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Short communication

A new perspective on the association between radio-immuno and ELISA progesterone assays in Indonesian goats

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Abstract

The Indonesian Kacang goat (*Capra hircus*) is fecund and is perfect for tropical countries but its production and population have not been optimised. Livestock reproductive improvement requires hormone monitoring. The enzyme-linked immunosorbent assay (ELISA) and radio-immuno assay (RIA) are required for precise progesterone quantification. To our knowledge, this is the first study to compare progesterone estimation methods in Indonesian Kacang goats and correlate them with corpus luteum size throughout an oestrus cycle standardized using oestrus synchronisation. All periods showed a high ELISA–RIA correlation. The data also showed that CL and progesterone concentrations were highly correlated until day 8 but uncorrelated thereafter. Our study found that the two methods were qualitatively similar but required different quantitative range standards. This research emphasizes that the comparison of progesterone hormone test results will differ when different assays are used and data on the range of deviation using these two methods is required.

Keywords: hormone analysis, Kacang goats, reproductive hormones, tropical goat breed
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Introduction

The Indonesian Kacang goat (*Capra hircus*) is fecund and is perfect for tropical countries but its production and population have not been optimised. Assessing reproductive and physiological data and monitoring reproductive hormone levels improve goat production and population (Santoso *et al*., 2021). Monitoring the reproductive hormone levels is essential for improvements in reproductive management (Sitaresmi *et al*., 2017; Kumala *et al*., 2022). Progesterone (P4) is a primary gonadal hormone produced by the corpus luteum of the ovaries and placenta during pregnancy and is a 21-carbon steroid hormone that is endogenously generated from cholesterol via pregnenolone. The measurement of P4 in animals at various physiological stages is regarded as one of the most essential indicators of reproductive status (Sitaresmi *et al*., 2017; Kolatorova *et al*., 2022). Consequently, precise quantification of P4 is essential for clinical or research practice. Immunoassay techniques (quantitative or qualitative assessment methods depending on the use of antibodies), such as the radio-immuno assay (RIA) and enzyme-linked immunosorbent assay (ELISA), have been widely used for steroid hormone analysis. RIA is highly accurate and precise; however, this approach is complex and time-consuming, necessitates the use of radioactive substances, and carries the risk of radiation exposure (Sakamoto *et al*., 2018; Benabdelaziz *et al*., 2020). ELISA is a more convenient and widespread approach than RIA to resolve these issues. ELISA is commonly used for the detection and

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quantification of proteins (Khatun *et al*., 2009; Aydin, 2015). A biochemical assay employing solid-phase enzyme immunoassay (EIA) is easy to perform and can handle a large number of samples in parallel through a single trough. An enzyme-labelled antibody specific to the analyte of interest combines with a substrate to produce a detectable and quantifiable response (Nemeth *et al*., 2014). RIAs and ELISAs have been widely utilized for the detection and quantification of biomarkers; there are distinctions between both approaches that should be considered when evaluating and choosing an assay protocol. Differences between data from different studies have occurred according to the use of different methodologies for quantifying goat progesterone and must be analysed further to determine which method should be used, in addition to clarifying the respective standard ranges (Warnken *et al*., 2016). Corpus luteum (CL) emergence by angiogenesis is strongly linked to plasma P4 levels and endothelial cell death is engaged through functional connectivity luteolysis (Goel and Kharche, 2012; Salzano *et al*., 2020). The primary goal of the current study was to compare the ELISA and conventional RIA methods for assessing P4 content to determine whether the ELISA approach could successfully substitute RIA. To the best of our knowledge, this is the first study to compare the different techniques for measuring P4 levels in Kacang goats in Indonesia.

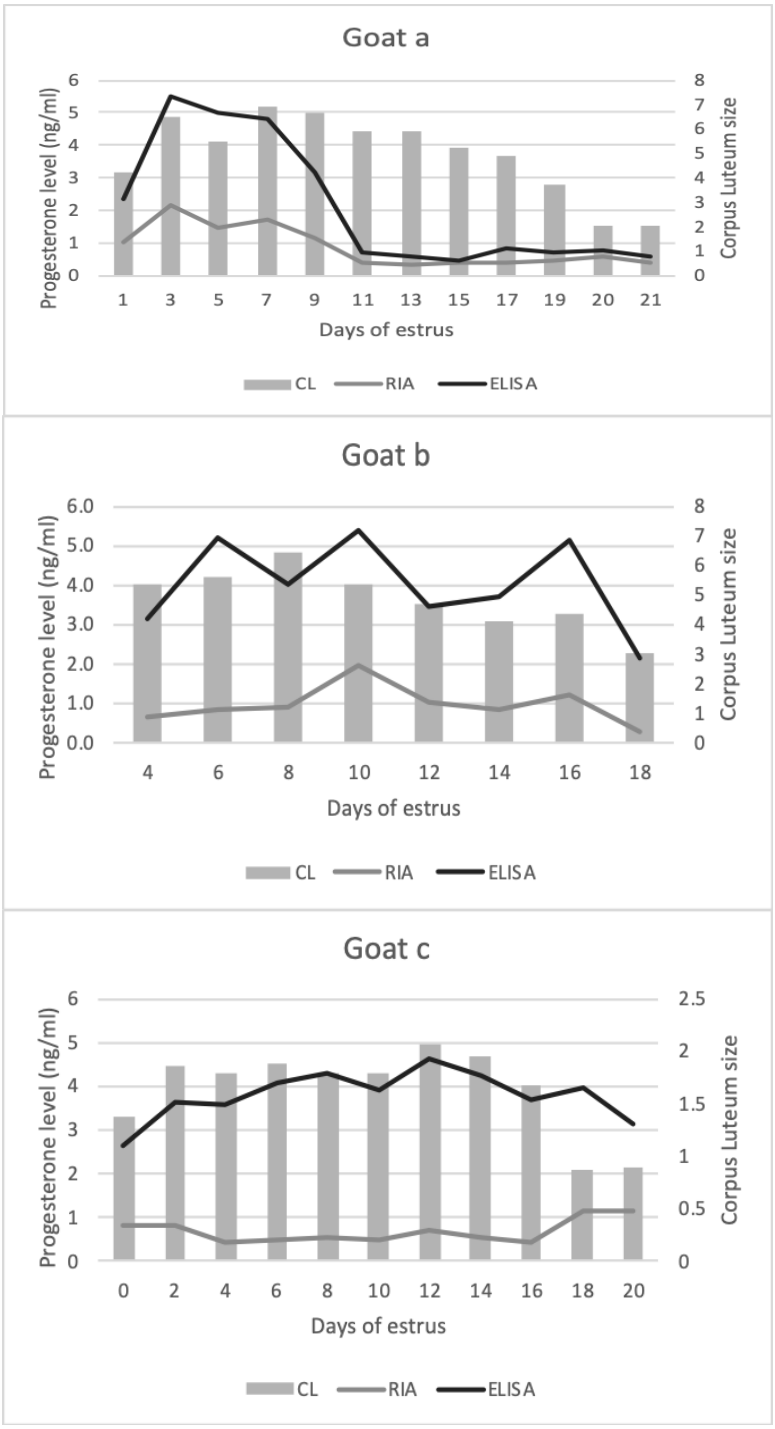
Materials and Methods

All procedures described in this manuscript were approved by the Ethics Committee of the National Research and Innovation Agency (BRIN) with clearance number EC BRIN 082/KE.02/SK/10/2022. The study was carried out using six female Kacang goats between 2–3 years of age, 15–20 kg body weight, that had given birth, and had a normal reproductive cycle. Synchronization of oestrus was performed by injecting PGF2α (Noroprost® 0.5%, Norbrook, UK) intramuscularly at a dose of 0.5 mg/kg body weight. Blood sampling (n:60) was conducted two days apart and intensified every day before the onset of oestrus (pro-oestrus). The plasma was poured into a 2-ml microtube, then immediately stored at -20 °C until analysis in the laboratory.

Progesterone hormone levels were analysed using the RIA Progesterone [125I] kit (Izotope, Hungary) and ELISA Progesterone Kit (DRG, Germany). The procedures for commonly-produced ELISA techniques (progesterone for goat KIT, DRG, Germany) were adjusted for use in this study (Sitaresmi *et al*., 2020) using completely automated enzyme immunoassay processing of microtiter plates. The RIA was performed in accordance with the procedures described in the Progesterone Kit - 25I (Izotope, Hungary) (Santoso *et al*., 2021). An ultrasound machine (ALOKA model SSD-500, ALOKA Co., Ltd, Japan) with 7.5 MHz linear probe (ALOKA Co., Ltd, Japan) was used. Observation records were printed using a thermal printer (SONY UP-895 MD, Japan). The daily amount and diameter of the CL were measured. The oestrus period in goats was categorized into three CL development stages (days 0–7; 8–12; >12), as described by Jiang *et al*. (2021). Progesterone levels determined using the two different methods, CL size, and days of the oestrus cycle were statistically compared to facilitate multiple comparisons. Two-tailed tests were considered statistically significant when the *P*-value was <0.05. The data were analysed using Pearson correlation coefficients to identify the correlation between CL size and P4 values using RIA and ELISA. All statistical analysis was performed using SPSS Ver. 25.

Results and Discussion

This study showed a correlation between ELISA and RIA in the measurement of P4 hormone levels during the oestrus cycle in Kacang goats, with ELISA levels being substantially higher than RIA levels, but the graph patterns were similar (Table 1; Figures 1–6). This pattern concurs with several similar investigations utilizing other hormones (Warnken *et al*., 2016) and is corroborated by the strong correlations on P4 concentrations along the developmental period of the CL (F_{prob} values: 0.72–0.78) (Goel and Kharche, 2012; Brunner *et al*., 2022). This variation in results is possibly due to the fact that ELISA detection technology permits the utilization of antibodies in greater amounts than those enabled by RIA's radiotracer, which gives ELISA a broader spectrum than RIA, especially for concentrations >2 ng/ml. This makes a difference in high P4 concentrations using the two approaches, although the pattern of response would be similar, as occurs in insulin analysis in mares (Warnken *et al*., 2016).


Goat a
Progesterone level (ng/ml)
Corpus Luteum size
Days of estrus
CL
RIA
ELISA
Goat b
Progesterone level (ng/ml)
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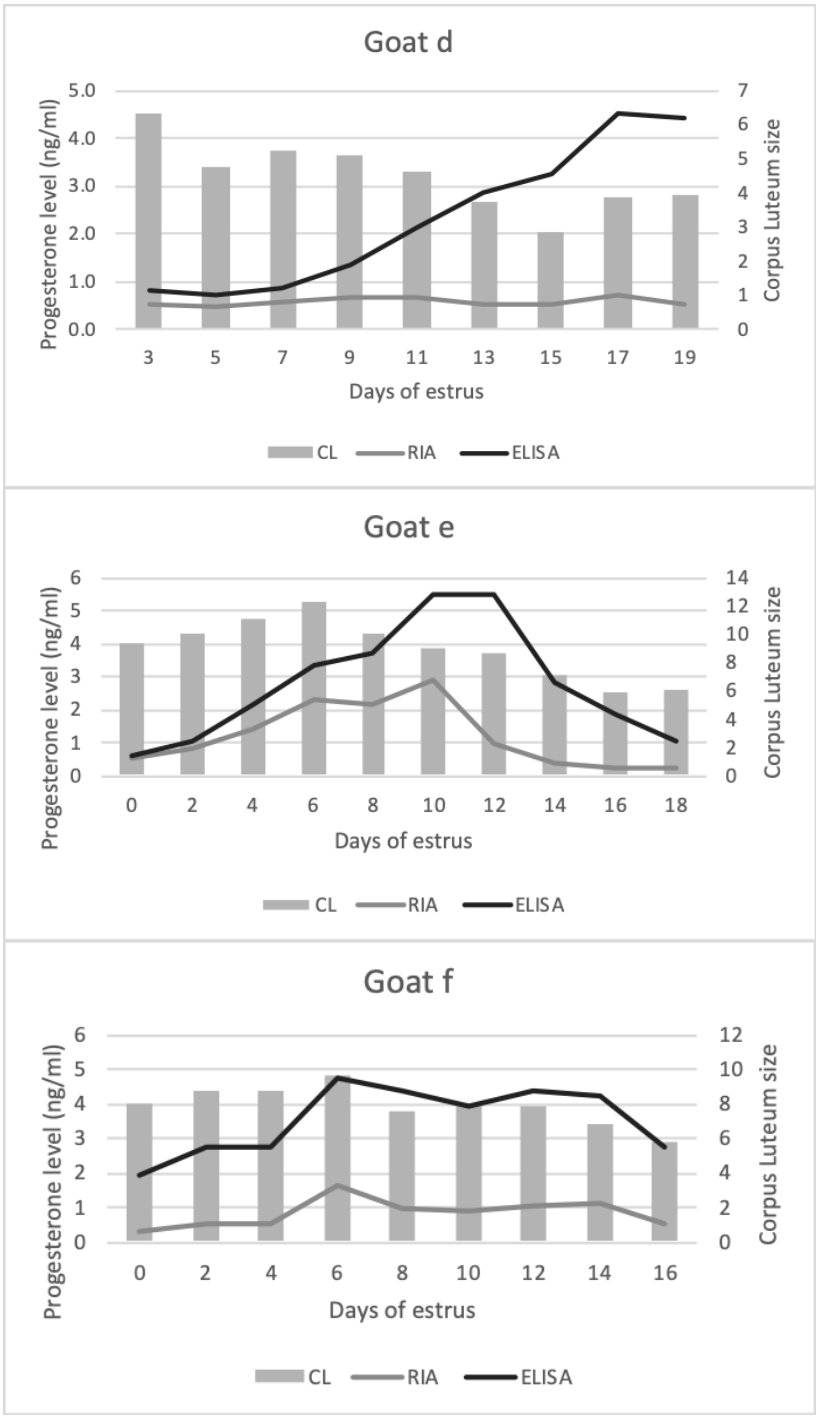

Figures 1–6. Luteal diameter and plasma progesterone concentration as determined using an enzyme-linked immunosorbent assay (ELISA) and radio-immuno assay (RIA) during the luteolysis period in each Kacang goat. Data are normalized to the time of luteolysis; *P <0.05; luteal diameter and plasma P_4 concentration

Table 1. Progesterone level (P4) and corpus luteum (CL) diameter in each period of CL development

	Days 0–7 (n = 42)		Days 8–12 (n = 32)		Days 13–20 (n = 46)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
CL (cm^2)	4.28 ± 0.56[b]	4.05–4.52	4.11 ± 0.5[b]	3.30–5.00	2.89 ± 0.93[a]	1.50–5.30
P4 (ng/ml)						
RIA	1.51 ± 1.30[ab]	0.19–5.37	1.87 ± 1.78[b]	0.21–6.81	0.72 ± 0.46[a]	0.19–2.21
ELISA	4.05 ± 2.86[ab]	1.09–9.57	5.77 ± 3.910[b]	1.02–9.57	3.34 ± 2.39 [a]	0.64–8.42

CL: corpus luteum diameter; P_4: progesterone content
[a, b] significant difference in data values on the same period in each row

The different results of these approaches also indicate that interference with a single form of investigation can result from the sensitivity of antibodies or even from the solution in which the substance is deposited (Frank, 2011; Borer-Weir *et al*., 2012). The current study found a strong correlation between CL size and P4 measured using ELISA and RIA on days 0–8 of oestrus (Table 1), but this correlation weakened the following day. CL size increased markedly from day 0 to day 8 and was positively correlated with P4 content determined using RIA and ELISA (Table 2; F values: 0.64 and 0.59, respectively). Nevertheless, no further growth was observed from days 8 to 12, indicating that by day 8, the CL had reached maturity and had begun to decrease (Jiang *et al*., 2016; Orita *et al*., 2000). Although the size of the embryonic CL barely increased from day 8 to day 12, the blood P4 content increased, indicating continued hormonal activity throughout this phase. According to previous studies, the CL reaches its maximum size on day 8, and its maximum progesterone level on day 12. At each time point, a wide variety of luteal weights and plasma progesterone concentrations were measured, highlighting the heterogeneous patterns of luteal growth and function in cows. The current study showed that CL ceased to develop 6–8 d after ovulation and continued to diminish 15 d after ovulation. The CL matures approximately 7 and 5 days post-ovulation in cattle (Cuervo-Arango *et al*., 2011) and ewes (Jiang *et al*., 2016; Orita *et al*., 2000), respectively, and regressed nearly 20 and 11 days after ovulation in cows and sheep, respectively. This is generally considered to correlate with elevated blood progesterone levels (Arashiro *et al*., 2010).

Table 2. Correlation of enzyme-linked immunosorbent assay (ELISA) and radio-immuno assay (RIA) progesterone levels (ng/ml) and CL diameter (mm) throughout oestrus in Indonesian Kacang goats

Covariate	Days 0–7 (n = 42)			Days 8–12 (n = 32)			Days 13–20 (n = 46)		
	RIA	ELISA	CL	RIA	ELISA	CL	RIA	ELISA	CL
RIA	-	0.73**	0.64**	-	0.78**	-0.18	-	0.78	0.44
ELISA	0.73**	-	0.59**	0.78**	-	-0.31	0.78	-	0.32
CL	0.64**	0.59**	-	-0.18	-0.31	-	0.44	0.32	-

** indicates had a statistically significant correlation of the concentration of progesterone and the size of the corpus luteum at the same period or day of oestrus

Conclusion

Considering that many studies have evaluated the association between CL size and systemic progesterone content, these results are inconsistent. Our study concluded that, qualitatively, the two methods were relatively similar (note that the spike and decline patterns in each of the methods were similar), but it is necessary to calculate the appropriate range standard quantitatively in each method.

Conflict of Interests

The authors declare that they have no conflict of interest

Authors' Contribution

S and PIS designed the study, interpreted the data, and drafted the manuscript; DAM, RIA, and FBL were involved in collection of data and also contributed to manuscript preparation. S, PIS, H, and FAP took part in preparation and critical checking of the final manuscript.

REFERENCES

Arashiro, E.K.N., Viana, J.H.M., Fonseca, J.F., Camargo, L.S., Fernandes, C.A., Brandão, F.Z., 2010. Luteal dynamics in goats: Morphological and endocrine features. Rev Bras Zootec. 39(9), 1937–1942. https://doi.org/10.1590/s1516-35982010000900011

Aydin, S., 2015. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides. 72, 4–15. https://doi.org/10.1016/j.peptides.2015.04.012

Benabdelaziz, A., Boudjemai, S., Khelili, R., Besbaci, M., Kaidi, R., 2020. Development of in-house RIA kit for progesterone in cow's skim milk. J Immunoass Immunochem. 41(2), 195–207. https://doi.org/10.1080/15321819.2019.1708386

Borer-Weir, K.E., Bailey, S.R., Menzies-Gow, N.J., Harris, P.A., Elliott, J., 2012. Evaluation of a commercially-available radioimmunoassay and species-specific ELISAs for measurement of high concentrations of insulin in equine serum. Am J Vet Res. 73(10), 1596–1602. https://doi.org/10.2460/ajvr.73.10.1596

Brunner, M., Moser, O., Raml, R., Haberlander, M., Boulgaropoulos, B., Obermayer-Pietsch, B., Svehlikova, E., Pieber, T.R., Sourij, H., 2022. Assessment of two different glucagon assays in healthy individuals and Type 1 and Type 2 diabetes patients. Biomolecules. 12(3), 1–8. https://doi.org/10.3390/biom12030466

Cuervo-Arango, J., García-Roselló, E., García-Muñoz, A., Valldecabres-Torres, X., Martínez-Ros, P., González-Bulnes, A., 2011. The effect of a single high dose of PGF2α administered to dairy cattle 3.5 days after ovulation on luteal function, morphology, and follicular dynamics. Theriogenology. 76(9), 1736–1743. https://doi.org/10.1016/j.theriogenology.2011.07.006

Frank, N., 2011. Equine metabolic syndrome. Vet Clin North Am - Equine Pract. 27(1). https://doi.org/10.1016/j.cveq.2010.12.004

Goel, A.K., Kharche, S.D., 2012. Ovulatory pattern and serum progesterone levels during oestrous cycle in Jamunapari goats. Indian J Anim Sci. 82(5), 468–471.

Jiang, Y.F., Hsu, M.C., Cheng, C.H., Tsui, K.H., Chiu, C.H., 2016. Ultrastructural changes of goat corpus luteum during the estrous cycle. Anim Reprod Sci. 170, 38–50. https://doi.org/10.1016/j.anireprosci.2016.04.001

Jiang, Y.F., Yu, P.H., Budi, Y.P., Chiu, C.H., Fu, C.Y., 2021. Dynamic changes in mitochondrial 3D structure during folliculogenesis and luteal formation in the goat large luteal cell lineage. Sci Rep. 11(1), 1–10. https://doi.org/10.1038/s41598-021-95161-w

Khatun, S., Nara, S., Tripathi, V., Rangari, K., Chaube, S.K., Kariya, K.P., Kumar, S., Shrivastav, T.G., 2009. Development of ELISA for measurement of progesterone employing 17 - OH-P-HRP as enzyme label. J Immunoass Immunochem. 30(2), 186–196. https://doi.org/10.1080/15321810902782889

Kolatorova, L., Vitku, J., Suchopar, J., Hill, M., Parizek, A., 2022. Progesterone: A steroid with wide range of effects in physiology as well as human medicine. Int J Mol Sci. 23(14), 1–36. https://doi.org/10.3390/ijms23147989

Kumala, S., Sitaresmi, P.I., Suranindyah, Y., Widayati, D.T., 2022. Profiles of steroid hormones on follicular and luteal phase in Saanen Etawah crossbred (SAPERA) does. In: Proc 6th Int Semin Anim Nutr Feed Sci (ISANFS 2021). Vol. 21. [place unknown]. https://doi.org/10.2991/absr.k.220401.041

Nemeth, A., Cziraki, A., Sulyok, E., Szabados, S., Horvath, I., Nasri, A., Rauh, M., Rascher, W., 2014. P205Adipokines in patients undergoing on-pump and off-pump coronary artery bypass grafting. Cardiovasc Res. 103(1), 1382–1388. https://doi.org/10.1093/cvr/cvu082.139

Orita, J., Tanaka, T., Kamomae, H., Kaneda, Y., 2000. Ultrasonographic observation of follicular and luteal dynamics during the estrous cycle in Shiba goats. J Reprod Dev. 46(1), 31–37. https://doi.org/10.1262/jrd.46.31

Sakamoto, S., Putalun, W., Vimolmangkang, S., Phoolcharoen, W., Shoyama, Y., Tanaka, H., Morimoto, S., 2018. Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. J Nat Med. 72(1), 32–42. https://doi.org/10.1007/s11418-017-1144-z

Salzano, A., Russo, M., Anglani, G., Licitra, F., Zullo, G., Cotticelli, A., Fatone, G., Campanile, G., 2020. Early prediction of corpus luteum functionality using an imaging software. Front Vet Sci. 7, 1–7. https://doi.org/10.3389/fvets.2020.00299

Santoso, S., Herdis, H., Arifiantini, R.I., Gunawan, A., Sumantri, C., 2021. Characteristics and potential production of frozen semen of Pasundan bull. Trop Anim Sci J. 44(1), 24–31. https://doi.org/10.5398/tasj.2021.44.1.24

Sitaresmi, P.I., Widyobroto, B.P., Bintara, S., Widayati, D.T., 2017. Progesterone and biochemical profile of Ettawa-Saanen crossbreed goats in Turi area, Yogyakarta-Indonesia. Int J Dairy Sci. 12(4), 289–294. https://doi.org/10.3923/ijds.2017.289.294

Sitaresmi, P.I., Widyobroto, B.P., Bintara, S., Widayati, D.T., 2020. Effects of body condition score and oestrus phase on blood metabolites and steroid hormones in Saanen goats in the tropics. Vet World. 13(5), 833–839. https://doi.org/10.14202/vetworld.2020.833-839

Warnken, T., Huber, K., Feige, K., 2016. Comparison of three different methods for the quantification of equine insulin. BMC Vet Res. 12(1), 1–10. https://doi.org/10.1186/s12917-016-0828-z