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Circulating level of pregnancy-associated glycoprotein (PAG) is affected by number of fetus during mid pregnancy in goats

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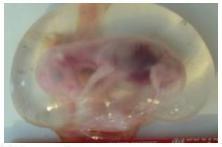
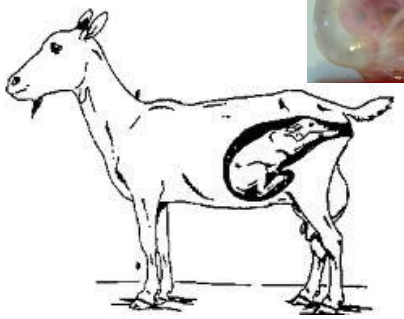
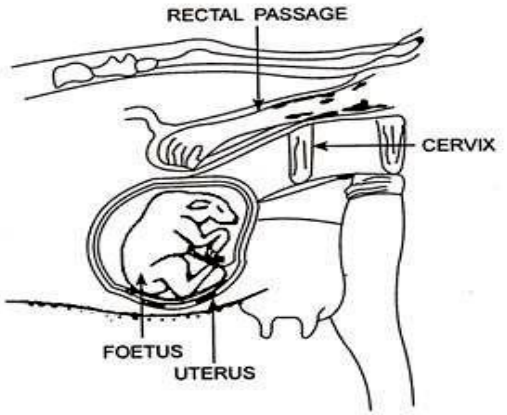
Background

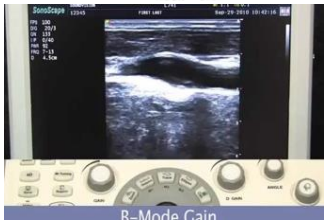
- An early and accurate diagnosis of pregnancy is an essential factor for optimizing reproductive performance of farm animals.



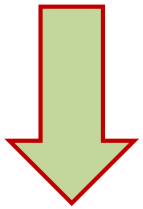




- Several methods of PD (transabdominal palpation, P4 estimation and USG) are being practiced for small ruminants.



Yet, none of the test qualifies as the ideal due to their inherent limitations of **sensitivity, speed and ease of performing the test**



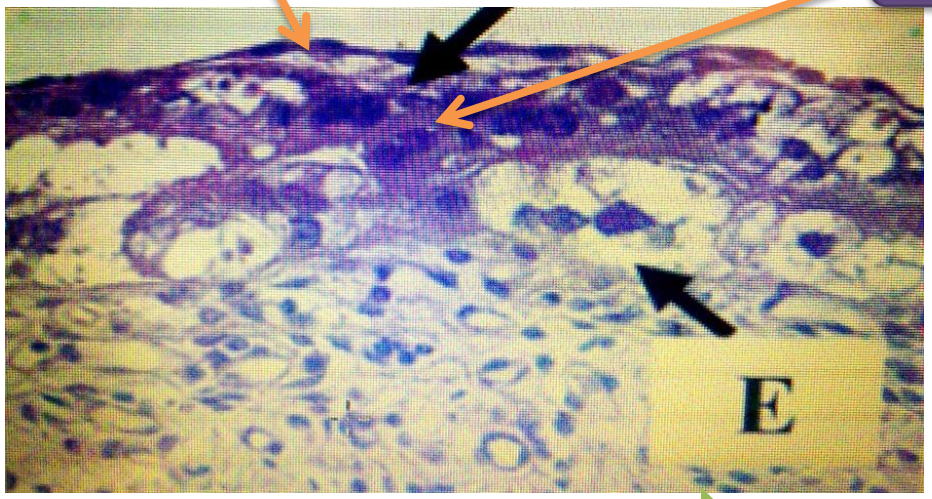
Large polymorphic placentally expressed proteins (PAGs) have been discovered in farm animals

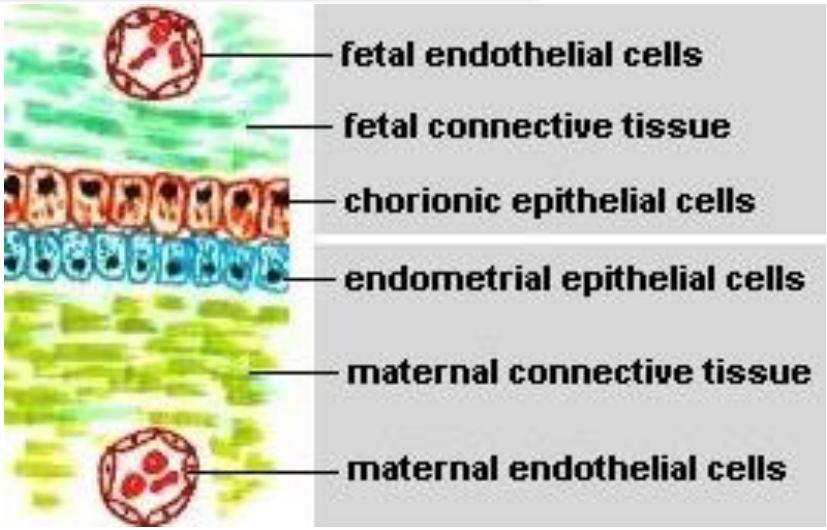
Source of PAGs

Mononucleate cells 80-85%

15-20%

Binucleate cells





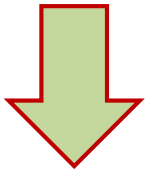
MNC → Acytokinetic mitosis → BNC

Bazer *et al.*, 2009

✓ PAGs are mainly expressed in **mono- and bi-nucleate trophoblast cells** in ruminants (Zoli *et al.*, 1998).

Physiological applications of PAGs

Quantification of PAG concentrations in circulation may be useful for different purposes



- Monitoring of placental secretory functions (Breukelman *et al.*, 2012)
- Detection of placental abnormalities (Chavatte-Palmer *et al.*, 2016)
- Investigation of embryonic and foetal mortalities (Szenci *et al.*, 2018)

Background cont…

Although, blood could be considered to be an ideal biological sample for evaluation of biomarkers relating to stress & inflammatory responses in farm animals

Little is known about how blood PAG can be influenced by the stage of pregnancy and number of fetus in goats

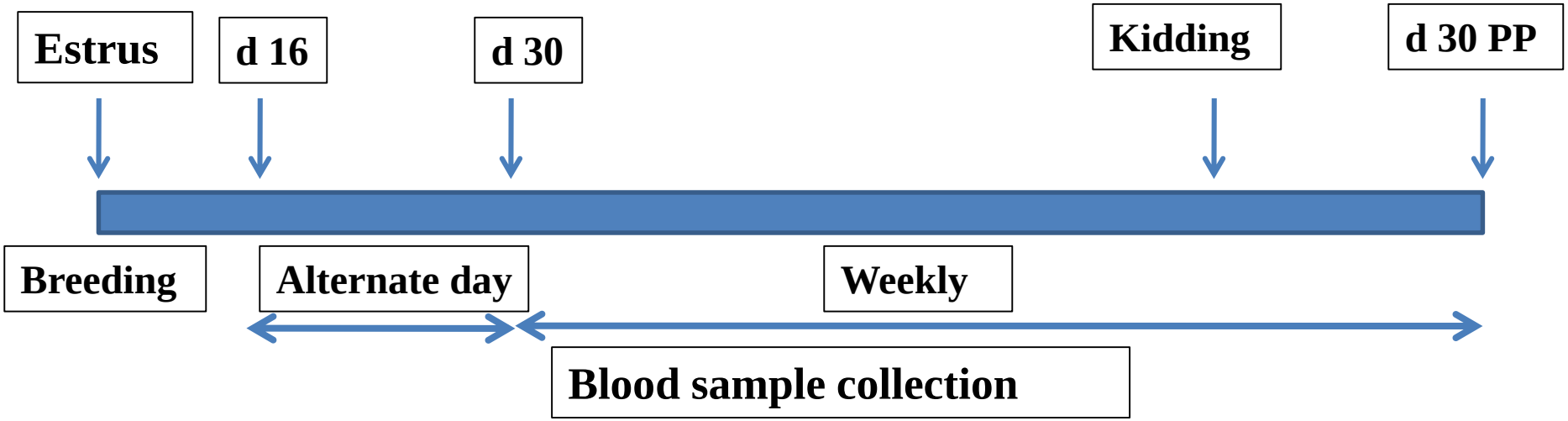
Objectives

1. To investigate time dependent changes in PAG in circulation during pregnancy in goats.

2. To identify plasma PAG as a potential biomarker for detection of number of fetus in pregnant goats.

Materials and Methods

- Barbari goats (n=20; 2-3 parity) were selected and observed regularly for estrous cycle. Once animal came in to estrus, breeding was done by natural mating.
- Blood sampling was started at d 16 from the day of mating, on alternate day for first month.

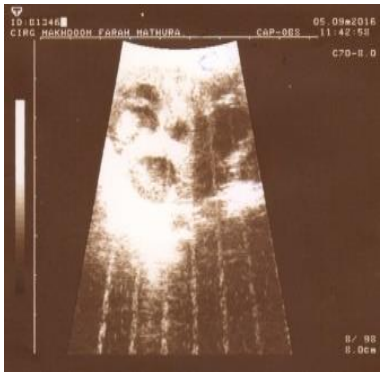


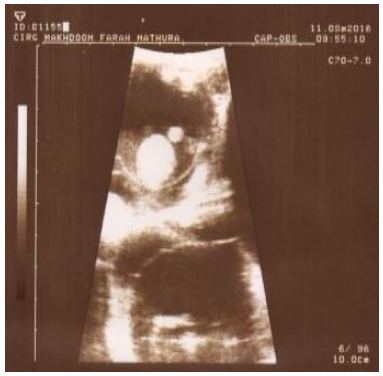
Materials and methods cont…

- Blood samples were collected in dipostasium (K2)-EDTA evacuated tubes (Vacutainer, BD, Franklin Lake, NJ) and immediately placed in the ice box.

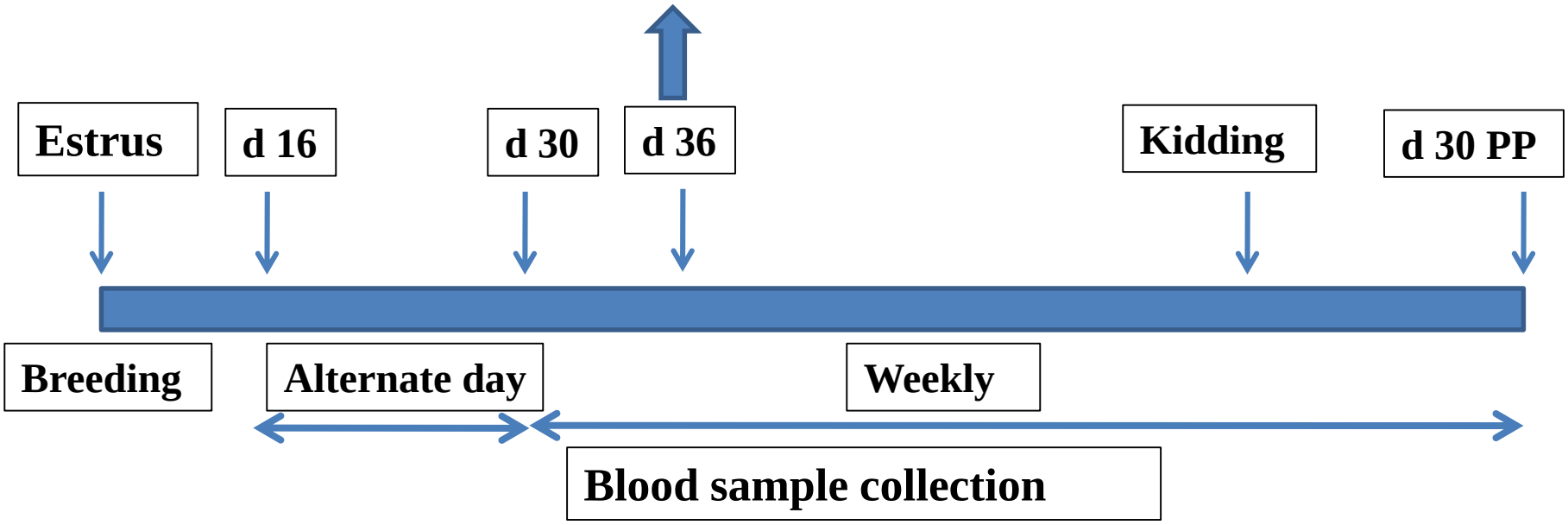
- After centrifugation at 2000 × *g*, 10 min at 4 ^{o}C, plasma was harvested and stored at -40 ^{o}C until thawed for assay.

Blood sampling and storage cont..





After confirmation of pregnancy by **non return into estrus** at about 21 days and then by **USG** (at d 36), blood sampling continued till d 30 PP



Blood PAG analysis

- PAG conc. in plasma samples was measured by in-house developed ELISA test for caPAG.

✓ Intra-assay CV: 4.54%

✓ Inter-assay CV: 8.65%

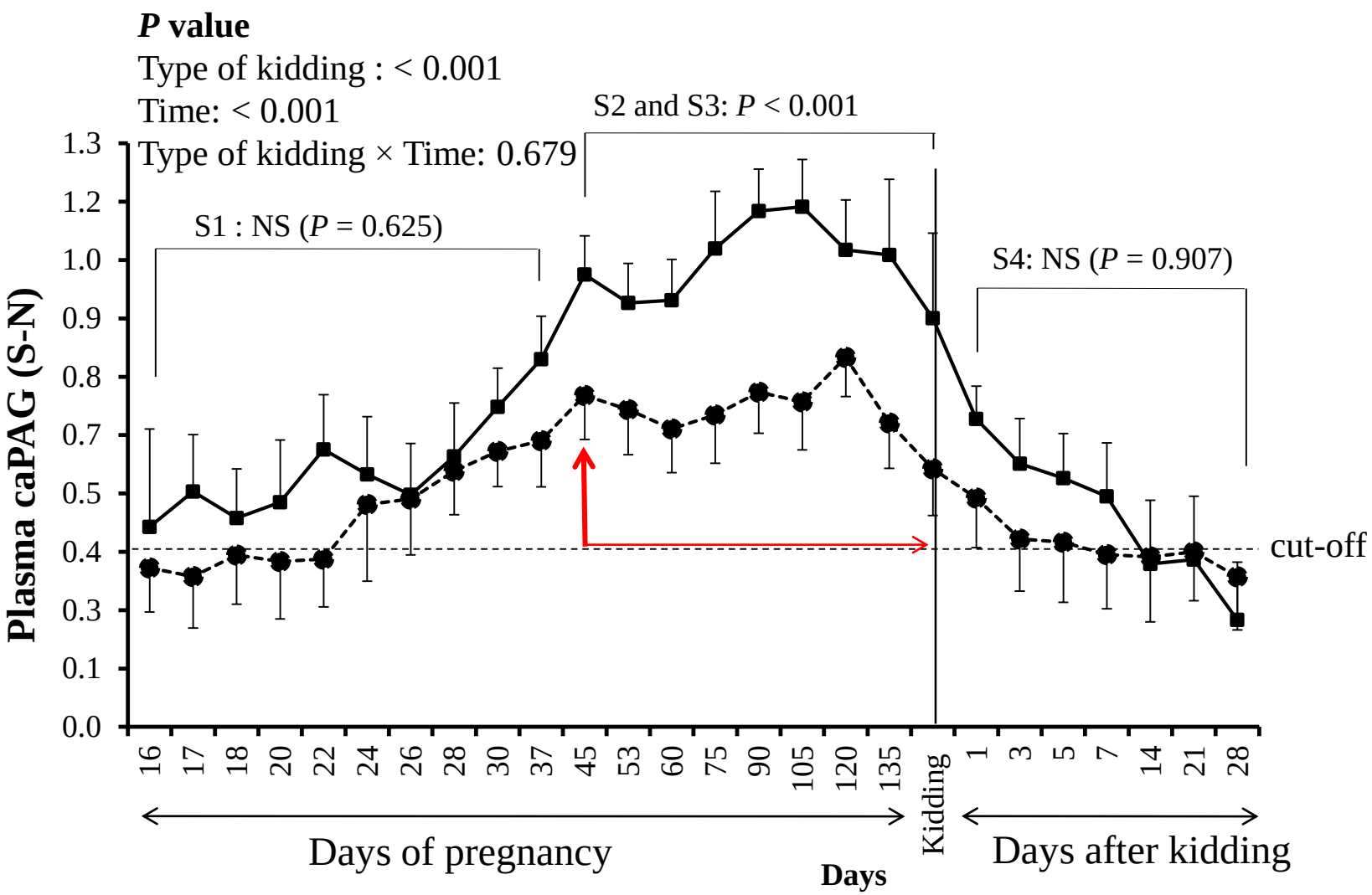
✓ Recovery: 99.22 ± 6.58 %

✓ Dilutional linearity: 6.8%

AS	PAG	N-terminal micro-sequence	Accession No.
707	$caPAG_{55kDa}$	ISSPVSXLTIHPLRNIMDMLYVGXITI	P80935
	$caPAG_{62kDa}$	RDSXVTIVPLRNMRDIVYVGXITIGTP	P80933
708	$caPAG_{55kDa}$	ISSPVSXLTIHPLRNIMDMLYVGXITI	P80935

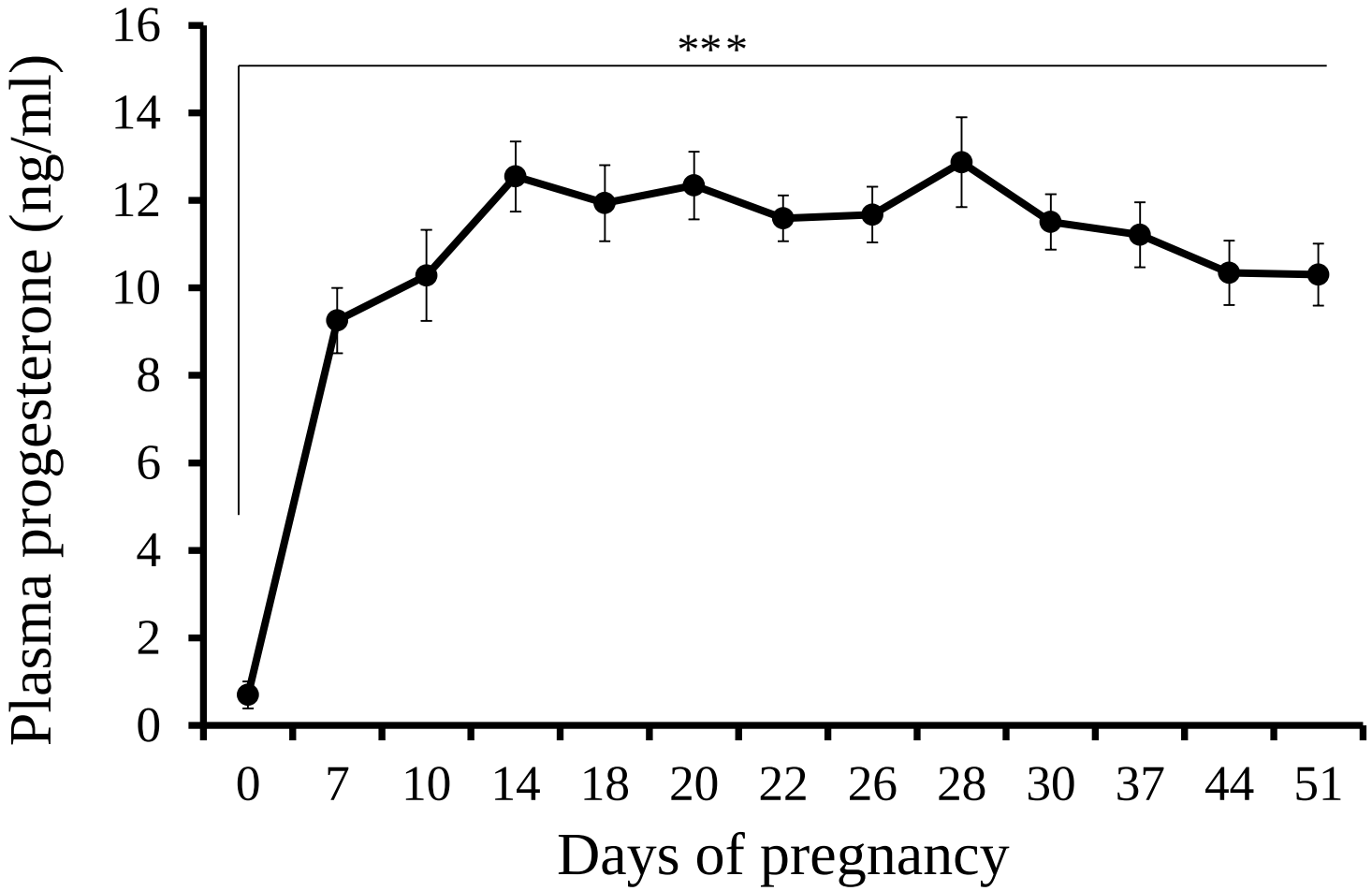
Statistical analysis

- The mixed model procedure was used for each variable as a dependent variable.
- Treatment (number of fetus) and days of pregnancy were considered as **fixed factor,** sampling time as repeated effects, and their respective interactions were included into the model.
- To account for multiple mean comparisons an independent sample **t-test** was performed.
- Relationships between blood PAG and P4 conc. was estimate by **Pearson's correlation coefficient**.

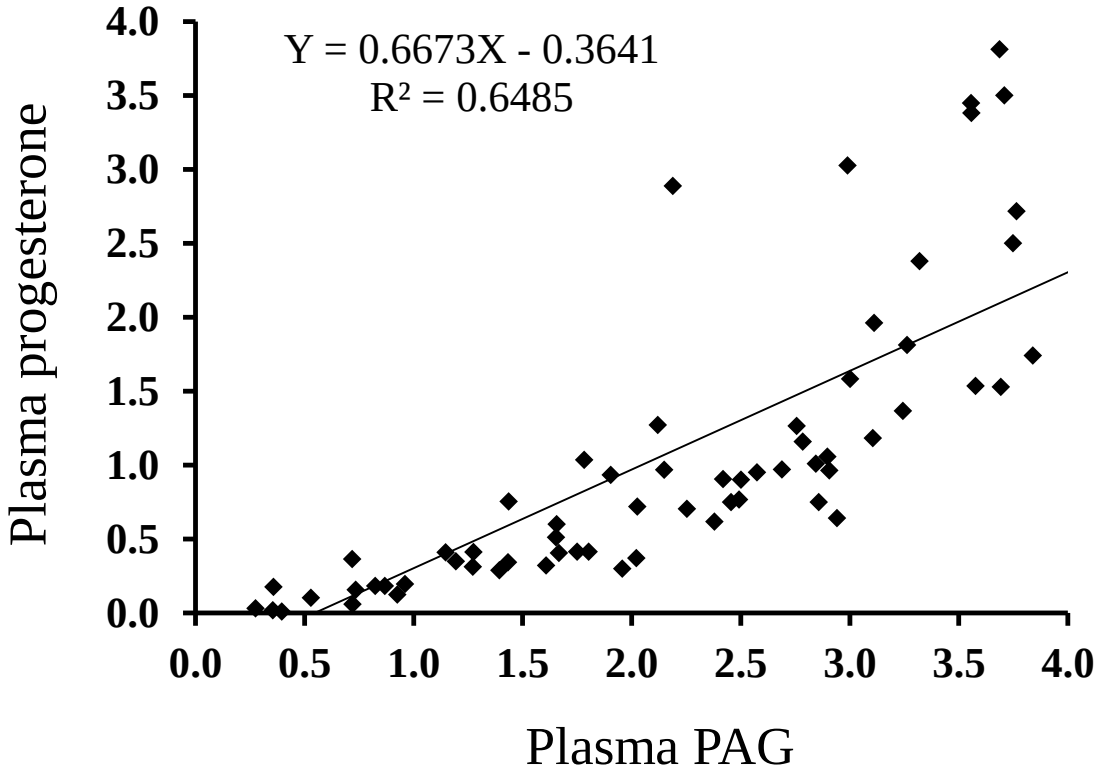


Dynamic profiles of caPAG in **single** (*dotted line and closed circle*) or **twin** (*solid line and closed square*) fetus bearing goats during and after gestation. Type of kidding represents single or twin birth.

Progesterone concentration during pregnancy



Relationship of plasma PAG with P4 concentration



r = 0.721

P < 0.001

Conclusions

Higher level of caPAG in does bearing twins compared to the does with single fetus on and after d 45 during pregnancy.

Strong significantly positive relationship of caPAG with P4 concentration in circulation

Suitability of caPAG as a biomarker for early PD & detection of number of fetus after d45 of pregnancy in goats

Future prospects

Future studies are required to establish effectiveness of such measurements (PAG in circulation) to identify early pathophysiological conditions and fetal well-being in goats and other farm animals.

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THANKS

FOR YOUR ATTENTION !!