



## **Assessment of blood and urine pregnancy associated glycoprotein 1 in pregnant and aborted Osseimi ewes**

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### **Abstract**

The early pregnancy diagnosis in ewes has economic importance for the sheep industry. The study aimed to determine the ovine pregnancy-associated glycoproteins (PAGs) profile in the serum and urine of Osseimi ewes from mating day till Day 90 of gestation at specific time periods, and to study the correlation between the maternal PAG levels in the serum of pregnant, non-pregnant and aborted ewes, and the number of feti. The results reported that the serum and urinary PAGs were significantly detected at Day 16 of gestation and increased gradually till reached to peak at Day 60 then decline at Day 90 of gestation. There was a positive correlation between the PAGS level and the number of feti and between PAGS and progesterone levels at Days 60- 90 of gestation. The concentration of PAGs in serum was declined at Day 45 of gestation and reached the basal line at Day 90. The concentration of PAGs was also higher in multiple pregnant than single pregnant ewes. In conclusion, the PAGs can be detected in the serum and urine at Day 16 of gestation in ewes and it may be used as a useful tool for the detection of both pregnancy in the early stage and the number of feti in pregnant ewes.

**Keywords:** pregnancy-associated glycoprotein, early pregnancy, number of feti, progesterone level

### **1. Introduction**

The early pregnancy diagnosis in ewes is a very important tool for sheep breeding, management, and nourishment. (Karen et al., 2003). Although, there are various methods for diagnosis of early pregnancy in sheep only a few of them are effective (Karen et al., 2001). The serological diagnosis of early pregnancy in ewes is considered a useful tool for diagnosis because it is more simple, sensitive, rapid, and accurate (Karen et al., 2001).

Recently many studies are interested in the detection of pregnancy markers, among them pregnancy-associated glycoproteins (PAGs), and pregnancy-specific protein B (PSPB) that belong to the aspartic proteinase family, which are released by trophoblastic binucleate cells during pregnancy (Xie et al., 1991). When the trophoblastic binucleate cells begin to migrate and fuse to the endometrial cells, producing the fetomaternal syncytium, they are visible in the maternal blood around the time of definitive attachment of the fetal placenta (Wooding, 1984). Thus, PAGs are excellent predictors of both pregnancy and fetal-placental health (Garbayo et al., 2008). There are more than 20 types of PAGs have already been identified (Telugu et al., 2009), and their

molecular weights might vary between 55 and 70 kDa (Klisch and Leiser, 2003).

The bovine (boPAG) and ovine pregnancy-associated glycoproteins (ovPAG) have a similarity in molecular structures (Xie et al., 1991). The ovPAG concentration fluctuates throughout the pregnancy period depending on the ewes breed, sex, and the number of fetuses (Gajewski et al., 1999, Ranilla et al., 1997). The PAGs detection in the blood is a useful method for pregnancy detection in sheep (Karen et al., 2003), due to PAGs can pass through the placental barrier and into the maternal circulation, and the fetal blood (Haugejorden et al., 2006). The maternal blood level of PAGs begins to decrease at the time of parturition and continued to decrease until 10 weeks post-partum (Roberts et al., 2017), however, it is still detectable for more than 63 days postpartum (Steckeler et al., 2019). It is also used for the detection of viable concepts (Zarrouk et al., 1999). Many studies reported that the PAGs detection accuracy for pregnancy is 93.5 % on day 22, but 100 % on day 29 of pregnancy (Karen et al., 2003), according to another study ovPAGs can be found around 28 to 42 days of pregnancy (El Amiri et al.,

2015). Through the second or third weeks of pregnancy, ovPAGs can be found in maternal blood (Willard et al., 1995).

The progesterone hormone is an important hormone for pregnancy diagnosis, moreover, the blood progesterone level can categorize litter size in sheep ranging from 63-79% at 90-108 days of gestation in ewes (Schneider and Hallford, 1996). Many studies revealed that progesterone and PAG levels were higher in multiple pregnancies than in single pregnancies in general (Barbato et al., 2009), and there is a high positive association between progesterone and PAGs (Roberts et al., 2017).

Up to our best knowledge, there is no study focusing on the detection of ovPAG in the urine of ewes during different stages of pregnancy so, The aims of the current study are to determine the PAGs level in serum and urine during early pregnancy (from day 0 to 90) focusing on specific time periods, evaluation if urine is a good alternative to serum for early pregnancy diagnosis in ewes, Investigate the link between maternal concentrations of ovine PAG1 and progesterone throughout pregnancy in ewes, and compare the effectiveness of this visual test to the gold standard of ultrasonography.

## 2. Materials and methods

### 2.1. Animals

The current study was carried out in Riwina Animal Production Station which is located in Kafrelsheikh Governorate (Northern Egypt, 31 degrees north latitude), Egypt during the autumn breeding season. A total of 90 Osseimi Egyptian ewes (3-5 years old age), and weighted (45-55 kg), with 1-3 parties, and a body condition score of 2-3). The ewes were kept in semi-intensive conditions, housed in an open yard, and fed on CFM and roughages following the National Research Council (NRC., 2007) guidelines (Freer, 2007). Furthermore, the ewes were fed a daily meal of 1 kg CFM containing 14 percent crude protein, 1 kg alfalfa hay, and fresh water was available ad libitum.

### 2.2. The animal groups and experimental design

The animals were divided into two groups. The 1st group for the time-mated pregnancy approach was used for breeding 80 ewes: for estrus detection, three vasectomized rams were placed with a mounting harness on their chests, and estrus was recognized by the presence of paint on the female's dorsum after mounting. Following the identification of estrus, all of the marked ewes were mounted by fertile rams. Blood and urine samples were taken from each ewe on days 0, 10, 16, 18, 20, 25, 30, 45, 60, and 90 following mating. Day 0 was defined as the day of mating. In the 2nd group; 10 ewes of identical genetic stock, age, and parity remained unmated to act as controls. Since they had been separated from rams for at least five months, PAG1 concentrations in these ewes were measured at the same time intervals of the first group and were utilized as non-pregnancy controls and to test the assay's specificity (Vandaele et al., 2005).

### 2.3. Blood and urine samples

The blood and urine samples were collected in terms of the ethics, of farm conditions in accordance with a good veterinary practice. The Blood samples (5 ml/ewe) were taken aseptically through jugular vein puncture and placed in anticoagulant-free vacutainer tubes in a cold box until centrifugation. After centrifugation at 2000 rpm for 20 minutes, the serum was collected and kept at -80 °C until Sheep PAG1 concentration was evaluated. The urine samples were collected in the morning using a sterile container and then centrifuged at 2000 rpm for 20 minutes, and the supernatant was stored at -20 °C until Sheep PAG1 concentration was evaluated.

### 2.4. Ultrasonographic assessment

The number of fetuses and fetal size were determined in all pregnant ewes at gestation days (GD) 30, 60, and 90 by using transabdominal and transrectal ultrasonography (Sonoscape M12 Ultrasound, Guangdong, China). The ewes were held in a standing position, the flank area was clipped, and ultrasonic gel optimized transducer contact. Once a fetus had been discovered, the biparietal diameter was determined by measuring the cross-section of the head, and crown-rump length as illustrated in Fig.1 also the fetal measurements were digitized and saved as digital photographs (Roberts et al., 2017).

### 2.5. Progesterone Assay

Progesterone concentrations were assessed by Enzyme-Linked Fluorescent Assay (ELFA) (≠30409. VIDAS® Progesterone, biomérieux SA) and followed up the manual procedure of kits.

### 2.6. Pregnancy-Associated Glycoprotein Assays

The serum and urine PAGs were measured by using Sandwich ELISA Kit (Catalog No: SG-70126; SinoGeneClon Biotech Co., Ltd, China) following the manual procedures of commercial kits. and measure the absorbance at 450nm, for the quantitative determination of Sheep PAG1 concentrations.

### 2.7. Statistical analysis

A T-test was used to calculate the differences among the experimental group, followed by a Mann-Whitney test (GraphPad Software Inc., San Diego, CA, USA). The data are presented as a mean ±. Statistical significance was defined as a value of  $P \leq 0.05$ .

## 3. Results

Of 80 Osseimi ewes, 52 ewes became pregnant as shown by the RIA analysis and ultrasonography, four were aborted. They gave PAG1 concentrations below the cut-off value (serum =9.00ng/ml & urine =0.00ng/ml). Among the pregnant ewes, 39 had single pregnancies while 9 carried multiple ones.

### 3.1. Progesterone concentration

The progesterone concentration was measured in the blood of single and multiple pregnant ewes by ELISA. The result showed that the progesterone level in single and numerous pregnant ewes on a mating day were (0.23±0.01, 0.4±0.01ng/ml) respectively. The progesterone concentration increased with the

time of pregnancy in which our result revealed that the progesterone level in single and multiple pregnant ewes at GD10 was  $(2.9\pm 0.01$  and  $3.07\pm 0.01$  ng/ml) respectively, and at GD 90 was  $(8.35\pm 0.22$  and  $9.52\pm 0.175$  ng/ml) respectively. The result shows that progesterone concentration was increased with the number of fetuses (Fig. 2).

### 3.2. Serum PAGs in pregnant and aborted ewes

The serum PAG1 levels were estimated in pregnant and aborted ewes from mating day until GD 90. The results showed that serum PAG1 levels gradually increased up to GD 60 ( $74.55\pm 0.35$  ng/ml) in the pregnant ewes, then decreased at GD 90 ( $54.28\pm 0.414$ ). At the same time, the serum PAG1 levels in aborted ewe declined to reach  $(8.20\pm 0.27$  ng/ml) at GD 60 and reached the basal line on GD 90 ( $3.55\pm 0.32$  ng/ml) as shown in Fig. 3.

### 3.3. Serum PAG1 levels in pregnant and non-pregnant ewes

The current results show that serum PAG1 levels in non-pregnant ewes have nearly the same level from GD 0 until GD 90 ( $4.35\pm 0.28$  and  $3.58\pm 0.22$  ng/ml) respectively. In comparison, the serum PAG1 levels in single pregnant and multiple pregnant ewes reached to peak at GD 60 ( $74.55\pm 0.35$  and  $80.84\pm 0.46$ ) respectively. The result indicated that the

serum PAGs in numerous pregnancies are higher than the serum PAGs in single pregnant on the all-time point of the experiment. So the level of PAGs is shown in Fig. 4.

### 3.4. Urine PAG1 levels in single pregnant, multiple pregnant and non-pregnant ewes

PAG1 levels in the urine of pregnant ewes (single and multiple) were measured on day 10 ( $6.76\pm 0.23$  and  $7.38\pm 0.28$  ng/ml) respectively, then reached the peak levels at day 60 ( $55.15\pm 0.32$  and  $70.73\pm 0.36$  ng/ml) respectively as shown in Fig. 5. It was reported that urine PAG1 levels in multiple pregnancies were higher than those of single pregnant ewes, While PAG1 levels in non-pregnant ewes were undetectable.

### 3.5. PAG1 and progesterone level in Aborted ewes

The results show that the PAG1 level was decreased after abortion at GD 60 and continue to decline at GD 90 ( $8.204\pm 0.270$ , and  $3.552\pm 0.231$  ng/ml) respectively Fig. 6. While the progesterone level not affect by abortion as shown in Fig. 7. This result indicated the PAG1 is good marker for pregnancy.

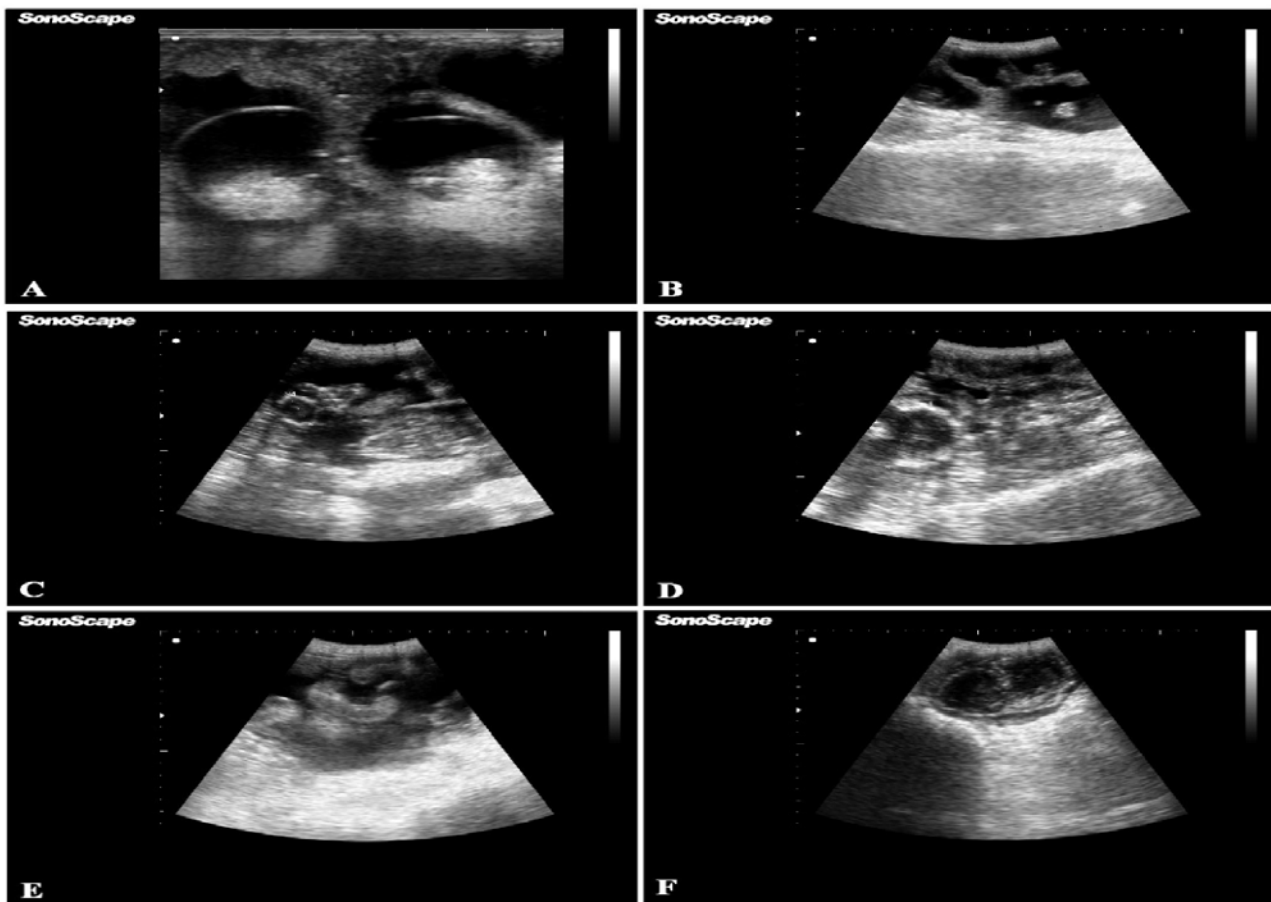


Figure 1. Trans-rectal ultrasound image of one month embryo (A), trans-abdominal of uterus containing 2 embryos (B), triplet (C), trans-abdominal ultrasound of 90 days old embryo (D), trans-abdominal ultrasound of c-shape placentom of 90 days old embryo (E) and trans-abdominal ultrasound of intrauterine fetal death (F).

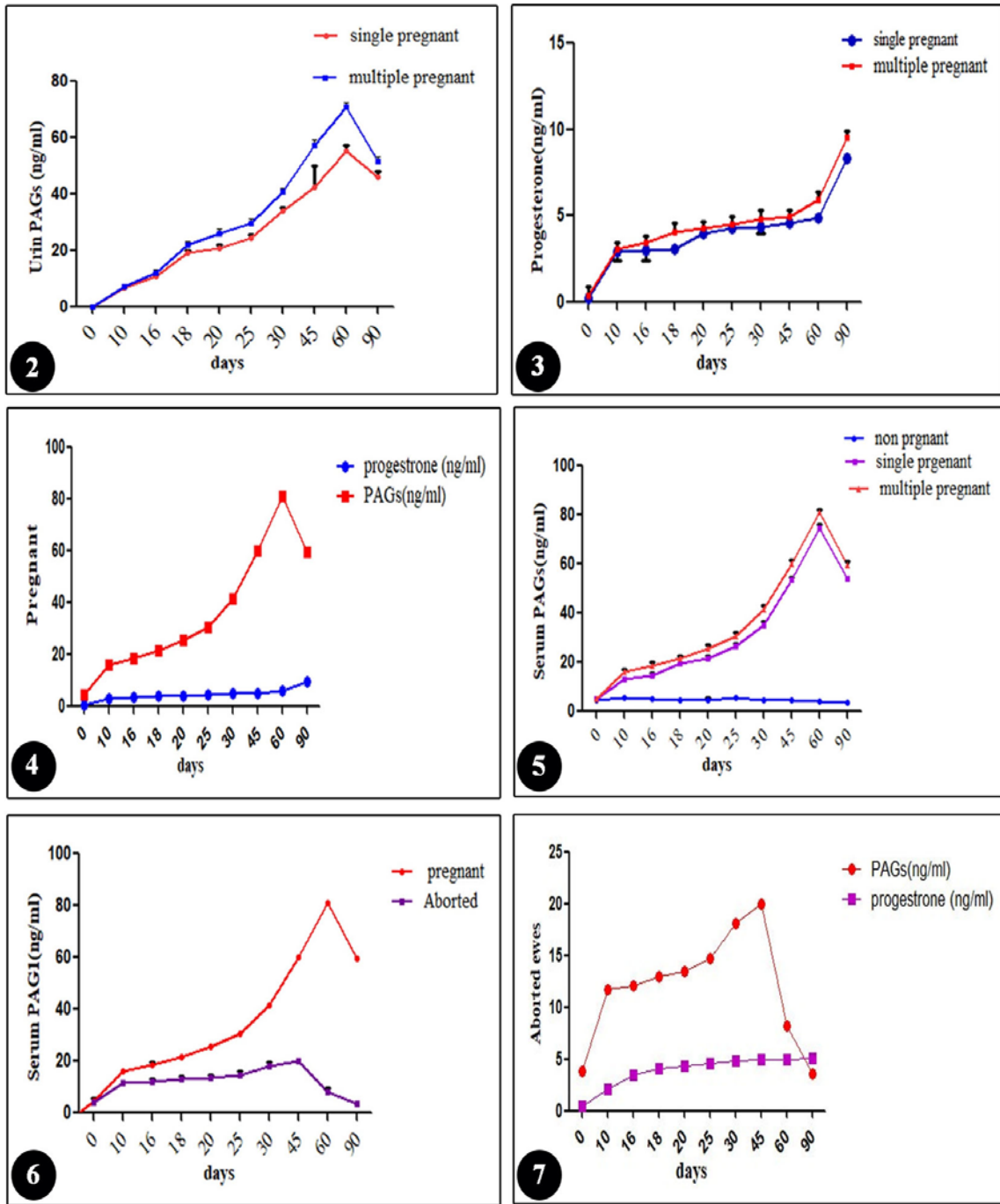


Figure 2: concentration of ovine pregnancy-associated glycoprotein (PAGs) in the urine of ewes carrying singles and multiples embryos on mating day to GD 90 of gestation

Figure 3: progesterone concentrations in ewes carrying singles and multiples embryos on mating day to 90 days of gestation

Figure 4: serum ovine pregnancy-associated glycoprotein (PAGs) concentrations (■), progesterone concentrations (●) in ewes carrying singles and multiples embryos on mating day to 90 days

Figure 5: serum ovine pregnancy-associated glycoprotein (PAGs) concentrations in nonpregnant (●), in single pregnant (■), and multiple pregnant (▲) ewes at mating day to 90 days

Figure 6: serum ovine pregnancy-associated glycoprotein (PAGs) concentrations in pregnant (●), and aborted ewes (■) at mating day to 90 days

Figure 7: serum ovine pregnancy-associated glycoprotein (PAGs) concentrations in aborted ewes (●), and progesterone concentration (■) in aborted ewes at mating day to 90 days.



#### 4. Discussion

The PAGs detection by the RIA systems is a reliable method for early pregnancy diagnosis better than progesterone determination and even ultrasound in sheep (Karen et al., 2003). The current study is the first to investigate the PAGs profile in the urine, to investigate the PAGs profile in the blood during the gestation period in Osseimi ewes beginning from GD 0 till GD 90. Our finding revealed that the PAGs in serum can be significantly detected at GD 16 of pregnancy and can differentiate between pregnant and pregnant ones in Osseimi ewes, and this disagrees with other studies that reported the PAGs can be detected by RIA in the plasma and can differentiate between pregnant and non-pregnant ewes at day 18 of pregnancy in Sarda and Lacaune Sheep (De Carolis et al., 2020). Another study documented that PAGs in the blood were significantly detected at 20 days of gestation period in Awassi, Merino ewes (Karen et al., 2003), and another study reported that PAGs could differentiate the pregnant and non-pregnant ewes at 24 days of gestation period in pluriparous Sarda ewes (Barbato et al., 2009). According to our research and previous studies, there are alterations between the accurate days of detection of PAGs in the blood, this may due to difference in the breed and age of ewes or due to difference in the pattern of ovine PAG concentrations that differs from species to another (Barbato et al., 2018). This may be due to the ability of the antisera to distinguish between various epitopes (Ranilla et al., 1997). The PAGs profile in our study increased at 60 days, and declined at 90 days in the blood. This is a similar trend to that described by Ledezma-Torres et al (Ledezma-Torres et al., 2006) for various sheep breeds, and by Ranilla et al., (1997) for Churra sheep.

Our result revealed an appositive relation between the PAGs concentration in the blood and the number of Feti. This agrees with another study that showed that PAG concentration was higher in multiple pregnancies than in single pregnancies, at deliveries in Sarda ewes (De Carolis et al., 2020). The increased concentrations in multiple deliveries compared to single delivery may be because the twin placentas' have a higher amount of attachment sites, and hence secretory activity (Ranilla et al., 1997). Furthermore, another study reported that the PAGs concentration is higher in twins than in single ones (Ledezma-Torres et al., 2006).

Our study reported that the PAGs concentration has more accuracy than progesterone concentration in pregnancy diagnosis in the early diagnosis because it can differentiate between pregnancy and prolonged inter-estrus intervals (Karen et al., 2003). The other study reported that PAGs could be found in other body fluid as milk (El Amiri et al., 2015). This encourage us to investigate PAGs in urine and our result reported that PAGs were significantly detected in urine in the early stage of pregnancy.

#### 5. Conclusion

In conclusion, the PAGs have more accuracy at 16 days of gestation in Osseimi ewes in blood for early pregnancy diagnosis. Furthermore, urine is an excellent alternative to plasma for pregnancy diagnosis in sheep at 16 days of gestation. In addition, the PAGs concentration is an indicator of the number of feti.

#### Acknowledgments

We gratefully acknowledge Dr. Mohey Ashour, Dr. Ibrahim Mansour, Dr. Tawfik Imam and Staff members of Riwina Animal Production Station in Kafr El-Sheikh Governorate

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