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Short Communication

Early Pregnancy Diagnosis using Pregnancy-Associated Glycoproteins in the Serum of Pregnant Ruminants

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ABSTRACT

Early pregnancy diagnosis is an essential tool in successful reproductive management of ruminants. We attempted to explore the effectiveness of a marketable ELISA test kit for assessment of Pregnancy Associated Glycoproteins (PAGs) in peripheral blood for early pregnancy identification in cattle, buffalo, goats and sheep. A total of 120 blood were taken from jugular vein of different breeds of cattle (Achai, Achai x Jersey, Holstein Friesian and Jersey), buffalo (Nilli Ravi, Aza Kheli and non-descript), goats (Beetal, Teddy and non-descript) and sheep (Bulkhi, Karri and non-descript). In cattle, the average sensitivity, specificity, false pregnancy prognostic value, false non-pregnancy prognostic value and accuracy of the PAG-ELISA test were 86.77%, 66.67%, 86.77%, 100% and 90%, respectively. Similarly, the current study indicated that in buffalo, average sensitivity, specificity, false pregnancy prognostic value, false non-pregnancy prognostic value and accuracy of the PAG-ELISA test were 62.22%, 74.6%, 62.22%, 88.57% and 76.67% respectively. The current study demonstrated that average sensitivity, specificity, false pregnancy prognostic value, false non-pregnancy prognostic value and accuracy of the PAG-ELISA test were 100%, 100%, 100%, 100% and 100% respectively in goat. Similarly, the results in sheep showed that the average sensitivity, specificity, false pregnancy prognostic value, false non-pregnancy prognostic value and accuracy were 95.23%, 91.67%, 95.23%, 100% and 100% respectively. The results showed that PAG is a valuable marker for early pregnancy diagnosis in ruminants.

Pregnancy maintenance is a clear economic factor in dairy animals since most of the pregnancy losses occur during the early stages of pregnancy (Garcıa-Ispierto *et al*., 2013; Commun *et al*., 2016). Therefore, timely pregnancy diagnosis is very important for sound reproductive management in herd health (Green *et al*., 2005; Kaya *et al*., 2016). Several techniques are being used to diagnose pregnancy in dairy herd such as ultrasound, palpation per rectum, estrone sulfate, milk progesterone assay, and blood tests. However, in most of the instances, accurate pregnancy diagnosis is hardly achieved due to one or the other reasons (Commun *et al*., 2016). Therefore, a simple but reliable pregnancy test for domestic animals have long been sought.

Finally, pregnancy can also be diagnosed through specific pregnancy-associated glycoproteins (PAGs). PAGs are considered powerful markers of pregnancy diagnosis since they are expressed during the early period of gestation (Commun *et al*., 2016). Among them, Pregnancy Associated Glycoprotein (PAG-1) has been used for pregnancy diagnosis because this molecule can be found in maternal blood soon after implantation as a marker of fetal well-being (Zoli *et al*., 1992). Importantly, PAG-1 is an essential component of binucleate trophoblast cells (Green *et al*., 2005) and can be easily monitored in serum since it can be test after at the end of one month of pregnancy and secondly interpretation of the assay does not require much knowledge. The aim of the present experiment was to evaluate the PAG as non-invasive diagnostic tool for early pregnancy marker in cow, buffalo, sheep and goat.

Materials and methods

All procedures in this experiment were approved by Committee on Ethics and Animal Welfare, The University of Agriculture, Peshawar, Pakistan.

A total of 120 pregnant animals including buffaloes (Nilli-Ravi, Aza-Khelli and cross bred), cows (Achai, Holstein Friesian and cross bred), sheep (Bulkhi, Karri, and cross bred) and goats (Beetal, Teddy and cross bred) were randomly selected which were recently inseminated. Each group of species consisted of 30 animals. The animals

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were synchronized by intramuscular injection of PGF2α. and estrus was detected. Animals with standing heat were inseminated artificially.

About 10 ml blood sample was taken on day 30 of the pregnancy of each animal inseminated. Blood was centrifuged at 3000 rpm for 10 min and then serum was separated and stored -20°C.

Pregnancy was confirmed on day 60 of each inseminated animal through abdominal ultrasonogrphy using a real-time B-mode ultrasound scanner equipped with a 5/7.5-MHz linear endorectal transducer. Serum PAG-1 was determined through commercial ELISA kit (IDEXX Laboratories, Westbrook, USA). The manufacturer instructions given in the kit were strictly followed.

The findings of the current study were subjected to statistical analysis in SPSS. In the current study (a) accurate pregnancy detection was done when both PAGs-ELISA analysis and ultrasound check were found affirmative. Likewise, accurate non-pregnancy detection was done when PAGs-ELISA analysis were affirmative but ultrasound check were found false. Also our finding further explored the false pregnancy prognostic assessment when PAGs-ELISA analysis were found false whereas ultrasound check were found affirmative. Lastly our results indicated false non-pregnancy prognostic assessment when both PAGs-ELISA analysis and ultrasound check were found negative. Furthermore, the sensitivity (100 × a / a + c), specificity (100 × d / d + b), positive predictive value (100 × a / a + b), negative predictive value (100 × d / c + d), and accuracy (100 × (a + d) / (a + b + c + d)) of the PAGs-ELISA analysis were measured from the current findings (Kaya *et al*., 2016).

Results and discussion

The results of pregnancy diagnosis in different breeds of cattle buffalos, goats and sheep are given in Table I. The average sensitivity, specificity, false pregnancy prognostic value, false non-pregnancy prognostic value and accuracy of the PAG-ELISA test were 86.77%, 66.67%, 86.77%, 100% and 90% for cattle, 62.22%, 74.6%, 62.22%, 88.57% and 76.67%, respectively for buffalos, 100%, 100%, 100%, 100% and 100%, respectively for goats and 95.23%, 91.67%, 95.23%, 100% and 100%, respectively for sheep.

Early pregnancy diagnosis is an important tool in livestock species and has traditionally been performed through transrectal palpation or ultrasonography. However, due to the limitations of the skilled technician and high demand of accurate pregnancy diagnosis, chemical pregnancy diagnosis is gaining more importance. In the current study, the accuracy was found to be 90%, 76.67, 100 and 96.66% in cattle, buffalo, goat and sheep respectively. The finding of the current study associated with PAGs in peripheral blood as pregnancy markers are in line with the literature (Zoli *et al*., 1992; Silva *et al*., 2007; Kaya *et al*., 2016). The slight difference in the results may be due to the differences among commercial kits, user experience, and individual variability of PAG concentration causing the limitations of pregnancy diagnosis. After successful conception, PAG may be detected in pregnant animals as early as 3 weeks of insemination. The concentration of PAG increases gradually until before calving, therefore, the sensitivity and accuracy of the test also increases with the advancement of the pregnancy (Szenci *et al*., 1998; Kaya *et al*., 2016).

In the current study, inaccurate positive pregnancy results were found more in cattle compared to the other species. In addition, no false positive pregnancy results were found in goat. According to Kaya *et al*. (2016) incorrect positive pregnancy results are most likely found during the early period of pregnancy. Incorrect positive results are most likely to be the result of embryonic death leading to the disappearance of maternal PAG in the blood due to the short half life of about 2.7 to 7 days (Semambo *et al*., 1992; Szenci *et al*., 2003). Another reason of false positive results may be due to the residual PAGs from the previous pregnancy since it takes almost 45 days before the residues of PAGs are cleared from the maternal blood (Kaya *et al*., 2015). Some authors have also reported missing PAG in cows near 100 days after calving following confirmed pregnancy (Silva *et al*., 2007). We infer that the incorrect positive results may be related to the cross-reaction with protein other than PAG resulting in lower sensitivity.

On the average, the accuracy of positive pregnancy diagnosis was severely affected in case of buffalo in this study. The average accuracy was 76.67% in buffaloes. In the past, attempts for the isolation of PAG in buffalo serum have been conducted, however, only partial purification have been achieved with a success rate of 68% (Jerome, 2012). Karen *et al*. (2007) found that the sensitivity of PAG test in buffalo serum was 11.1% during 19-24 days of pregnancy and reached 100% on day 31. It indicates that the best results through PAG test could be achieved after 31 days in buffalo.

In case of ewes and goats, in the present study, the results were satisfactory in terms of accuracy. On the average, the accuracy was 96.66% and 100% in sheep and goats, respectively. In ewes and goats, the concentration of PAG increases rapidly from week 3 of gestation. Maximum concentration reaches during the 9th week and then declines (Haugejorden *et al*., 2006).

Table I. Assessment of pregnancy associated glycoproteins in plasma sample of different breeds of cattle, buffalos, goat, and sheep for early pregnancy diagnosis.

	a	b	c	d	Se %	Sp %	FPPV	FNPV	ACC
Cattle breed									
Achai X Jersey	6	1		3	85.71	75	85.71	100	90
HF X Jersey	8	1		1	88.89	50	88.89	100	90
Average	20	3		7	86.77	66.67	86.77	100	90
Buffalo breed									
Nilli Ravi	3	2	0	5	60	71.42	60	100	80
Aza Kheli	2	1	1	6	66.67	85.71	66.67	85.71	80
Non-descript Breed	3	2	1	4	60	66.67	60	80	70
Average	8	5	2	15	62.22	74.6	62.22	88.57	76.67
Goat breeds									
Beetal	08	00	00	02	100	100	100	100	100
Teddy	07	00	00	03	100	100	100	100	100
Non-descript Breed	06	00	00	04	100	100	100	100	100
Average	21	00	00	09	100	100	100	100	100
Sheep breeds									
Bulkhi	6	1	3		85.71	75	85.71	100	90
Karri	8	0	2		100	100	100	100	100
Non-descript Breed	5	0	5		100	100	100	100	100
Average	19	1	10		95.23	91.67	95.23	100	96.66

a, accurate pregnancy detection (pregnant); b, inaccurate pregnancy detection (non-pregnant); d, accurate non-pregnancy detection (non-pregnant); Se%, sensitivity percentage; Sp%, specificity percentage; FPPV, false pregnancy prognostic value; FNPV, false non-pregnancy prognostic value; ACC, accuracy.

Conclusion

The results indicated that Pregnant Associated Glycoproteins (PAG) is a potential biomarker in early pregnancy in domestic ruminants.

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Statement of conflict of interest

Authors declare no potential conflict of interest

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