



## Temporal changes in circulating progesterone and pregnancy-associated glycoprotein concentrations in Jakhrana goats with failed pregnancy

N SHARMA, S P SINGH\* and A BHARADWAJ<sup>1</sup>

ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura, Uttar Pradesh 281 122 India

Received: 15 August 2019; Accepted: 11 October 2019

### ABSTRACT

This study aimed to evaluate the changes in circulating Progesterone (P4) and Pregnancy-Associated Glycoprotein (PAG) during pregnancy interrupted by abortion and to identify the better predictor of abortion risk in Jakhrana goats. Pluriparous goats (18) were involved in the study. Out of 15 pregnant goats, 3 goats were aborted during the third or fourth months of pregnancy. In the normal pregnancy, mean P4 concentration ( $13.96 \pm 0.27$  ng/mL) remained unchanged throughout gestation whereas, the PAG level increased during early pregnancy and reached to the highest level ( $S-N=2.14 \pm 0.40$ ) on day 51 of gestation. In non-pregnant goats, the P4 ( $0.3 \pm 0.03$  ng/mL) and PAG ( $0.06 \pm 0.03$ ) were significantly lower compared to the goats with maintained or failed pregnancy. In goats with the failed pregnancy, the PAG level started to decline about 12 days before the drop in P4 concentration (day  $33.0 \pm 2.1$  vs  $21.3 \pm 3.5$ , before the occurrence of abortion). The mean PAG level in goats with failed pregnancy ( $0.33 \pm 0.03$ ) was 4.8-folds lower compared to the animals with maintained pregnancy ( $1.58 \pm 0.08$ ). In conclusion, the P4 and PAG concentrations in maternal circulation are changed by the pregnancy status and abortion. Plasma PAG is a more reliable predictor for the high risk of pregnancy failure than the P4 concentration in goats.

**Keywords:** Abortion, Goats, Pregnancy-associated glycoprotein, Progesterone

Pregnancy failure or abortion significantly influences the reproductive efficiency of goats (Menziez 2011). Diverse unfavourable ecological conditions can make an interruption for pregnancy expressed by fetal demise and abortion. According to the previous studies, goats appear to be more vulnerable to abortion than ewe (Goossens *et al.* 1997 and Osaer *et al.* 1999) and cow (Jourdain *et al.* 2005). It is suggested that non-infectious causes such as nutritional and environmental factors may be important for the abortion in goat and causes a substantial loss to the goat keepers (Waldeland *et al.* 1991). Thus a suitable biomarker for identifying animals with a high risk of abortion may provide a practical tool for better reproductive management of goat herds.

In ruminants, placenta works as an endocrine and paracrine organ that releases a wide range of biomolecules into maternal circulation during pregnancy. These pregnancy-associated glycoproteins (PAG) have a place with a vast group of latent aspartic proteinase family expressed by the placenta of ruminants including goats and sheep (Haugejorden *et al.* 2006). The PAG are considered as an important biomarker for pregnancy since placental cells are the exclusive source in farm animals including goats (Garbayo *et al.* 1998; Robert *et al.* 2017).

Progesterone (P4) plays a pivotal role in the implantation, embryonic development and maintenance of pregnancy in

mammals. The principle site of P4 secretion in goat is the ovary through both estrous cycle and pregnancy (Charallah *et al.* 2010). Thus, the information related to the changes in the concentration of pregnancy hormones such as P4 and PAG in the maternal bloodstream might provide a valuable tool for monitoring of pregnancy status and identification of animals with a high risk of pregnancy failure. However, limited information is available on the relationship of dynamic changes in the circulating concentrations of PAG and P4 with the pregnancy loss in goats.

Therefore, the objective of this study was to evaluate the temporal dynamic changes in circulating concentrations of P4 and PAG during the maintained or interrupted pregnancy in goats and identify the relative importance of plasma P4 and PAG as a biomarker of pregnancy failure in goats.

### MATERIALS AND METHODS

*Experimental animals and their management:* Total eighteen healthy and clinical disease free pluriparous (3<sup>rd</sup> to 5<sup>th</sup> parity) Jakhrana goats were selected and kept at the institutional flock of ICAR-Central Institute for Research on Goats, Makhdoom, Mathura (UP) with the semiarid climate. During the breeding season (from May to June), the estrus activities were observed twice a day (morning and evening) and goats were bred 10 to 12 h after the onset of estrus by superior breeding bucks.

<sup>1</sup>Department of Biotechnology, GLA University, Mathura.  
\*Corresponding author e-mail: shiva.singh@icar.gov.in

**Blood sampling and storage:** Blood samples (~ 6 mL) were collected from the jugular vein at various time points from day 10 post-mating until kidding or 1 to 2 weeks after abortion. After centrifugation at  $2,500 \times g$  for 10 min at 4°C, plasma was harvested and stored at -20°C until assayed for P4 and PAG using ELISA. Among 18 animals, three animals sustained an abortion because of the undefined and non-infectious causes.

To ascertain the pregnancy status, ultrasonography was performed in all the animals at day 40 after mating by using an ultrasonic device (Just Vision 200-Model SSA-320 A, Diagnostic Ultrasonography System, Toshiba, Japan) equipped with a real-time convex array transrectal transducer (PVF-738 F) of variable frequency (5–7 MHz).

**P4 assay:** Plasma P4 concentration was measured using ELISA kit following the manufacturer's instructions (DRG Diagnostic, Germany). Briefly, 25 µL of standards, controls, and samples were dispensed into the antibody-coated microtiter plate. After 5 min incubation at room temperature (RT), and addition of 200 µL of the enzyme conjugate, microtiter plate was incubated for 1 h at 25°C. After 3 washings (plate washer, Hydroflex, Tecan, Austria), the substrate solution (200 µL) was added and then incubated for 15 min. at RT. Thereafter, stop solution (50 µL) was added and the absorbance was recorded by a microplate reader (Sun Rise, Tecan, Austria). The intra and inter-assay coefficients of variations (CVs) ranged between 5.40 to 6.99 and 4.34 to 9.96%, respectively. The analytical sensitivity and measuring range of the assay were 0.045 ng/mL and 0 to 40 ng/mL, respectively.

**PAG assay:** The level of PAG in plasma samples was determined by sandwich ELISA as recommended by the manufacturer (IDEXX Laboratories, Westbrook, ME, USA). Briefly, 100 µL of samples and controls along with 25 µL sample diluent were added into the 96-well plate and incubated for 60 min at 37°C. After 4 washings and the addition of detector solution (100 µL), plates were incubated at RT for 30 min. Thereafter, plates were washed (4×), before the addition of 100 µL of the conjugate solution and incubated for 30 min at RT. After addition of substrate solution (100 µL) and incubation for 15 min at RT, the reaction was stopped and absorbance was recorded. The

intra- and inter-plate CVs for positive controls were 5.7% and 10.5%, respectively.

Results were calculated for each sample by subtraction of corrected mean sample absorbance with corrected mean absorbance of negative controls (n=4) (corrected absorbance = absorbance at 450 nm – absorbance at 630 nm) and expressed as a sample-negative (S–N). Pregnancy outcomes were determined based on the cut-off value (S–N = 0.30), as provided by the manufacturer. For the values (S–N)  $\geq 0.3$ , samples were classified as positive (pregnant), and those  $< 0.3$  were classified as negative (non-pregnant).

**Statistical analyses:** The relationship among plasma P4 and PAG concentrations, and day of gestation were determined by Pearson's correlation coefficient. A stepwise multiple linear regression analysis was conducted for estimation of the predictive value of plasma P4 and PAG to determine the pregnancy status or abortion in goats. Comparison of means among different groups of pregnancy status was analysed by Kruskal-Wallis test and the differences were evaluated by the  $P < 0.05$  (SPSS 20.0).

## RESULTS AND DISCUSSION

As revealed by the ultrasonographic examination conducted at day 40 of gestation, from a total of 18 goats, 15 were pregnant and three were non-pregnant. Subsequently, 3 animals aborted at day 70, day 79 and day 120 of pregnancy. The characteristics of plasma profiles of P4 and PAG in goats with failed pregnancy are presented in Table 1. The mean concentrations of P4 (ng/mL) and PAG (S–N) in pregnant goats ( $13.93 \pm 0.29$  and  $1.58 \pm 0.08$ ) was significantly ( $P < 0.001$ ) higher than in the non-pregnant goats ( $0.30 \pm 0.03$  and  $0.06 \pm 0.03$ ). The significantly higher level of P4 in animal with maintained pregnancy throughout gestation compared to the non-pregnant goats is in accordance with the earlier reports suggesting constantly higher P4 concentration in peripheral blood plasma from day 10 to 140 of pregnancy in goats (Sawada *et al.* 1994, Singh *et al.* 2019)

The highest plasma P4 and PAG in the animals with failed pregnancy was observed about 3 and 5 weeks prior to the abortion, respectively. Thereafter, a gradual decline in the concentration of both P4 and PAG was observed until

Table 1. Characteristics of plasma progesterone (P4) and pregnancy-associated glycoprotein (PAG) profiles in goats with the failed pregnancy

Animal No.	Day of abortion*	Highest concentration of P4 (ng/mL) and day of pregnancy	Day of decline in P4 concentration (before abortion)**	Highest level of PAG (S–N) and day of pregnancy	Day of decline in PAG concentration (before abortion)**	Rate of decline	
						P4 (ng/mL/day)	PAG (S–N/day)
JK 1450	70	20.81 (d58)	22	0.80 (d44)	36	0.95	0.02
JK 1125	120	33.37 (d93)	27	0.63 (d86)	34	1.24	0.01
JK 1352	79	21.63 (d50)	15	0.85 (d44)	29	1.44	0.02
Mean $\pm$ SEM	90.6 $\pm$ 14.8	25.27 $\pm$ 4.06	21.3 $\pm$ 3.5	0.76 $\pm$ 0.06	33.0 $\pm$ 2.1	1.21 $\pm$ 0.14	0.02 $\pm$ 0.00

\*Day of abortion with respect to the day of pregnancy; \*\*First day of decline, the first day at which decline in P4 and PAG concentration was observed before abortion.

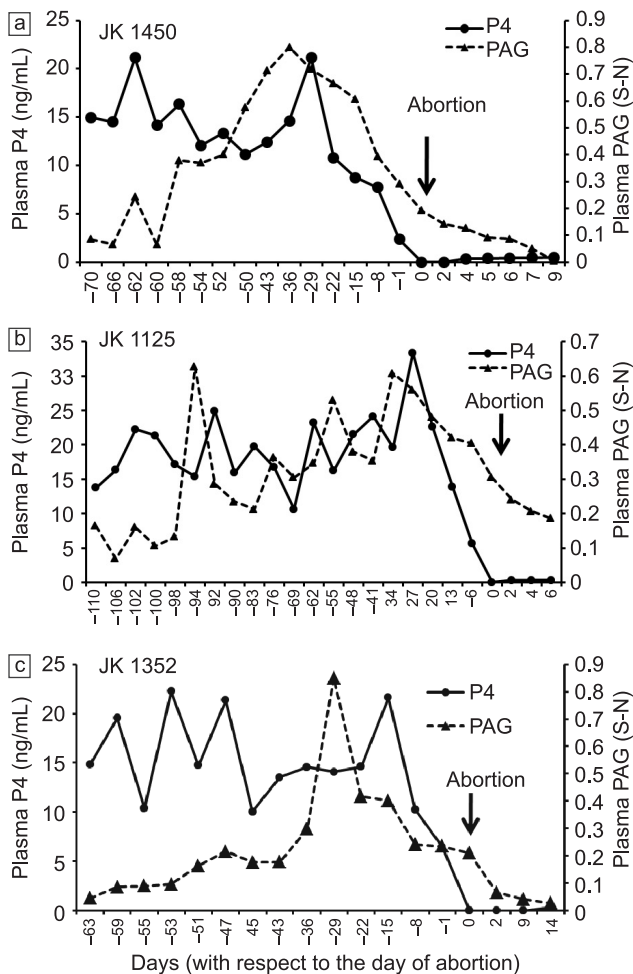


Fig. 1. Circulating profiles of progesterone (P4) and pregnancy-associated glycoprotein (PAG) in goats (1a: JK 1450, 1b: JK 1125, 1c: JK1352) with pregnancy interrupted by the abortion.

the day of abortion (Fig.1). Overall, the decline in the PAG concentration starts at about 12 days prior to the decline in P4 concentration (21.3±3.5 vs 33.0±2.1). This indicates that the placental functions are impaired well before the changes in ovarian functions in goats which will soon abort. Thus, PAG could be a more appropriate biomarker for early identification of animals with the problem of maintaining the pregnancy. Although, PAG started decreasing 12 days earlier than P4 concentration, the rate of decline, as mentioned in the Table 1, was 0.02±0.00/day for PAG and 1.21±0.14/day for P4 which will also be considered while establishing the marker of fetal distress as more useful for identifying pregnant animals with the high risk of abortion in goat flocks.

In Bedouin goats, decline in PAG started at 9.2±1.2 days before abortion (Charallah *et al.* 2010). Whereas, in the present study, PAG concentration in Jakhrana goats started to decline at 33.0±2.1 days before the abortion. This difference may likely be due to the breed-specific variation in placental functions, expression, and release of PAG into the circulation or difference in the assay systems for estimation of PAG (RIA/ELISA). Further, we observed that

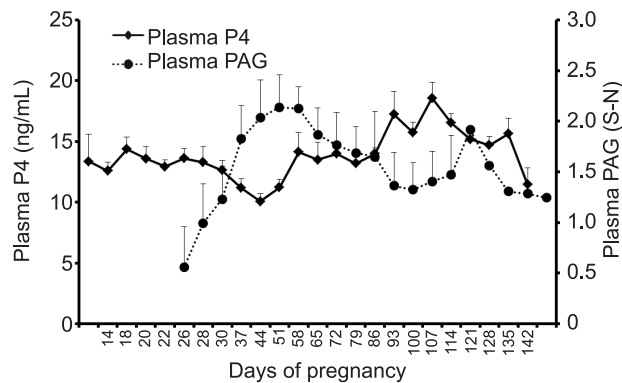


Fig. 2. Circulating profiles of progesterone (P4) and pregnancy-associated glycoprotein (PAG) during the maintained pregnancy in goats from d 10 until d 142 of gestation.

the concentration of P4, but not of the PAG, reached its lowest level on the day of abortion. This might be due to the long half-life of PAG (7.4 to 9 days; Ali *et al.* 1997) compared to P4 (about 7 min; Short *et al.* 1963), in circulation.

The kinetic profiles of plasma concentration of P4 and PAG in goats with maintained pregnancy are presented in Fig. 2. A positive relationship between plasma P4 and PAG (r=0.445, P<0.001) was observed. Considering the pooled values, positive associations of gestation day with the plasma P4 (r=0.443, P<0.001) and PAG (r=0.588, P<0.001) were detected. Overall, the PAG concentration in animals with the failed pregnancy was 4.8-folds lower compared to the goats with maintained pregnancy. However, the mean concentration of plasma P4 in goats with maintained or failed pregnancy was not different (Table 2). This variation may be because of the distinct sources of both the pregnancy hormones and probably due to the decline in number and/or activity of PAG secreting placental cells (mainly bi-

Table 2. Plasma concentration (mean±SEM) of progesterone (P4) and pregnancy-associated glycoprotein (PAG) in goats with different pregnancy status

Plasma variable	Goats with successful pregnancy (n =12)	Goats with failed pregnancy (n =3)	Non-pregnant goats (n =3)
P4 (ng/mL)	13.96 <sup>a</sup> ±0.27	16.18 <sup>a</sup> ±0.86	0.30 <sup>b</sup> ±0.03
PAG (S-N)	1.54 <sup>a</sup> ±0.07	0.33 <sup>b</sup> ±0.03	0.06 <sup>c</sup> ±0.03

Means with different superscripts (a, b, c) in the same row differ significantly (P<0.05).

Table 3. Multiple linear regression analyses results for the relationship of plasma progesterone (P4) and pregnancy-associated glycoprotein (PAG) with the failed or successful pregnancy in goats (from day 26 to day 72 of pregnancy)

Predictor variable	Unstandardized coefficient	Standardized β coefficient	P-value	Adjusted R <sup>2</sup>
PAG	0.168	0.520	< 0.001	0.333
P4	-0.024	-0.258	< 0.001	

nucleate trophoblastic cells) in goats with unsuccessful pregnancy. Further investigations, to find out the differences in the cellular population and their secretory activities in animals with failed or maintained pregnancy may provide a better solution to apply this information for early identification of animals with the high risk of pregnancy failure.

The results of stepwise multivariate linear regression analysis showed higher unstandardized and standardized  $\beta$ -coefficients for plasma PAG compared with the P4 concentration for the likelihood of abortion during pregnancy in goats (Table 3). These results coupled with the observation that the decline in PAG concentration starts much earlier than the decline in P4 concentration, suggest that the PAG can be preferred over P4 estimation for identification of animals with difficulty in maintaining pregnancy. This information has general concurrence with the study (Wallace *et al.* 1997) according to that PAG could be a more significant marker of fetal viability than P4 concentration in ewes.

The decline in circulating P4 and PAG during pregnancy exhibits occurrence of the abortion in goats. The overall mean plasma PAG, but not the P4, was significantly lower in goats with the failed pregnancy compared to those with the maintained pregnancy. The results of multivariate analysis and the observation that the decline in circulating PAG starts 12 days earlier than the change in P4 concentration, suggest PAG as a better biomarker of fetal distress and may be more useful for identifying pregnant animals with the high risk of abortion in goat flocks.

#### ACKNOWLEDGEMENTS

The study was supported by a grant from SERB-DST, Govt. of India, New Delhi (Grant No.: YSS/2015/001518). The authors are thankful to the support extended by Director of the Institute for providing necessary facilities to carry out this study. The scientific contribution of Dr N. Ramachandran, skilled technical assistance by Mr C. M. Sharma and help from Mr Ranjeet Singh, to conduct the study, are duly acknowledged.

#### REFERENCES

- Ali M, Gebbie F E, Sinclair K D, Broadbent P J, Hutchinson J S M, Beckers J F and Sulon J. 1997. Half-life of serum bovine pregnancy-associated glycoprotein (bPAG), postpartum anoestrus and performance of beef cows. *Journal of Reproduction and Fertility Abstract Series* **20**: 25.
- Charallah S, Amirat Z, Sulon J, Khammar F and Beckers J F. 2010. Pregnancy-Associated glycoprotein and progesterone concentrations during pregnancy failure in bedouin goat from the Southwest of Algeria. *Reproduction in Domestic Animals* **45**: 231–38.
- Garbayo J M, Remy B, Alabart J L, Folch J, Wattiez R and Falmagne P. 1998. Isolation and partial characterization of a pregnancy-associated glycoprotein family from the goat placenta. *Biology of Reproduction* **58**: 109–15.
- Goossens B, Osaer S and Kora S. 1997. Long term effects of an experimental infection with *Trypanosoma congolense* on reproductive performance of trypanotolerant Djallonke ewe and West African Dwarf does. *Research in Veterinary Science* **63**: 169–73.
- Haugejorden G, Waage S, Dah E, Karlberg K, Beckers J F and Ropsta E. 2006. Pregnancy associated glycoproteins (PAG) in postpartum cows, ewes, goats and their offspring. *Theriogenology* **66**: 1976–84.
- Jourdain E, Gibert P, Gauthier D, Fromont E, Jullien J M and Hars J. 2005. Sondagesurles maladies abortives chez les ongulés sauvagesetdomestiquesenalpages. Enquêtemene dans la RNCFS des Bauges. *Gibier Faune Sauvage* **268**: 24–32.
- Menzies P I. 2011. Control of important causes of infectious abortion in sheep and goats. *Veterinary Clinic: Food Animal Practice* **27**: 81–93.
- Osaer S, Goossens B, Kora S, Gaye M and Darboe L. 1999. Health and productivity of traditionally managed Djallonke sheep and West African Dwarf goats under high and moderate trypanosomosis risk. *Journal of Veterinary Parasitology* **82**: 101–19.
- Roberts J N, May K J and Veiga-Lopez A. 2017. Time-dependent changes in pregnancy-associated glycoproteins and progesterone in commercial crossbred sheep. *Theriogenology* **89**: 271–79.
- Sawada T, Nakatani T, Tamada H and Mori J. 1994. Secretion of progesterone and 20 alpha-dihydroprogesterone during pregnancy in goats. *Steroids* **59**: 468–71.
- Short R V and Rowell J G. 1963. The half-life of progesterone in the peripheral blood of ewe at two stages of gestation. *Journal of Endocrinology* **25**: 369–74.
- Singh S P, Natesana R, Sharma N, Goel A K, Singh M K and Kharche S D. 2019. Pregnancy-associated glycoprotein profile in milk and its relationship with the circulating level during early pregnancy in goats. *Small Ruminant Research* **173**: 81–87.
- Waldeland H and Loken T. 1991. Reproductive failure in goats in Norway: an investigation in 24 herds. *Acta Veterinaria Scandinavica* **32**: 535–41.
- Wallace J M, Aitken R P, Cheyne M A and Humblot P. 1997. Pregnancy-specific protein B and progesterone concentrations in relation to nutritional regimen, placental mass and pregnancy outcome in growing adolescent ewes carrying singleton fetuses. *Journal of Reproduction and Fertility* **109**: 53–58.