqi Local Ewes

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A B S T R A C T

Early pregnancy detection is an important tool in successful reproductive management in ewes. The objective of this study was to estimate the concentration of serum pregnancy-associated glycoprotein (PAG) during gestation using sheep ELISA kit in order to early distinguish between pregnant and non-pregnant ewes and the possible association with litter size, and fetal gender. Estrus was synchronized and blood samples were collected at day 0 (day of mating), 18, 30, 60, and 120 after mating from 18 Iraqi cross breed synchronized ewes. The result of PAG level (ng/ml) during estrus has shown insignificant difference between G1(CIDR group), G2(vaginal sponge group) and G3 (control group), while at day 18, 30, 60 and 120 of pregnancy was significantly ($p<0.05$) higher in G1 and G2, in comparison with G3. The discrimination value (cutoff point) for detection of pregnancy by PAG test was > 0.915 ng/ ml (using ROC Curve) and based on breeding data from day 18 PAG values were detectable in all pregnant ewes. It was also proved that mean values were significantly ($P<0.05$) higher in twin than single pregnancies and significantly ($P<0.05$) higher in male single than female single pregnancy in crossbred Iraqi ewes according to the data collected from day 0 to the end of pregnancy and follow the pregnancy up to the day of birth. Altogether, it is concluded that, ovine pregnancy may be reliably detected with fetal number and gender from day 18 after mating onward by using sheep ELISA kit of pregnancy associated glycoprotein.

Keywords: sheep, early pregnancy detection, pregnancy associated glycoprotein, litter size, fetal gender

INTRODUCTION

The economic factors in animals are maintain pregnancy because most losses of pregnancy occur at the first pregnancy stages (1). Thus, especially valuable tool is early detection of pregnancy and consider a tool that particularly important which used in management of herd to ensure good reproductive health of livestock herds (2). There are several tools for pregnancy detection used in animals but particularly important tools including: testing milk or blood progesterone, Ultrasound, estrone sulfate and testing milk or blood PAG. An earlier pregnancy diagnosis may be achieved by using trans rectal ultrasonography, but it takes a long time and experience (3). Progesterone testing is accurate and sensitive as early as Days 17 to 19 (4). Specific pregnancy-associated glycoproteins (PAG) are considered key indicators for the purpose of early pregnancy diagnosis given that they are produced within the first few days of the pregnancy (1). ELISA-PAG test may be considered as an alternative for rapid and accurate detection of PAG, as well as the identification non-pregnant ewes and it showed similar or better sensitivity as trans-rectal ultrasonography for pregnancy detection (5). Radioimmunoassay of plasma using sheep and goat antisera mixture to detect Pregnancy associated glycoprotein (PAG) results in about 95.3% of early expected pregnancy confirmation as early as Day 18 of pregnancy (6). Studies of PAG are not only for pregnancy detection,

however also for viability of pregnancy, fetal gender and fetal number (7, 8). A new color-binding polyclonal antibody-based enzyme-linked immunosorbent assay kit (CER-6900; Marloie) was assessed for pregnancy diagnosis in the Rasa Aragonesa breed, exhibiting sensitivity and specificity of 100% on Day 25 and forward (9).

From extremely low isoelectric points, the glycoproteins associated with pregnancy were first discussed as acidic (67-kelo Dalton). They are isolated from the cattle placenta and they were also found in the maternal blood serum shortly after embryo implantation (10). After that PAG were isolated from other species such as sheep, cat, pig , horse, buffalo and goat. Currently, more than 100 exons of the PAG gene family are known, showing an extremely diverse family of glycoproteins, which show various levels of expression at different days during gestation from day 7 post-fertilization onwards. The expression is largely in the pre-placental trophoblast and post-implantation trophectoderm (11). Hypothesis that these proteins help to suppress immunity so that the dam is acceptance of embryo, the maternal circulation has been revealed to contain chemical compounds of binucleate cells, suggesting that the development of the placenta and remodeling of placenta might be formation (12). PAG, is a placental derived, a multigenic, aspartic proteinase family and is produced and secreted from placenta (13). In ruminants, the family of aspartic protease is represented by more than ten similarity antigenic proteins related to each other with distinct levels of glycosylation according to findings (13). All sheep have day related with identification of PAG in blood circulation of mothers within 20 days after mating (4). PAG is test of choice as biomarkers of pregnancy and serves as the basis for pregnancy diagnosis in ruminants (14). Throughout the gestation period, ruminant PAG profile varies based on the breed (15) or other factors such as singleton or twinning (14, 16), gender of newborn and birth weight (17), environment and particular kit used (18). Quantification changes of PAGs in maternal circulation are reflection of the binucleate cell number in placenta (19).

The variable nature of sheep blood PAG levels (14), therefore it is important for a standardization of blood PAG level in any breed before reliance on PAG profile for confidently diagnosis of ewes pregnancy status from the data provided. The pregnancy-associated glycoproteins is able to cross the placental barrier, then transported on the fetal circulation. Nevertheless, the important of these proteins which enter the circulatory system pre and post birth is ill defined. The studies on the fact that PAG goes into newborn's circulation through trans placental route in utero, also it is passively absorbed from the intestinal epithelium after colostrum ingestion due to its higher permeability after ingestion of colostrum (20). ELISA tests are more practical. The ELISA method which was used was confounded by the antibody and types the blood samples used which led to reported sensitivity to range from 93.5 % on day 23 (8) to 100 % on day 24 post mating (21) and the specificity was reported as 98.9% in varying time of pregnancy (8).

Between days 18 and 32–35, ovPAG concentrations rise. Subsequently, there is a drop at day 42, and then, there is an increase once again at day 49 (21). To distinguish between pregnant and nonpregnant ewes, these compounds may be detected in maternal plasma at amounts detectable with RIA techniques as early as 18–20 days after conception and beginning from day 18 of pregnancy (6, 21-23) and using the enzyme immune assay (EIA) method at day 18-20 of pregnancy (5, 8, 24). and in other ruminant species (25,26). Breed, fetal number, sex, and birth weight are the factors that influence PAG concentrations in these animals during pregnancy (15, 17). There have been numerous studies in the field of reproduction science that have sought to analyze pregnancy-associated glycoprotein. These studies have not only information regarding early pregnancy diagnosis, but also fetal number, pregnancy viability, fetal sex (7,8, 27,28). Breukelman et al. (29) and Engelke et al. (30) found that PAG levels may also detected fetal viability, which was previously only determined using ultrasonography. Therefore, elevated levels of serum PAG may be associated with a healthy pregnancy, while low levels suggest a higher risk of fetal loss or late embryonic death. This makes of PAG as a tool for early pregnancy detection (31). The main object of current study to estimate the concentration of blood PAG during early gestation in crossbred Iraqi ewes to determine pregnancy from non-pregnancy as well as fetal number and fetal gender.

Generally, sheep considered seasonal polyestrous animals and short day breeders (32, 33). The breeding season started in autumn and continued until med or late winter (34,35). Synchronization of estrous can be achieved via different methods including manipulation of photoperiod, effect of ram and administration of exogenous hormones (prostaglandin, melatonin progesterone, eCG, kisspeptin and Bromocriptine) during both non breeding and breeding season in ewes (36-39) and in goat (40). The administration of equine chorionic gonadotropin with progesterone during both nonbreeding and breeding season can improve estrus response and gestation rate (41). The ram effect may be improving the effectiveness of progesterone and serve as substitution for eCG in sheep (42). The response of estrus and birth rate were significant higher in ewes using Controlled internal drug release (CIDR) and Kisspeptin injection than those used CIDR alone (43), but other authors showed no significant difference between two groups when used same protocols (39).

MATERIALS AND METHODS

Ethical Approval

Ethical approval was granted according to the local committee of care and use of animals in research at the College of Veterinary Medicine, University of Baghdad (Approval number P.G/995).

Animals

This study was conducted at the farm belong to the College of Veterinary Medicine, University of Baghdad,

Baghdad, Iraq. The experiment extended from January 2023 to September 2023. Eighteen cross adult Iraqi ewes aged between 3-4 years determined by breeding recorded data of farmer. Three males were also assigned for current study theses males were sexually mature and fertile according to the pervious breeding recorded of data. The animals are subjected to careful clinical examination and ultrasonography examination to determine that they are non-pregnant/healthy and free from diseases. Preventive health measures applied such as vaccination against enterotoxaemia (against clostridia infection) at dose of 2 ml S/C and treatment against internal &external parasites (Rafoxanide (VAPCO, Jordan) 7.5 mg/kg orally and Ivermectin (Pfizer, USA) 200µg/kg injection.

Experimental Design

The animals were randomly divided into three groups (G1, G2 and G3), six ewes for each group. Group1 (G1) has received CIDR (contain 300 mg progesterone) for12 days (44) it had been done according to the instructions of company , with i.m. injection 400 IU of PMSG at withdrawal, group 2 (G2) has received vaginal sponge (60 mg Medroxyprogesterone acetate) for 12 days with i.m. injection 400 IU of PMSG at withdrawal of the sponges, while group3 (G3) was not treated and served as control group. The animals kept at semi-opened shade shelter supplemented with drinking water ad libitum, provided with 1kg concentrated grains diet (barley 40%, wheat 51%, soya been 5%, limestone 2%, NaCl 1%, minerals and vitamins 1%) (45-48) daily per ewe.

Blood Collection

Blood was collected from jugular vein using vacutainer gel tubes and numbered by hand approximately 5ml, the lower part of the neck of the ewe was held firmly by the left hand so that jugular vein could be visible. The skin on the jugular vein was cleaned by 70% alcohol. The needle was inserted in jugular vein and collected the blood for determination of PAG in the serum of pregnant ewes at the following days of pregnancy: day of mating, 18, 30, 60, and 120 days after mating (according to the experimental plan). Serum was harvested following centrifugation of samples at 3000 RPM for 10 minutes and then stored at -20°C until the assay (49).

PAG assessment

Determination of the concentration of PAG during gestation, at the following days: At the time of mating (0), 18, 30, 60 and 120 of pregnancy. Blood samples were collected during estrus and pregnancy from jugular vein and placed into gel vacutainer tubes to detect PAG level by sheep PAG ELISA- kit in order to distinguish between pregnant and non-pregnant ewes, also the number and gender of fetuses. The tubes were transported to the laboratory of college of veterinary medicine, Baghdad University in a cool box, centrifuged for 10 minutes at 3000 RPM, placed into 1.5ml Eppendorf tubes and stored at -20 C until analysis.

PAG ELISA Kit: Pregnancy associated glycoproteins were measured using Sheep PAG ELISA KIT (Shanghai Ideal Medical Technology Co., Ltd, China). Catalogue No. YZ-Y21955, chemicals consist of samples diluent solution, Standard diluent solutions, Stop solution, chromogen solution A, chromogen solution B, Wash buffer Concentrate (2x) and conjugate reagent.

Whole blood samples were collected kept in freezer until. For this assay, added 50 µl in first column of kit plate as standers for test and added 10 µl of sample in all other column, anti-PAG antibody coated plates along with 40 µL of diluent sample and 100µl of conjugate reagent enzyme (anti-IgG- horseradish peroxidase) was added for 96-well with standers and incubated for 60 min at 37ºC in a forced air incubator. After incubation, plates were washed four times with 350 µl of wash solution. The detector solution (chromogenic A and chromogenic B) 100 µL was added to each well, covered, and incubated for 15 min at 37ºC. The plates were washed and added 100 µL of tetramethylbenzidine as a substrate solution and late 15 min in room temperature. In the end, the color change in formula was detected by ELISA blat. Sample values were reported as serum sample minus negative controls after subtracting the mean. At the end of test the results were depended on the color of samples the positive test observed that well was stained blue, while negative test the color of well remained transparent.

Statistical Analysis

The Statistical Analysis System- SAS, (50) program was used to detect the effect of different factors in study parameters. Least significant differences (LSD) test was used to significant compare between means in this study. Receiver operation characteristic curve (ROC curve) also one and Two-way ANOVA and Chi square tests were used to identify the validity of markers as an indicator of the pregnancy and type of offspring. The markers were compared according to area under curve. The analysis was submitted by using MedCalc, (51) Software. $P<0.05$ is considered statistically significant.

RESULTS

Results of pregnancy associated glycoprotein level (ng/mL)during estrus and different stage of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in Table1 The results proved no significant difference ($P<0.05$) between the values of PAG level during estrus in G1, G2, and G3, while during pregnancy the results indicate that at days 18, 30, 60, and 120 of pregnancy show that PAG level significantly higher in G1 and G2 in comparison with G3.

Concerning the differences in PAG concentration at days of estrus and different period of pregnancy (day 18, day30, day60, and day 120), these results revered that in G1and G2 treatment the concentration of PAG recorded highest value at day 120 of pregnancy which differed significantly ($P<0.05$) from other pregnancy periods of study and lowest significant value was found in day 18 of pregnancy and at estrus period. While the results of G3 show no significant

differences among all periods of pregnancy and estrus phase.

Results of PAG level (ng/mL) during different stage of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in Table 2. The results proved significant difference (P<0.05) between the values of PAG level in single and twin pregnant ewes at day 18 of pregnancy highest values in twin pregnant ewes, which differed significantly (P<0.05) from single pregnant ewes, which show lowest significant value, the same trend of PAG have been proved at day 30 of pregnancy, day 60 of pregnancy and day 120 of pregnancy means highest values was recorded in twin pregnant ewes in comparison with single pregnant ewes in all above mentioned period.

Concerning the differences in PAG concentration at different period of pregnancy (day 18, day30, day60 and day 120), these results revered that in single pregnant ewes the concentration of PAG recorded highest value at day 120 of pregnancy which differed significantly (P<0.05) from other pregnancy periods of study and the lowest significant value was found at day 18 of pregnancy and recorded that PAG level significantly (P<0.05) higher at day 30 of pregnancy than day 60 of pregnancy , also in twin pregnant ewes result of current study regarding PAG concentration recorded the same trend in which highest significant value found at day 120 of pregnancy and lowest value at day 18 of pregnancy and recorded that PAG level significantly (P<0.05) higher at day 30 of pregnancy than day 60 of pregnancy.

Table 1. Values of pregnancy associated glycoprotein during different periods of pregnancy in estrus synchronized ewes (ng/mL)

Group	Day of estrus (mating)	Day of pregnancy			
		18	30	60	120
G1	0.556±0.07 ^{Ad}	6.05±0.53 ^{Ac}	11.35±0.74 ^{Ab}	10.3±0.52 ^{Ab}	13.8±0.60 ^{Aa}
G2	0.609 ±0.09 ^{Ae}	4.94±0.40 ^{Ad}	11.55±0.71 ^{Ab}	9.63±0.12 ^{Ac}	13.9±0.55 ^{Aa}
G3 (control)	0.653 ±0.06 ^{Aa}	0.46±0.08 ^{Ba}	0.653±0.06 ^{Ba}	0.52±0.12 ^{Ba}	0.79±0.02 ^{Ba}

Means (±SEM) with different capital letters in the same column and small letters in the same row are significantly different (P≤0.05), LSD value=1.265

Table 2. Changes in the concentration of pregnancy associated glycoprotein (PAG) during single and twin pregnancy in ewes of study (ng/mL)

Group	Sampling (Day of pregnancy)			
	18	30	60	120
PAG level in single pregnancy	4.81±0.21 ^{Bd}	10.80±0.32 ^{Bb}	9.20±0.12 ^{Bc}	13.2±0.30 ^{Ba}
PAG level in twin pregnancy	7.33±0.37 ^{Ad}	13.78±0.36 ^{Ab}	12.1±0.25 ^{Ac}	15.6±0.03 ^{Aa}

Means (±SEM) with different capital letters in the same column and small letters in the same row are significantly different (P≤0.05), LSD value=1.335

Table 3. Cutoff point of glycoprotein at different days of pregnancy

Status	Cutoff point (Day of Pregnancy)			
	18	30	60	120
Glycoprotein pregnant singleton	> 0.915	≤ 11.829	≤ 9.603	≤ 14.488
Glycoprotein in pregnant twins	> 5.963	> 11.829	> 9.603	> 14.488

Results of cutoff point of PAG level (ng/mL) at different days of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in Table 3. The results proved the cut of point of PAG to confirm single and twin pregnancy in different day of pregnancy in studied animals, which recorded that when cut of point of PAG is greater than 0.915 ng/mL which confirm that the ewe is single pregnant at day 18 but greater than 5.963 ng/mL which confirm that the ewe is twin pregnant at day 18 also recorded at day 30,60, and120, which show the cut of point of PAG is greater than 11.829 ng/ml which confirm that the ewe is twin pregnant at day 30, while greater than 9.603 ng/mL which confirm that the ewe is twin pregnant at day 60 and greater than 14.488 ng/ml which confirm that the ewe is twin pregnant at day 120.

Results of PAG level (ng/mL) during different stage of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in Table 4. The results proved significant difference (P<0.05) between the values of PAG level in male and female pregnant ewes ,at day 18 of pregnancy highest values in male pregnant ewes, which differed significantly (P<0.05) from female pregnant ewes, which show lowest significant value, the same trend of PAG have been proved at day 30 of pregnancy, day 60 of pregnancy and day 120 of pregnancy means highest values was recorded in male pregnant ewes in comparison with female pregnant ewes in all above mentioned period. These results possible due to higher body weight of male than female lead to increase placental mass.

Concerning the differences in PAG concentration at different period of pregnancy (day 18, day30, day60 and day 120), these results revered that in male pregnant ewes, the concentration of PAG recorded highest value at day 120 of pregnancy which differed significantly (P<0.05) from other pregnancy periods of the study. The lowest significant value was found at day 18 of pregnancy and recorded that PAG level significantly (P<0.05) higher at day 30 of pregnancy than day 60 of pregnancy, also in twin pregnant ewes, the result of PAG concentration recorded the same trend in which highest significant value found at day 120 of pregnancy and lowest value at day 18 of pregnancy and

recorded that PAG level significantly (P<0.05) higher at day 30 of pregnancy than day 60 of pregnancy.

To support this study, here we are mentioning the other results published recently (52) of reproductive parameters (estrus response %, duration of response (hrs.) and estrus phase length (hrs.) of estrus synchronized ewes of present study are depicted in Table 5. The results indicate highest values of estrus response % was recorded in G1 and G2, which differed significantly (P<0.05) from result of G3, which recorded lowest value. Regarding duration of response, the result proved highest values was recorded in G3, which differed significantly (P<0.05) from G1 and G2, on the other hand the length of estrus phase shows highest values in G3 which differed significantly (P<0.05) from G1 and G2.

Table 4. Changes in the concentration of pregnancy associated glycoprotein (ng/mL) in pregnant ewes of study in male and female fetus (in singleton pregnancy)

		Day of pregnancy			
Sex of fetus	**Number**	**18**	**30**	**60**	**120**
Male	5	5.35±0.19 Ad	11.47±0.15 Ab	9.53±0.02 Ac	13.99±0.31 Aa
Female	5	4.26±0.16 Bd	9.94±0.39 Bb	8.98±0.18 Bc	12.42±0.12 Ba

Means (±SEM) with different capital letters in the same column and small letters in the same row are significantly different (P≤0.05), LSD value=0.518

Table 5. Values of estrus response(%), duration of response (h) and estrus phase length (h) in animals of study. Al-Rawi and Hussain (52)

Group	**Number**	**Estrus response (%)**	**Duration of response (h)**	**Length of estrus phase (h)**
G1	6	100±0.00 [a]	39.17±2.74 [b]	31.67±1.97 [b]
G2	6	100±0.00 [a]	43.50±3.19 [b]	26.33±2.85 [c]
G3 (control)	6	17 ±0.52 [b]	48.00±0.75 [a]	38.00±0.52 [a]
LSD value		9.437	4.902	3.283

Means (±SEM) with different letters in the same column are significantly different (P≤0.05)

DISCUSSION

The results of the present study regarding PAGs as pregnancy markers in blood of pregnant ewes were in line with other author studies (2, 53, 54), with slight variation in these results possible due to differences in commercial kits or type of assay also due to differences in breed, age, season, nutrition and location of study. The pregnancy associated with glycoprotein continue to increase with progresses of pregnancy and increase placental function this agree with Ward et al. (19) who recorded that quantification changes of PAGs in maternal circulation is possible reflection of the number of binucleate cells in the fetal trophectoderm. Also agree with García-Ispiertol et al. (55) show that the increasing PAG1 level during pregnancy up to delivery is essential role of this group of these proteins for maintaining of pregnancy until parturition. Wallace et al. (56) proved that sheep PAG level peak at day 120 of pregnancy which similar of current study. Our study proved and agreed with several authors in order to distinguish between pregnant and nonpregnant ewes, these compounds may be detected in maternal blood as early as 18–20 days after conception and beginning from day 18 of pregnancy with high accuracy (6, 21-23).

Current study proved that the concentration of PAG increase between day 18 and 30 then slightly decrease between day 30 and 60 of pregnancy, this decrease may be due to changes in gene expression of PAG from one to other or due to at this period the placenta begin to produce and secrete progesterone lead to decrease of its function toward the PAG, this result quite similar to the finding which proved that between days 18 and 32–35, ovPAG concentrations rise (21), the same authors also proved a drop at day 42, and showed a decrease and increase in the concentration of PAG depending in the week of pregnancy and significantly differed from week to week . More than 100 exons of the PAG gene family are known, showing an extremely diverse family of glycoproteins, which show various levels of expression at different days during gestation from day 7 post-fertilization onwards. The expression is largely in the pre-placental trophoblast and post-implantation trophectoderm (11). Ranilla et al. (57) show in Merino's ewes that PAG values begin to increase then decrease around mid-pregnancy. A decrease in PAG at day 42 of pregnancy has been seen by using ELISA (58). As proved by Lopez-Gatius et al. (59) that PAG molecules are a closely related protein family and that expression of these molecules vary temporarily during early and late gestation periods (60); day 42 could be the begin of a switch off of some genes coding for PAG and begin a switch on of other PAG groups detected by antiserum.

The current results are similar to El Amiri et al. (22) who proved that at Starting from day 18, all ewes that were not pregnant had ovPAG values that were undetectable to those seen in pregnant females. Furthermore, it is worth noting that all pregnant ewes had ovPAG levels that were beyond the designated threshold level for diagnosing pregnancy, which is less than 1.0 ng/ml. Although the number of experimental animals was small, these findings suggest that pregnant and non-pregnant ewes may be distinguished at an earlier stage (day 18). Also, in agreement with Alabart et al. (9) showed that PAG values above 0.8 ng/mL at day 18 of pregnancy in Aragonesa breed sheep. Barbato, et al. (6) and De Carolis et al. (23) proved that cutoff of P4 and PAG were 1.0 ng/ml at day 18 of pregnancy can be used to distinguish between pregnant and non-pregnant ewes.

Early known the lamb's number during pregnancy allows to divide ewes in two feeding groups according to

their expected number of fetuses lead to improve birth weight, weaning weight and lambs' survival rate, as well as prevent dystocia and pregnancy toxemia (21). Our study proved these results and agreed with numerous studies who show that breed and foetal number are the major factors that influence PAG concentrations in these animals during pregnancy (15, 17). Other studies confirm that PAG analyses have not only information regarding early pregnancy diagnosis, but also fetal number, pregnancy viability, fetal sex (7, 8, 21, 27, 28). Throughout the gestation period, ruminant PAG profile varies based on factors such as singleton or twinning (15, 16). The level of PAG shows higher in ewes with twin pregnancy than single pregnancy up to day 79 of gestation due to the rise in the number of main sources of PAG (binucleate cell) because the increase in the mass of placenta in ewes but did not relate with specific breed (14). Kaulfub et al. (61) and Ranilla et al. (62) suggested that higher level of PAG in twin pregnancy in comparison with single may be due to increase number of attachment points and increase cotyledons surface with increasing litter size and thus increase secretory activity of twin placentas. Singh et al. (63) show there was higher blood PAG level in does bearing twin pregnancy than does with single pregnancy, also this show in cattle (64) and in sheep (65). Higher PAG values in ewes with twin fetuses in comparison with single pregnant ewes may be attributed to higher number of attachment points. Therefore, improved synthetic activity of twin placentas (21). De Carolis et al. (23) detected that PAG levels were higher in ewes with twin fetuses than those in single fetuses may be because increase in number of attachments point lead to increase the activity secretion of twin placenta. Barbato et al. (6) detected the cutoff point of PAG values in twin pregnancy at day 18 is 4.4±1.0 ng /ml in ewes.

Numerous studies agree with our study in which to detect ovine PAGs throughout the gestation period, ruminant profile varies based on the number of factors, one of them gender of newborn and birth weight (15, 17). Analyses of pregnancy-associated glycoprotein have not only information regarding early pregnancy diagnosis, but also fetal number, pregnancy viability, fetal sex (7,8,21, 27,28). Singh et al. (63) observed that there is a positive correlation between PAG level and sex of fetus with higher level in male than female. De Carolis et al. (23) detected that PAG values tended to be affected by the gender of fetus with higher significantly values in male fetuses than female fetuses, this variation possible due to placental weight which differs depending on gender. In contrast to our results Vandeale et al. (66) who did not show any variation between ewes with male or female fetuses, may be because they detected at early stage of pregnancy or they used different kit from us, also due to differences in breed, age, season, nutrition and location of study. It is concluded that ovine pregnancy can be reliably detected with fetal number and gender from day 18 after mating onward by using sheep ELISA kit of PAG.

The present study is in agreement with several authors such as Godfrey et al. (67) proved that giving CIDR (300 mg) for 12 days combination with ram introduction obtain estrus response 100%. Letelier et al. (68) show that decline the amount of progesterone in vaginal sponges or time of treatment from 12 to 6 days did not effect on growth of follicular and ovulation. Kaylan et al. (69) proved that vaginal sponge's insertion for 12 days then injection 200 IU PMSG at sponge withdrawal stimulates estrus response 84%. Naderipour et al. (70) proved estrus response was 90% in ewes with CIDR insertion and 85% in ewes with sponge insertion. the results of present study was very closed to and in agreement with Ozyurtlu et al. (71) who show that insertion of CIDR, sponge for 12 days then i/m injection of 400IU PMSG at time of withdrawal and control results estrus response 90%, 87.5% and 16.7% respectively, which significantly ($P<0.05$) higher in treated groups in comparison with control group also proved that the onset of estrus 35.22±1.6, 38.48± 1.5 and 49.33±3.5 respectively and duration of estrus 30.17±0.7, 29.43±0.8 and 36.67±0.7, respectively which significantly ($P<0.05$) lower in treated groups in comparison with control group in Awassi ewes. Current study agrees with Hameed et al. (42) Yu et al. (72) who proved that CIDR device containing 300mg progesterone is similar effective to 60 mg MAP sponge with 12 days long protocol on reproductive performance.

This research is important to breeders since it is a tool to maximize fertility by detecting early pregnancy and isolation non pregnancy followed finding the causes of pregnancy failure and giving the appropriate treatment.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Commun L, Velek K, Barbry JB, Pun S, Rice A, Mestek A, et al. Detection of pregnancy-associated glycoproteins in milk and blood as a test for early pregnancy in dairy cow. J Vet Diagn Invest. 2016;28(3):207-213. 10.1177/1040638716632815
2. Kaya MS, KÖSE M, Bozkaya F, Mutlu H, Uçar EH Atli MO. Early pregnancy diagnosis using a commercial ELISA test based on pregnancy-associated glycoproteins in Holstein-Friesian heifers and lactating cows. Turk J Vet Anim Sci. 2016;40:694-699. 10.3906/vet-1602-41
3. González-Bulnes A, Pallares P, Vazquez MI. Ultrasonographic imaging in small ruminant reproduction. Reprod Domest Anim. 2010; 45(2):9–20. 10.1111/j. 1439-0531.2010.01640.x
4. Karen A, Beckers JF, Sulon J, de Sousa NM, Szabados K, Reczigel J, et al. Early pregnancy diagnosis in sheep by progesterone and pregnancy associated glycoprotein tests. Theriogenology. 2003;59:1941–8. 10.1016/s0093-691x(02)01289-x
5. de Miranda ESCC, Dias da Costa RL, Roncato Duarte KM, Machado DC, Paro de Paz CC, Beltrame RT. Visual ELISA for detection of pregnancy-associated glycoproteins (PAGs) in ewe serum. Theriogenology. 2017;97:78-82. 10.1016/j.theriogenology.2017.04.026
6. Barbato O, Sousa NM, Debenedetti A, Canali C, Todini L, Beckers JF. Validation of a new pregnancy-associated glycoprotein radioimmunoassay method for the detection of early pregnancy in ewes. Theriogenology. 2009;72:993–1000. 10.1016/j.theriogenology.2009.06.020

7. Karen A, Sousa NM, Beckers JF, Bajcsy AC, Tibold J, Mádl I, et al. Comparison of a commercial bovine pregnancyassociated glycoprotein ELISA test and a pregnancy-associated glycoprotein radioimmunoassay test for early pregnancy diagnosis in dairy cattle. Anim Reprod Sci. 2015;159:31–37. 10.1016/j.anireprosci.2015.05.005
8. Rovani MT, Skrebsky Cezar A, Lazzari Rigo M, Garziera Gasperin B, da Nobrega JE, Dias Torres F, et al. Evaluation of a bovine pregnancy glycoprotein enzyme-linked immunosorbent assay kit for serological diagnosis of pregnancy in sheep. Cienc Rural. 2016;46:362–367. 10.1590/0103-8478cr20150270
9. Alabart JL, Lahoz B, Folch J, Martí JI, Sánchez P, Delahaut P, et al. Early pregnancy diagnosis in sheep by plasmatic pregnancy associated glycoprotein (PAG) enzyme immune-assay (EIA) kit. XXXV Congreso de la SEOC. Valladolid, Spain Instituto Tecnológico Agrario, Junta de Castilla y Leó. 2010; p. 199–202.
10. Zoli AP, Beckers JF, Wouters-Ballman P, Closset J, Falmagne P, Ectors F. Purification and characterization of a bovine pregnancyassociated glycoprotein. Biol Reprod. 1991;45:1–10. 10.1095/biolreprod45.1.1
11. Garbayo JM, Green JA, Mannikam M, Beckers JF, Kiesling DO, Early AD, Roberts M. Caprine pregnancy associated glycoproteins (PAG): their cloning, expression, and evolutionary relationship to other PAG. Mol Reprod Dev. 2000;57:311–322. 10.1002/1098-2795
12. Hashizume K, Ushizawa K, Patel OV, Kizaki K, Imai K, Yamada O, et al. Gene expression and maintenance of pregnancy in bovine roles of trophoblastic binucleate cell specific molecules. Reprod Fert Dev. 2007;19:79–90. 10.1071/rd06118
13. Sousa NM, Ayad A, Beckers JF, Gajewski Z. Pregnancy associated glycoproteins (PAG) as pregnancy markers in the ruminants. J Phy Pharm. 2006;57(8):153–171.
14. Redden R, Passavant C. Efficacy of pregnancy-specific protein B assay to detect pregnancy and lambing rates in sheep. Sheep Goat Res J. 2013;28:21–24.
15. Mercadante PM, Waters KM, Mercadante VR, Lamb GC, Elzo MA, Johnson SE, et al. Subspecies differences in early fetal development and plasma pregnancy-associated glycoprotein concentrations in cattle. J Anim Sci. 2013;91:3693–701. 10.2527/jas2012-6130
16. Serrano B, Lopez-Gatius F, Santolaria P, Almeria S, Garcia-Ispierto I, Bech-Sabat G, et al. Factors affecting plasma pregnancy-associated glycoprotein 1 concentrations throughout gestation in highproducing dairy cows. Reprod Domest Anim. 2009;44:600–605. 10.1111/j.1439-0531.2007.01025.x
17. Lobago F, Bekana M, Gustafsson H, Beckers JF, Yohannes G, Aster Y, et al. Serum profiles of pregnancy-associated glycoprotein, oestrone sulphate and progesterone during gestation and some factors influencing the profiles in Ethiopian Borana and crossbred cattle. Reprod Domest Anim. 2009;44:685–92. 10.1111/j.1439-0531.2007.01049.x
18. Thompson IM, Tao S, Branen J, Ealy AD, Dahl GE. Environmental regulation of pregnancy-specific protein B concentrations during late pregnancy in dairy cattle. J Anim Sci. 2013;91:168–73. 10.2527/jas.2012-5730
19. Ward JW, Wooding FB, Fowden AL. The effects of cortisol on the binucleate cell population in the ovine placenta during late gestation. Placenta. 2002;23:451–458. https://doi.org/10.1053/plac.0834
20. Haugejorden G, Waage S, Dahl E, Karlberg K, Beckers JF, Ropstad E. Pregnancy associated glycoproteins (PAG) in postpartum cows, ewes, goats, and their offspring. theriogenology. 2006;66:1976–84. 10.1016/j.theriogenology. 2006.05.016
21. El Amiri B, Sousa NM, Alvarez Oxiley A, Hadarbach D, Beckers JF. Pregnancy-Associated glycoprotein (PAG) concentration in plasma and milk samples for early pregnancy diagnosis in Lacaune dairy sheep. Res Vet Sci. 2015;99:30–36. 10.1016/j.rvsc.2014.12.016
22. El Amiri B, Sulon J, Karen A, Alvarez AV, Cogniè Y, Sousa NM, et al. Measurement of ovine pregnancy-associated glycoprotein (PAG) during early pregnancy in Lacaune sheep. Reprod Domest Anim. 2007;42:257–262. 10.1111/j.1439-0531.2006.00761.x
23. De Carolis M., Barbato O. , Acuti G., Trabalza-Marinucci M., de Sousa N.M., Canali C, et al. Plasmatic Profile of Pregnancy-Associated Glycoprotein (PAG) during Gestation and Postpartum in Sarda and Lacaune Sheep Determined with Two Radioimmunoassay Systems. animals. 2020;10:1502. 10.3390/ani10091502
24. Steckeler P, Weber F, Zerbe H, Rieger A, Voigt K. Evaluation of a bovine visual pregnancy test for the detection of pregnancy-associated glycoproteins in sheep. Reprod Domest Anim. 2019;54:280–288. 10.1111/rda.13356
25. Beriot M, Tchimbou AF, Barbato O, Beckers JF, de Sousa NM. Identification of pregnancy-associated glycoproteins and alpha protein in fallow deer (Dama dama) placenta. Acta Vet. Scandinava. 2014;56(1):4. 10.1186/1751-0147-56-4
26. Barbato O, Menchetti L, Sousa NM, Malfatti A, Brecchia G, Canali C, Beckers JF, Barile VL. Pregnancy-Associated glycoproteins (PAGs) concentrations in water bu aloes (Bubalus bubalis) during gestation and the postpartum period. theriogenology. 2017;97:73–77. 10.1016/j.theriogenology.2017.04.021
27. Karen A, Bajcsy AC, Minoia R, Kovács R, Sousa NM, Beckers JF, et al. Relationship of progesterone, bovine pregnancy-associated glycoprotein-1, and nitric oxide with late embryonic and early fetal mortalities in dairy cows. J Reprod Dev. 2014;60:162–167. 10.1262/jrd.2013-033
28. Piechotta M, Bollwein J, Friedrich M, Heilkenbrinker T, Passavant C, Branen J, et al. Comparison of commercial ELISA blood test s for early pregnancy detection in dairy cows. J Reprod Dev. 2011;57:72–75. https://doi.org/10.1262/jrd.10-022t
29. Breukelman SP, Perényi Z, Taverne MAM, Jonker H, Van der Weijden GC, Vos PLAM, et al. Characterisation of pregnancy losses after embryo transfer by measuring plasma progesterone and bovine pregnancy-associated glycoprotein-1 concentrations. Vet J. 2012;194:71–76. 10.1016/j.tvjl.2012.02.020
30. Engelke J, Knaack H, Linden M, Feldmann M, Gundling N, Gundelach Y, et al. Identification of embryonic/fetal mortality in cows by semiquantitative detection of pregnancy-associated glycoproteins. Livest Sci. 2015;178:363–370. 10.1016/j.livsci.2015.05.030
31. Pohler KG, Pereira MH, Lopes FR, Lawrence JC, Keisler DH, Smith MF, et al. Circulating concentrations of bovine pregnancy-associated glycoproteins and late embryonic mortality in lactating dairy herds. J Dairy Sci. 2016;99(2):1584–1594. 10.3168/jds.2015-10192
32. Younis LS, Hatif SA. AA-NAT melatonin gene polymorphism in ewes lambing out of season. Onl J Vet Res. 2017;21(3):118-125.
33. Ajafar MH, Kadhim AH, AL-Thuwaini TM. The reproductive traits of sheep and their influencing factors. Reviews in Agric Sci. 2022;10:82-89. 10.7831/ras.10.0 82
34. Hatif SA, Younis LS. Nocturnal and diurnal plasma melatonin in season and non-seasonal sheep during spring. Onl J Vet Res. 2018a;22(6):513-517.
35. Hatif SA, Younis LS. Effect of aryl alkyl amine-N-acetyl-transferase gene polymorphism on melatonin in non-seasonal ewes. Onl J Vet Res. 2018b;22(5):356-361.
36. Al-Hamedawi TM, Khammas DJ, Mohammed AH. Effect of using various doses of Bromocriptine in estrus induction and subsequent fertility in lactating anestrus Iraqi Ewes. Iraqi J Vet Med. 2016;40(2):14-19. 10.30539/iraqijvm.v40i2.104
37. Al-Hamedawi TM, Mohammed AH, Al-Yasiri EA, Athab ML. Comparative study between melatonin hormone and vaginal sponges plus eCG on effect of fertility in lactating Iraqi Nuaimy ewes. Plant Archives. 2020; 14(93.3): 4-82.
38. Abas HKEAA, Al-Hamedawi TM, Ajeel HM. Pregnancy Rate in Synchronized Iraqi Awassi Ewes Inseminated Artificially and Naturally. Indian J Forensic Med Toxicol. 2022;16(1):1669-1673.
39. Kadhim Q, Hussain SO. Assessing the Influence of Administration of Kisspeptin-10 on LH Release and Reproductive performance in estrus synchronized ewes. Egypt J Vet Sci. 2024a; 55(2):499-505. 10.21608/EJVS.2023.239749.1632
40. Kadhim QJ, Hussain SO. Determination the serum oestrogen Concentration and Alkaline phosphatase activity during pregnancy and parturition in oestrus synchronized goat. Iraqi J Vet Med. 2014;38(2):95-99. 10.30539/iraqijvm.v38i2.229

41. Hussein KA, Omran SN, AL-Rawi HM. The Use of Ultrasonography to Detect the Response of Local Awassi Ewes to Synchronization of Estrous and Superovulation to Different Intravaginal Progestagen sponge and eCG. Iraqi J Vet Med. 2007;31(1):41-49. 10.30539/iraqijvm.v31i1.802
42. Hameed N, Khan MIUR, Zubair M, Andrabi SMH. Approaches of estrous synchronization in sheep: Developments during the last two decades: A review. Trop Anim Health Prod. 2021;53(5):485. 10.1007/s11250-021-02932-8
43. Abdulkareem TA, Muhammad SJ, Yousif AN. Effect of kispeptin-10 as an alternative to eCG in estrus synchronization protocol on improving the reproductive performance of karadi ewes. Iraqi J Agric Sci. 2021;52(3):535-546. 10.36103/ijas.v52i3.1340
44. Kadhim Q, Hussain SO. The Impact of Administering Kisspeptin-10 Peripherally on FSH Release and Reproductive Performance in Estrus Synchronized Ewes. Indian Vet J. 2024b;101(2):15–22. 10.62757/IVA.2024.101.2.15-22
45. Abood HK. Clinical study of experimentally induced vitamin E and selenium deficiency in Awassi ewes and their newborn lambs: HK Abood, AMH Judi and AA AL-Ani. Iraqi J Vet Med. 2012;36(2):158-162. 10.30539/iraqijvm. v36i2.458
46. Abdulkareem TA, Eidan SM, Al-Maliki LA, Al-Saidy FK, Mahdi MR. Reproductive performance of Iraqi Awassi ewes owned by sheep owners and extension farms in response to flushing and estrus synchronization regimes. Iraqi J Agric Sci. 2014;45:328-334.
47. Hussain SO, Hussain KA, Al-Ani AA. Study on post-partum uterine involution by Ultrasonography and progesterone profile in local goats in Iraq. Iraqi J Vet Med. 2016;40(1):151-156. 10.30539/iraqijvm.v40i1.153
48. Alrawy IY, Hussain SO. Efficacy of Gonadotropin Releasing Hormone on Puberty in Iraqi Awassi Cross Breed Ewe Lambs. Egypt J Vet Sci. 2025;56(1):61-68. 10.21608/EJVS.2024.262822.1782
49. Hussain SO, Badry KA, Zalzala SJ, Zakri AM. Activity of transaminase enzyme and testosterone hormone in blood of Awassi rams during different season. Asian Pac J Reprod. 2017;6(5):217-220. 10.4103/2305-0500.215932
50. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. 2018;Inst. Inc. Cary. N.C. USA.
51. MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; 2016; https://www.medcalc.org)"
52. Al-Rawi MAO, Hussain SO. Impact of CIDR-PMSG and MAP sponges-PMSG treatment of reproductive performance of ewes with assessment of progesterone concentration during treatment and pregnancy. O&G Forum. 2024;34(3):1220-1240.
53. Khan D, Khan H, Ahmad N, Tunio MT, Tahir M, Khan MS, Khan RU. Early pregnancy diagnosis using pregnancy-associated glycoproteins in the serum of pregnant ruminants. Pakistan J Zool. 2020;52(2):785-788. 10.17582/journal.pjz/20190527080541
54. Silva E, Sterry RA, Kolb D, Mathialagan N, McGrath MF, Ballam JM. Accuracy of a Pregnancy-Associated Glycoprotein ELISA to Determine Pregnancy Status of Lactating Dairy Cows Twenty-Seven Days After Timed Artificial Insemination. J Dairy Sci. 2007;90:4612-4622. 10.3168/jds.2007-0276
55. García-Ispierto I, Almería S, Serrano B, de Sousa NM, Beckers JF, López Gatius F. Plasma Concentrations of Pregnancy-Associated Glycoproteins Measured Using Anti-Bovine PAG-2 Antibodies on Day 120 of Gestation Predict Abortion in Dairy Cows Naturally Infected with Neospora caninum. Reprod Dom Anim. 2013;48(4):613-618. 10.1111/rda.12134
56. Wallace JM, Da Silva P, Aitken RP, Cruickshank MA. Maternal endocrine status in relation to pregnancy outcome in rapidly growing adolescent sheep. J Endocrinol. 1997;155(2):359-368. 10.1677/joe.0.1550359
57. Ranilla MJ, Sulon J, Carro MD, Mantecon AR, Beckers JF. Plasmatic profiles of pregnancy–associated glycoprotein and progesterone levels during gestation in Churra and Merino sheep. theriogenology. 1994;42(3):537–545. 10.1016/0093-691x(94)90691-b
58. Lawson BC, Shahzad AH, Dolecheck KA, Martel EL, Velek KA, Ray DL, et al. A pregnancy detection assay using milk samples: evaluation and considerations. J Dairy Sci. 2014;97(10):6316–6325. 10.3168/jds.2014-8233
59. López-Gatius F, Garbayo JM, Santolaria P, Yániz J, Ayad A, de Sousa NM, et al. Milk production correlates negatively with plasma levels of pregnancyassociated glycoprotein (PAG) during the early fetal period in high producing dairy cows with live fetuses. Domest Anim Endocrinol. 2007;32(1):29–42. 10.1016/j.domaniend.2005.12.007
60. Green JA, Xie S, Quan X, Bao B, Gan X, Mathialagan N, et al. Pregnancyassociated glycoproteins exhibit spatially and temporally distinct expression patterns during pregnancy. Biol Reprod. 2000;62(6):1624–1631. 10.1095/biolreprod62.6.1624
61. Kaulfuß KH, Schram D, Berttram M. Effect of genotype, age of dams, litter size, birthweight, and rams on morphological parameters of the placenta in sheep. Dtsch Tierarztl Wochenschr. 2000;107(7):269–275.
62. Ranilla MJ, Sulon J, Mantecon AR, Beckers JF, Carro MF. Plasma pregnancy-associated glycoprotein and progesterone concentrations in pregnant Assaf ewes carrying single and twin lambs. Small Rumin Res. 1997;24:125–131. 10.1016/s0921-4488(96)00922-4
63. Singh SP, Ramachandrana N, Sharmaa N, Goela AK, Gururajb K, Kharchea SD. Temporal changes in plasma profile of pregnancy-associated glycoprotein, progesterone and estrone sulfate associated with fetal number during early- and mid-pregnancy in goats. Anim Reprod Sci. 2019;205:115–125.
64. Serrano-Pérez B, Garcia-Ispierto I, Sousa NM, Beckers JF, Almeida S, López-Gatius F. Gamma interferon production and plasma concentrations of pregnancy-associated glycoproteins 1 and 2 in gestating dairy cows naturally infected with Neospora caninum. Reprod Dom Anim. 2014;49(2):275–280. 10.1111/rda.12267
65. Ledezma-Torres RA, Beckers JF, Holtz W. Assessment of plasma profile of pregnancy-associated glycoprotein (PAG) in sheep with a heterologous (anticaPAG55+ 59) RIA and its potential for diagnosing pregnancy. Theriogenology. 2006;66(4):906–912. 10.1016/j.theriogenology. 2006.02.031
66. Vandaele L, Verberckmoes S, El Amiri B, Sulon J, Duchateau L, Van Soom A, et al. Use of homolougous radioimmunoassay (RIA) to evaluate the effect of maternal and foetal parameters on pregnancy-associated glycoprotein (PAG) concentrations in sheep. Theriogenology. 2005;63(7):1914-1924. 10.1016/j.theriogenology.2004.08.009
67. Godfrey RW, Collins JR, Hensley EL, et al. Estrus synchronization and artificial insemination of hair sheep ewes in the tropics. Theriogenology 1999;51(5):985–97. 10.1016/s0093-691x(99)00044-8
68. Letelier CA, Contreras-Solis I, Garcı´a-Ferna´ndez RA, et al. Ovarian follicular dynamics and plasma steroid concentrations are not significantly different in ewes given intravaginal sponges containing either 20 or 40 mg of fluorogestone acetate. Theriogenology. 2009;71(4):676–82. 10.1016/j.theriogenology.2008.09.030
69. Kalyan De, Kumar D, Balaganur K, Gulyani R, Naqvi SMK. Effect of breeding season on fertility of sheep following estrus synchronization and fixed-time artificial insemination under field conditions in semi-arid tropical region, Biol. Rhythm Res. 2016. 10.1080/09291016.2016.1197497
70. Naderipour H, Yadi J, Shad A, Sirjani MA. The effects of three methods of synchronization on estrus induction and hormonal profile in Kalkuhi ewes: a comparison study. Afr J Biotechnol. 2012;11(2):530-533. 10.5897/AJB11.2718
71. Ozyurtlu N, Kucukaslan I, Cetin Y. Characterization of oestrous induction response, oestrous duration, fecundity, and fertility in Awassi ewes during the non-breeding season utilizing both CIDR and intravaginal sponge treatments. Reprod Dom Anim. 2010;45(3):464-467. 10.1111/j.1439-0531.2008.01246.x
72. Yu XJ, Wang J, Bai YY. Estrous synchronization in ewes: The use of progestogens and prostaglandins, Acta Agriculturae Scandinavica, Section A—Anim Sci. 2019. 10.1080/09064702.2019.1674373

مستوى البروتين السكري المرتبط بالحمل في الكشف المبكر عن الحمل مع التنبؤ بعدد وجنس الجنين في النعاج المحلية العراقية

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الخلاصة

يعد الكشف المبكر عن الحمل اداة مهمة في ادارة الانتاج الناجحة بالنعاج. كان الهدف من هذه الدراسة هو تقدير تركيز البروتين السكري المرتبط بالحمل (PAG) في مصل الدم خلال الحمل باستخدام طبق ELISA الخاص بالأغنام من اجل التمييز المبكر بين النعاج الحامل وغير الحامل وكذلك امكانية الارتباط مع عدد الاجنة وجنس الجنين. تمت مزامنة الشبق في نعاج الدراسة وتم جمع عينات الدم في الايام صفر (يوم الجماع) و١٨،١٢٠،٦٠ بعد الجماع من ١٨ نعجة عراقية من سلالات مهجنة. تم تحليل عينات المصل للبروتين السكري المرتبط بالحمل باستخدام طبق ELISA التجاري الخاص بالأغنام. اثبتت نتيجة مستوى البروتين السكري المرتبط بالحمل (نانوغرام/مل) اثناء الشبق عدم وجود فرق معنوي بين المجاميع الثلاثة بينما في اليوم ١٨،٣٠،٦٠و١٢٠ من الحمل كانت هناك زيادة معنوية ($P<0.05$) في المجموعة الاولى والثانية مقارنة بالمجموعة الثالثة. كانت قيمة التمييز للكشف عن الحمل بواسطة فحص PAG أكبر من ٩١٥و٠ نانوغرام/ مل بناءً على بيانات الولادة. من يوم ١٨ تم الكشف عن قيمة PAG في جميع النعاج الحوامل. كما اثبت ان القيم المتوسطة كانت بزيادة معنوية ($P<0.05$) في الحمل التوأم مقارنة بالحمل المفرد وزيادة معنوية ($P<0.05$) في الحمل المفرد الذكر مقارنة بالحمل المفرد الانثى في النعاج العراقية المهجنة حسب البيانات المجمعة من اليوم صفر حتى نهاية الحمل وتم متابعة الحمل حتى الولادة. مما تقدم يمكن الاستنتاج انه نستطيع الكشف عن الحمل بالاغنام بشكل موثوق ونستطيع تحديد عدد الاجنة وجنس الجنين من اليوم ١٨ بعد الجماع فصاعدا بواسطة استخدام طبق PAG-ELISA الخاص بالحمل.

الكلمات المفاحية: أغنام، كشف الحمل المبكر، البروتين السكري المرتبط بالحمل، عدد الأجنة، جنس الجنين