

Association of Plasma Progesterone and Pregnancy Associated Glycoproteins in Post-Insemination Conceived and Non-conceived Gir Cows and Jaffarabadi Buffaloes Detected by BovEasy Rapid PD Kits

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ABSTRACT

This research focused on healthy 75 Gir cows and 75 Jaffarabadi buffaloes, 60-65 days postpartum that were inseminated during the detected estrus and did not return to repeat estrus till day 22 post-AI. They underwent pregnancy diagnosis by plasma progesterone on Days 21, 28, 36, and 45; pregnancy-associated glycoprotein (PAG) on Days 28, and 36 post-AI; PAG based BovEasy rapid PD kits on day 28 and 32, and per rectal confirmation on day 45 post-AI. The study assessed and compared conception rates, and the levels of progesterone and PAG by RIA and ELISA, respectively, in conceived and non-conceived animals. The BovEasy bovine rapid PD kits showed very high (100%) accuracy, sensitivity, and specificity in detecting early pregnancy. In all 37 cows & 25 buffaloes were found pregnant by day 32 with PD kits, and the overall actual pregnancy rate at day 45 was 36.7% (55/150,) indicating 4.7% (7/150) embryonic mortality. There was a significant increase in plasma P₄ levels in both cows and buffaloes from Day 21 (2.92 ± 0.27 and 2.40 ± 0.23 ng/mL) to Day 45 (7.61 ± 0.71 and 5.75 ± 0.67 ng/mL) and PAG from day 28 (5.27 ± 0.87 and 4.48 ± 0.9 ng/mL) to day 36 (9.93 ± 1.63 and 6.96 ± 1.39 ng/mL) in conceived animals, while in non-conceived animals the P₄ was unstable, with significant fluctuations over the days measured, but PAG values always remained below 1 ng/mL. In both the species, a highly significant differences (p<0.000) in P₄ and PAG levels were noticed between pregnant and non-pregnant animals on all test days with strong positive correlations (p<0.01) between P₄ and PAG levels on all days, but in non-pregnant animals the changes in PAG were not strongly related to changes in progesterone. Thus, BovEasy rapid pregnancy kit or plasma PAG estimates can serve as effective markers for early pregnancy detection in bovines as it reflects the trophoblast activity and presence of viable fetus in uterus.

Key words: BovEasy rapid pregnancy kit, Bovines, Correlations, PAG, Plasma progesterone, Pregnancy status.

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INTRODUCTION

Ensuring optimal reproductive performance is critical for sustainable and profitable livestock farming. Pregnancy diagnosis plays a vital role in the livestock sector, particularly in evaluating the reproductive efficiency over time providing a framework for effective reproductive management, treatment, and rebreeding programmes. Accurate early pregnancy diagnosis is essential for boosting farm productivity and profitability (Barik and Yadav, 2022). Transrectal palpation is among the oldest and most widely practiced methods for diagnosing pregnancy in large animals around days 45-50 post-AI (Zemjanis, 1970), however, this method cannot accurately assess foetal viability, leading to variability in its diagnostic precision. The real-time B-mode transrectal ultrasonography though regarded as the "gold standard" for early pregnancy diagnosis in cows, has limitations of portability in the field, cost, skill and experience of its use with user (Devmurari *et al.*, 2025). Similarly plasma progesterone assay though highly accurate in detecting non-pregnant animals, has limitations of confirming positive pregnancy for

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uterine infections, persistent CL, early embryonic mortality or pathological pregnancy status.

Hence, ELISA-based assays kits to measure pregnancy-associated glycoprotein (PAG) levels in maternal blood have been developed recently for cattle and buffaloes (Barbato *et al.*, 2022). However, these tests are costly and not feasible for small farms. To address these issues, researchers have developed on-farm PAG-based early pregnancy detection (PD) strip test kits with accuracy of 95% to 99%, and are made available in markets (Devmurari *et al.*, 2025; Parmar *et al.*, 2025). Therefore, this study was planned to validate, under controlled farm conditions, the effectiveness of the pregnancy diagnostic methods like plasma progesterone assay, pregnancy-associated glycoproteins (PAG) and PAG based BovEasy rapid PD kits with traditional pregnancy detection technique by rectal palpation for detection of early pregnancy/embryonic mortality in cattle and buffaloes and to correlate them with positive and negative pregnancy status.

MATERIALS AND METHODS

The present study was conducted from December 2023 to October 2024, following approval of Institutional Animal Ethics Committee (IAEC) of the College (Protocol No.: KU-JVC-IAEC-LA-124-23) at Cattle Breeding Farm, Kamdhenu University, Junagadh (Gujarat, India).

Experimental Animals

This research focused on Gir cows and Jaffarabadi buffaloes, aged 4-14 years, with a voluntary waiting period (VWP) of 60-65 days postpartum. Each animal underwent a comprehensive reproductive evaluation to detect any palpable genital abnormalities, verify the presence of functional ovarian structures, and identify any outward signs of estrus. Only those animals showing normal cyclic activity and no detectable reproductive abnormalities were included in the study. The animals detected in estrus were subjected to artificial insemination (AI) using frozen-thawed semen during the latter half of the estrus. In all 75 Gir cows and 75 Jaffarabadi buffaloes, which did not return to repeat estrus on day 22 post-AI were included in the study to check them for pregnancy by various methods, *viz.*, plasma progesterone on days 21, 28, 36 & 45; plasma PAG on days 28 and 36; BovEasy rapid PD kits on day 28 and 32, and actual rectal palpation on day 45 post-AI.

Management of Animals and Blood Sampling

Cattle and buffaloes were housed in different sheds having a loose housing system with unrestricted access to wholesome drinking water. They were fed according to the farm's standard schedule, which included seasonal fodder, hay, compounded concentrates and mineral mixture according to their production and reproduction status. Blood samples (2 mL) were collected by Jugular veni-puncture in heparinized

vacutainers on days 21, 28, 32, 36 and 45 post-AI for various pregnancy detection tests using kits on fresh whole blood or plasma as per the manufacturer's instructions.

Lab Assay Procedures

The BovEasy bovine rapid pregnancy detection kits (supplied by Prompt Equipments Pvt. Ltd., Ahmedabad) were used to check whether the cow/buffalo was pregnant or not on day 28 and 32 post-AI using fresh blood on the same day of collection as per the manufacturer's guidelines. The stored separated plasma samples were used to determine progesterone concentrations on Days 21, 28, 36 & 45 by using standard RIA kits and technique of Beckman & Coulter, supplied by Immunotech, France, and the assay of PAGs levels on Days 28 and 36 was performed by using ELISA kits procured from Puregene (Catalogue No.PG-0349Bo) according to manufacturer's instructions.

Statistical Analysis

The animals were classified as conceived and non-conceived in both Gir cattle (37 vs 38) and Jaffarabadi buffaloes (25 vs 50). The data generated on plasma P₄ and PAG were analysed and expressed as mean ± SEs for conceived and non-conceived, and compared using one-way analysis of variance, Duncan's NMRT or Student's 't' test as per Snedecor and Cochran (1994). Pearson's correlation analysis of plasma PAG and progesterone (P₄) was performed in pregnant and non-pregnant Gir cows and Jaffarabadi buffaloes to correlate these parameters with pregnancy status.

RESULTS AND DISCUSSION

The BovEasy bovine rapid PD kits showed very high (100%) accuracy, sensitivity, and specificity in detecting early pregnancy by day 32. In all 37 cows & 25 buffaloes were found pregnant by day 32 with PD kits, and the overall actual pregnancy rate at day 45 was 36.7% (55/150), indicating 4.7% (7/150) embryonic mortality (Devmurri *et al.*, 2025). Almost similar results were noted with BovEasy rapid PD kits in crossbred cows by Parmar *et al.* (2025). The average peripheral plasma progesterone (P₄) levels recorded on Days 21, 28, 36, and 45 post-AI, and pregnancy-associated glycoprotein (PAG) recorded on Days 28 and 36 post-AI for both conceived and non-conceived animals of Gir cows and Jaffarabadi buffaloes are presented in Table 1 and 2, respectively. The analysis of variance revealed highly significant effects of pregnancy status ($p < 0.01$) on plasma levels of PAG and progesterone (P₄) in Gir cows and Jaffarabadi buffaloes. On Day 28 and 36 post-AI, PAG levels differed significantly ($p < 0.000$) between pregnant and non-pregnant animals of both species. Progesterone levels also showed significant differences ($p < 0.000$) between pregnant and non-pregnant animals on Days 21, 28, 36, and 45 post-AI. Specifically, on Day 21, progesterone levels differed significantly ($p < 0.000$) between pregnant and non-pregnant



Gir cows and Jaffarabadi buffaloes, and persisted on Days 28, 36, and 45.

Plasma Progesterone (P₄) Profile

Among the 37 conceived Gir cows and 25 conceived Jaffarabadi buffaloes, the progesterone levels showed a steady and significant increase over time, starting at 5.16 ± 0.85 and 4.95 ± 0.99 ng/mL on Day 21 and rising to 13.25 ± 2.18 and 13.46 ± 2.69 ng/mL by Day 45. This increase between the days was significant ($p < 0.05$), suggesting that as pregnancy progresses, P₄ levels continue to rise. In contrast, among the 38 non-conceived cows, the progesterone levels remained relatively lower and fluctuated, between 0.73 ± 0.12 and 2.46 ± 0.40 ng/mL. Similarly in 50 non-conceived buffaloes, the progesterone levels were lower and less variable, starting at 1.13 ± 0.18 ng/mL on Day 21, peaking slightly at 2.32 ± 0.37 ng/mL on Day 28, and then declining to 1.89 ± 0.30 ng/mL by Day 45. The variations between these days were also significant ($p < 0.05$), but the levels remained consistently lower than those in the conceived animals. When averaging the results across all 75 Gir cows, progesterone levels significantly increased from 2.92 ± 0.27 ng/mL on Day 21 to 7.61 ± 0.71 ng/mL on Day 45 in Gir cows and from 2.40 ± 0.23 ng/mL on Day 21 to 5.75 ± 0.67 ng/mL on Day 45 in Jaffaranadi buffaloes, with significant differences between these time points ($p < 0.05$). Additionally, the comparison between cows and buffaloes revealed that the buffaloes generally exhibited lower ($p < 0.05$) progesterone levels than Gir cows, indicating species-specific differences. These results underscore the importance of progesterone measurement for detecting pregnancy status in cows and buffaloes, with lower levels being a reliable indicator of unsuccessful conception.

Overall, the data illustrate the critical role of progesterone in maintaining pregnancy in both species. The progressive rise in progesterone levels among conceived animals suggests its potential as a biomarker for early pregnancy detection and monitoring in dairy animals. Similar findings were reported by Thasmi *et al.* (2017) and Nyman *et al.*

(2018). These findings are also in close agreement with those reported by Ghanem and Nishibori (2015), Saini *et al.* (2021), and Thanh *et al.* (2023), who observed that plasma progesterone levels were significantly higher in pregnant buffaloes with advancing pregnancy compared to their non-pregnant counterparts. Abdullah *et al.* (2017) investigated in pregnant crossbred and zebu cows, P₄ levels peaked on days 24 and 30, respectively, while in non-pregnant cows, P₄ peaked on day 15 post-AI, and remained below 1.5 ng/mL for the rest of the study period. According to Daghash *et al.* (2022), plasma progesterone levels in pregnant buffaloes showed a significant increase ($p < 0.05$) from day 20 to 40, and non-pregnant ones exhibited a significant decline in P₄ concentrations on days 25-30. Abu El-Hamd *et al.* (2019), Husnurrizal *et al.* (2020), and Peixoto *et al.* (2021) opined those animals with embryonic losses exhibit lower progesterone levels compared to those that maintain pregnancy. Contrarily, the results aligned with findings from Lopez-Gatius *et al.* (2007) and Karen *et al.* (2014), who also observed no significant differences in progesterone levels between pregnant animals and those with embryonic mortality. This consistently highlights the potential variability in progesterone dynamics across different studies and emphasizes the need for further investigation to clarify these patterns.

Plasma Profile of Pregnancy-Associated Glycoprotein (PAG)

The PAG levels showed a significant increase from Day 28 to Day 36 in both conceived cows (5.27 ± 0.87 and 9.93 ± 1.63 ng/mL) and buffaloes (4.48 ± 0.9 and 6.96 ± 1.39 ng/mL), with overall mean values across both days as 7.60 ± 1.25 and 5.72 ± 1.15 ng/mL, respectively (Table 2). PAG levels rose significantly ($p < 0.05$) as the pregnancy progressed. Conversely, in the non-conceived cows, PAG levels remained consistently low, measured at 0.53 ± 0.09 ng/mL on Day 28 and 0.51 ± 0.08 ng/mL on Day 36, with an overall average of 0.52 ± 0.08 ng/mL, and in non-conceived buffaloes, the corresponding values were 0.49 ± 0.08 , 0.70 ± 0.11 and 0.60 ± 0.10 ng/mL. The data indicate that PAG levels in non-

Table 1: Average plasma progesterone levels (ng/mL) in Gir cows and Jaffarabadi buffaloes on various days after AI in conceived and non-conceived animals (Mean \pm SEM)

Species	Status of Animals	No. of Animals	Days Post-AI				Overall
			Day 21	Day 28	Day 36	Day 45	
Gir Cow	Conceived	37	$5.16 \pm 0.85^{\text{Aa}}$	$7.51 \pm 1.23^{\text{Ba}}$	$10.24 \pm 1.68^{\text{Ca}}$	$13.25 \pm 2.18^{\text{Da}}$	$9.04 \pm 1.49^{\text{a}}$
	Non-conceived	38	$0.73 \pm 0.12^{\text{Ab}}$	$2.46 \pm 0.40^{\text{Db}}$	$1.17 \pm 0.19^{\text{Bb}}$	$2.11 \pm 0.34^{\text{Cb}}$	$1.62 \pm 0.26^{\text{b}}$
	Overall	75	$^{\text{X}}2.92 \pm 0.27^{\text{A}}$	$^{\text{X}}4.95 \pm 0.32^{\text{B}}$	$^{\text{Y}}5.64 \pm 0.54^{\text{B}}$	$^{\text{X}}7.61 \pm 0.71^{\text{C}}$	$^{\text{XY}}5.28 \pm 0.46$
Jaffarabadi Buffalo	Conceived	25	$4.95 \pm 0.99^{\text{Aa}}$	$8.10 \pm 1.62^{\text{Ba}}$	$10.70 \pm 2.14^{\text{Ca}}$	$13.46 \pm 2.69^{\text{Da}}$	$9.30 \pm 1.86^{\text{a}}$
	Non-conceived	50	$1.13 \pm 0.18^{\text{Ab}}$	$2.32 \pm 0.37^{\text{Cb}}$	$1.05 \pm 0.17^{\text{Ab}}$	$1.89 \pm 0.30^{\text{Bb}}$	$1.6 \pm 0.26^{\text{b}}$
	Overall	75	$^{\text{Y}}2.40 \pm 0.23^{\text{A}}$	$^{\text{Y}}4.25 \pm 0.34^{\text{B}}$	$^{\text{Z}}4.27 \pm 0.54^{\text{B}}$	$^{\text{Y}}5.75 \pm 0.67^{\text{C}}$	$^{\text{Y}}4.17 \pm 0.45$

Values with different superscripts in upper case within row (A, B, C, D) differ significantly ($p < 0.05$).

Values with different superscripts (a, b) within the column differ significantly for a particular species ($p < 0.05$), and those (X, Y, Z) within the column differ significantly between breed/species ($p < 0.05$).

Table 2: Average peripheral levels of pregnancy-associated glycoprotein (ng/mL) in Gir cows and Jaffarabadi buffaloes on days 28 and 36 after AI in conceived and non-conceived animals (Mean \pm SEM)

Species	Status of Animals	No. of Animals	Days Post-AI		Overall
			Day 28	Day 36	
Gir cow	Conceived	37	5.27 \pm 0.87 ^{Aa}	9.93 \pm 1.63 ^{Ba}	7.60 \pm 1.25 ^a
	Non-conceived	38	0.53 \pm 0.09 ^{Ab}	0.51 \pm 0.08 ^{Bb}	0.52 \pm 0.08 ^b
	Overall	75	2.87 \pm 0.33^{AY}	5.16 \pm 0.63^{BX}	4.02 \pm 0.48^X
Jaffarabadi buffalo	Conceived	25	4.48 \pm 0.9 ^{Aa}	6.96 \pm 1.39 ^{Ba}	5.72 \pm 1.15 ^a
	Non-conceived	50	0.49 \pm 0.08 ^{Ab}	0.7 \pm 0.11 ^{Bb}	0.60 \pm 0.10 ^b
	Overall	75	1.82 \pm 0.25^{AZ}	2.79 \pm 0.42^{BZ}	2.31 \pm 0.46^Z

Values with different superscripts in upper case within row (A, B) differ significantly ($p < 0.05$).

Values with different superscripts (a, b) within the column differ significantly for a particular species ($p < 0.05$), and those (X, Y, Z) within the column differ significantly between breed/species ($p < 0.05$).

conceived animals were significantly ($p < 0.05$) lower than those in conceived animals, reflecting that higher PAG levels are associated with viable pregnancy. When looking at all 75 animals, the overall PAG levels increased significantly ($p < 0.05$) from Day 28 to Day 36 in Gir cows (2.87 \pm 0.33 to 5.16 \pm 0.63 ng/mL) and in Jaffarabadi buffaloes (1.82 \pm 0.25 to 2.79 \pm 0.42 ng/mL). These values also differed significantly ($p < 0.05$) between species, being higher in cows, highlighting a species-specific difference in PAG levels. This trend suggests that PAG measurement can be a useful biomarker for early pregnancy detection in both cows and buffaloes, as the levels were markedly higher in pregnant individuals and showed a clear increase over time.

These findings were consistent with prior research highlighting PAG as a vital biomarker for early pregnancy detection in cattle and buffaloes (El-Battawy *et al.*, 2009). The observed rise in PAG levels in conceived Jaffarabadi buffaloes aligned with similar patterns reported by Barile *et al.* (2021) and Borakhatariya *et al.* (2024). Similarly, Shahin *et al.* (2014) and Ayad *et al.* (2021) recorded significantly higher PAG concentrations ($p < 0.001$) in pregnant HF cattle compared to non-pregnant ones between days 25 and 50 post-AI using an ELISA. The findings of Barbato *et al.* (2017) also indicate that pregnancy status has a profound impact on plasma PAG in buffaloes with values of 2.5 \pm 0.0 ng/mL and 0.0 \pm 0.0 ng/mL on day 28 in pregnant and non-pregnant buffaloes, respectively. The reports by Pohler *et al.* (2016) and Filho *et al.* (2019) collectively indicate that animals experiencing successful conception exhibited higher levels of pregnancy markers such as pregnancy-specific protein B and PAGs compared to those undergoing embryonic mortality. Conversely, studies by Karen *et al.* (2014) and Reese *et al.* (2018) reported no significant differences in pregnancy markers levels between conceived animals and those that experienced embryonic mortality. These differing outcomes suggest that while elevated pregnancy markers levels are commonly associated with conception success, individual variations and experimental conditions may influence the relationship between these markers and pregnancy outcomes.

Effect of Post-Insemination Interval on PAG & P₄ in Cows and Buffaloes

The data in Table 3 reveal highly significant effects of post-insemination intervals ($p < 0.01$) on plasma levels of PAG and progesterone (P₄) in pregnant and non-pregnant animals. In pregnant Gir cows and Jaffarabadi buffaloes, PAG levels differed significantly ($p = 0.000$) between Days 28 and 36 post-AI. Conversely, non-pregnant animal showed no significant difference ($p = 0.620$, $p > 0.05$) in PAG levels between these two-time points. Progesterone levels, however, showed significant differences ($p = 0.000$) between Days 21, 28, 36, and 45 post-AI in both pregnant and non-pregnant cows and buffaloes. These findings highlight the importance of considering time-dependent changes when using these parameters as diagnostic markers for pregnancy. The findings reported by Utt *et al.* (2009) highlight the same findings of progesterone in early pregnancy of cattle with the significant rise in serum levels from Days 7 to 33 post-AI. This progressive increase is crucial for preparing the uterine environment for implantation and sustaining the pregnancy. As per Lopes *et al.* (2007) plasma progesterone concentration was continuously increasing from day 0, 8, 15, 22, 29, 36 and 64 in pregnant cows compared to non-pregnant cows which is in line with the trend of our findings. Progressive rise of PAG levels in cows and buffaloes during early to mid-pregnancy was noticed by Breukelman *et al.* (2005), Green *et al.* (2010) and Casano *et al.* (2022), which proved the effect of post-insemination interval on PAG.

Correlation between PAG & P₄

The findings of correlation analysis of plasma PAG and P₄ in pregnant and non-pregnant animals in both Gir and Jaffarabadi breeds are shown in Table 4. In pregnant cows and buffaloes, the PAG levels on Day 36 and 28 post-AI were positively correlated ($r = 0.769$, 0.796 ; $p < 0.01$). No significant correlations were observed between PAG levels on Days 28 and 36 post-AI with plasma P₄ profile on any of the days studied, except in buffaloes, PAG levels on Day 28 and P₄ levels on Day 36 post-AI had significant positive correlation



Table 3: Analyses of variance showing effects of post-insemination intervals on plasma levels of PAG (Days 28, 36) and progesterone (P_4 , Days 21, 28, 36, 45) in pregnant and non-pregnant cows and buffaloes

Spp.	Parameters and Days post-AI	Source of Variance	Pregnant				Non-pregnant					
			Degree of Freedom	Sum of Squares	Mean Square	'F' value	Sig. 'P vs NP'	Degree of Freedom	Sum of Squares	Mean Square	'F' value	Sig. 'P vs NP'
Gir cow	PAG (D 28, 36)	Between periods	1	401.88	401.88	40.44	0.000	1	0.010	0.10	0.25	0.620
		Within period	72	715.45	9.94	-	-	74	3.05	0.04	-	-
		Total	73	1117.32	-	-	-	75	3.06	-	-	-
	P_4 (D 21, 28, 36, 45)	Between periods	3	1353.45	451.15	106.01	0.000	3	73.58	24.53	77.97	0.000
		Within period	144	612.82	4.26	-	-	148	46.55	0.31	-	-
		Total	177	196.27	-	-	-	151	120.13	-	-	-
Jaffri buffalo	PAG (D 28, 36)	Between periods	1	77.00	77.00	9.75	0.003	1	1.094	1.094	19.06	0.000
		Within period	48	379.05	7.90	-	-	98	5.63	0.06	-	-
		Total	49	456.06	-	-	-	99	6.72	-	-	-
	P_4 (D 21, 28, 36, 45)	Between periods	3	991.10	330.37	82.65	0.000	3	56.19	18.73	114.15	0.000
		Within period	96	383.74	3.99	-	-	196	32.16	0.164	-	-
		Total	99	1374.83	-	-	-	199	88.34	-	-	-

Table 4: Correlations of plasma PAG and progesterone on different days post-AI in pregnant and non-pregnant Gir cows and Jaffarabadi buffaloes

Species	Parameters	PAG-28	PAG-36	P_4 -21	P_4 -28	P_4 -36	P_4 -45
Correlations: Non-Pregnant cows							
Gir cows	PAG Day 28	1	0.011	-0.029	0.156	0.019	0.060
	PAG Day 36	0.769**	1	-0.153	0.266	0.087	0.245
	P_4 Day 21	0.142	0.046	1	-0.185	-0.425**	-0.093
	P_4 Day 28	0.013	0.041	-0.332*	1	0.373*	0.060
	P_4 Day 36	-0.060	0.049	-0.300	0.494**	1	0.422**
	P_4 Day 45	-0.141	-0.128	0.077	0.190	0.091	1
Correlations: Pregnant cows							
Correlations: Non-Pregnant buffaloes							
Jaffarabadi buffaloes	PAG Day 28	1	-0.060	-0.044	0.027	0.526**	0.028
	PAG Day 36	0.796**	1	-0.043	0.088	-0.004	0.054
	P_4 Day 21	0.712**	0.572**	1	0.357*	0.086	-0.055
	P_4 Day 28	0.645**	0.583**	0.748**	1	0.205	0.512**
	P_4 Day 36	0.788**	0.711**	0.878**	0.848**	1	0.076
	P_4 Day 45	0.683**	0.550**	0.827**	0.818**	0.908**	1
Correlations: Pregnant buffaloes							

Correlation * & ** significant at $p < 0.05$ and $p < 0.01$ level (2-tailed), respectively.

($r = 0.788$, $p < 0.01$). In Gir cows, P_4 levels on Day 28 were negatively correlated ($r = -0.332$, $p < 0.05$) with P_4 levels on Day 21 post-AI, and those on Day 36 were positively correlated ($r = 0.494$, $p < 0.01$) with P_4 levels on Day 28 post-AI. Similarly in buffaloes, progesterone levels on Day 45 post-AI were strongly correlated ($r = 0.908$, $p < 0.01$) with P_4 levels on Day 36 post-AI.

In non-pregnant cows, P_4 levels on Day 36 post-AI were negatively correlated ($r = -0.425$, $p < 0.01$) with P_4 levels on

Day 21 post-AI. Progesterone levels on Day 45 post-AI were high significantly correlated ($r = 0.422$, $p < 0.01$) with P_4 levels on Day 36 post-AI, and those on Day 36 were positively correlated ($r = 0.373$, $p < 0.05$) with P_4 levels on Day 28 post-AI. In non-pregnant buffaloes, P_4 levels on day 28 post-AI were significantly correlated ($r = 0.357$, $p < 0.05$) with P_4 levels on day 21 post-AI. Progesterone levels on day 45 post-AI were high significantly correlated ($r = 0.512$, $p < 0.01$) with P_4 levels on day 28 post-AI. These results suggest that plasma

PAG and progesterone levels can provide valuable insights into pregnancy status and reproductive physiology in Gir cows. The correlations observed in non-pregnant cows highlight the importance of considering hormonal dynamics in reproductive management. The positive correlation between PAG levels on Days 28 and 36 post-AI in pregnant cows supports previous findings (Green *et al.*, 2010). Also, the negative correlation between P₄ levels on Days 28 and 21 post-AI in pregnant cows may indicate a shift in hormonal dynamics during early pregnancy.

CONCLUSION

The findings of the current study revealed that the BovEasy bovine rapid PD kits give very high (100%) accuracy, sensitivity, and specificity in detecting early pregnancy in bovine at 32 days Post-AI and that 4.7% embryos were lost between days 32 and 45 of gestation. The plasma P₄ and PAG levels were significantly higher in conceived than non-conceived animals in both Gir cows and Jaffarabadi buffaloes suggesting that PAG can serve as a reliable biomarker for early pregnancy detection, with levels rising as pregnancy progresses from Day 28 to Day 36. Pregnancy status significantly affects P₄ and PAG levels in both the species, with consistent differences between pregnant and non-pregnant animals. Both PAG (notably on Days 28 and 36) and P₄ (on Days 21, 28, 36, and 45) showed strong discrimination ability, making them effective biomarkers for early pregnancy detection, showing close positive association with the results of BovEasy rapid early PD kit on day 32 and as confirmed by actual rectal palpation on day 45 post-AI. The differences in P₄ and PAG levels become more pronounced as pregnancy advances.

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