Short Communication

Relationship between luteal size and plasma progesterone concentration in pregnant and non-pregnant Indian crossbred dairy cows

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Abstract

The relationship between luteal size and plasma progesterone concentration in purebred cows has been investigated previously. However, there is limited information on this topic in Indian crossbred cows. This study aimed to evaluate the relationship between luteal size parameters (diameter and area) and plasma progesterone concentration in healthy pregnant and non-pregnant Indian crossbred cows. Fifty healthy lactating crossbred cows were artificially inseminated after estrus detection and retrospectively classified into pregnant (n=29) and non-pregnant (n=21) based on the results of ultrasonographic pregnancy diagnosis. Data on luteal diameter, luteal area, and plasma progesterone concentration collected on Days 7, 14, and 20 in non-pregnant cows and on Days 7, 14, 20, 25, 30, 35, 40, and 60 in pregnant cows were analyzed. Spearman’s correlation test was used to evaluate the association between the corpus luteum size parameters and the plasma progesterone concentration. Luteal area was positively correlated with plasma progesterone concentration in both pregnant (ρ=0.48, P<0.001) and non-pregnant (ρ=0.43, P=0.001) cows. Luteal diameter had a relatively weaker correlation with plasma progesterone concentration in both pregnant (ρ=0.45, P<0.001) and non-pregnant (ρ=0.24, P=0.058) cows.

These results suggest that luteal area is a better indicator of CL function than luteal diameter in healthy Indian crossbred cows.

Keywords: Bovine, corpus luteum, ultrasonography, pregnancy, progesterone
Introduction

Corpus luteum (CL) plays a pivotal role in the regulation of the estrous cycle and maintenance of pregnancy in cattle. Therefore, it is not surprising that luteal size and circulating progesterone concentration are frequently studied endpoints in bovine reproduction research. Luteal size in cattle is measured using transrectal ultrasonography (Pierson et al., 1988) and commonly expressed in terms of either luteal diameter (Taylor and Rajamahendran, 1991; Ricci et al., 2017; Zwiefelhofer et al., 2020) or luteal area (Kastelic et al., 1990; Ginther et al., 2012; Pugliesi et al., 2012; Rocha et al., 2019). Circulating progesterone concentrations are commonly determined using enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) of plasma or serum harvested from peripheral blood samples (Ginther et al., 2012; Pugliesi et al., 2012; Broes and LeBlanc, 2014). Several studies on the association between luteal size and circulating progesterone have been reported in pregnant and non-pregnant purebred cattle (Kastelic et al., 1990; Siqueira et al., 2009; de Tarso et al., 2016; Berger et al., 2017; Vrisman et al., 2018; Rocha et al., 2019). Similar information is lacking in Indian crossbred cattle that constitute about 26.6% of the total cattle population in the country as per the latest census report published by the Department of Animal Husbandry and Dairying in 2019. Therefore, the objective of this study was to evaluate the relationship between luteal size parameters (diameter and area) and plasma progesterone concentration in healthy pregnant and non-pregnant Indian crossbred cows.

Materials and methods

The Institutional Ethics Committee (IAEC/VGO/CVASC/181) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved all animal handling protocols and experimental procedures used in this study.
Animals and breeding management

Fifty healthy cyclic Indian crossbred dairy cows (4 to 7 years old, at least 60 days in milk, BCS 3 to 4) were enrolled. The enrolled cows were selected from a research herd after conducting transrectal ultrasonography and the white side test in order to only include cows that did not have any apparent reproductive abnormalities. Daily visual observation for behavioral signs of standing estrus was conducted in the morning and evening. Transrectal ultrasonography was performed to examine cows that were suspected to be in estrus. Cows with a preovulatory follicle (≥ 10 mm diameter) were bred twice through AI at 12 and 24 hrs after standing estrus. Pregnancy diagnosis was conducted using ultrasonography and the cows were retrospectively classified as pregnant (n=29) or non-pregnant (n=21). All cows were maintained under uniform husbandry conditions (housing and feeding) during the study.

Corpus luteum size and plasma progesterone concentration

Corpus luteum diameter and area were measured using built-in measurement functions in a portable ultrasound scanner (DIGI-600 M, PROVET; S.S. Medical Systems (I) Private Limited, India) equipped with a 5.0 MHz linear-array transducer. Progesterone concentration was measured using plasma samples harvested from blood collected in heparinized test tubes through jugular venipuncture. Commercial radioimmunoassay kits (M/s Beckman Coulter IM 1188) with analytical sensitivity of 0.03 ng/mL and including a highly specific progesterone antibody were used for progesterone estimation. Data on luteal diameter, luteal area, and plasma progesterone concentration collected on Days 7, 14, and 20 in non-pregnant cows and on Days 7, 14, 20, 25, 30, 35, 40, and 60 in pregnant cows were used to evaluate the association between corpus luteum size and plasma progesterone concentration.
Statistical analysis

Commercial statistical software (SPSS Version 16.0, SPSS Inc., Chicago, IL, USA) was used to conduct statistical analysis of the data. The data were tested for normality through the Shapiro-Wilk test, the results of which indicated that the data were not normally distributed. The relationships between corpus luteum size parameters and plasma progesterone concentration were evaluated using Spearman’s correlation test. Statistical significance was set at P-values less than 0.05. Results are presented as Spearman’s correlation coefficients and their associated P-values.

Results and Discussion

For each of the three parameters (luteal diameter, luteal area, and plasma progesterone concentration), a total of 63 observations in non-pregnant cows and 232 observations in pregnant cows were analyzed. There was a significant positive correlation between luteal area and plasma progesterone concentration in non-pregnant cows. In contrast, luteal diameter had a weak positive correlation with plasma progesterone concentration that approached significance. Luteal diameter and luteal area were positively correlated with each other, and the correlation was highly significant (Table 1). In pregnant cows, relatively stronger positive correlations were observed between luteal diameter, luteal area, and plasma progesterone concentration (Table 2). Similar to the observation in non-pregnant cows, luteal area was slightly more strongly correlated with plasma progesterone concentration than luteal diameter.

The associations between luteal size parameters and plasma progesterone concentration observed in the Indian crossbred cows in this study are similar to those reported previously in purebred dairy and beef cattle (Kastelic et al., 1990; Siqueira et al., 2009; de Tarso et al., 2016; Berger et al., 2017; Vrisman et al., 2018; Rocha et al., 2019). Our results suggest that luteal area
may be a better indicator of CL function than luteal diameter in pregnant and non-pregnant
Indian crossbred cows. Future studies including luteal size parameters and blood flow are
required to further our understanding of the relationship between luteal structure, vascularity, and
function in Indian crossbred cows.

Conflict of interest
The authors declare that they have no conflict of interest.

Data availability statement
The data that support the findings of this study are available from the corresponding author upon
reasonable request.

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Author contributions
Sanjay Agarwal: Conceptualization, Methodology, Investigation. Harihar Prasad Gupta: Funding
Acquisition, Project Administration, Supervision. Shiv Prasad: Project Administration,
Resources, Supervision. Irfan Ahmad Khan: Writing – Review and Editing. Afroza Khanam:
Data Curation, Visualization, Writing – Original Draft. Firdous Ahmad Khan: Conceptualization,
Formal analysis, Software, Writing – Review and Editing.
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Vrisman, DP; Bastos, NM; Rossi, GF; Rodrigues, NN; Borges, L; Taira, AR; de Paz, C; Nogueira, GP; Teixeira, P; Monteiro, FM and Oliveira, M (2018). Corpus luteum dynamics after ovulation induction with or without previous exposure to progesterone in prepubertal Nellore heifers. Theriogenology. 106: 60–68.

### TABLE 1. Correlations between luteal diameter, luteal area, and plasma progesterone concentration in non-pregnant Indian crossbred cows (n=21)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Spearman’s rho</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteal diameter and progesterone</td>
<td>0.24</td>
<td>0.058</td>
</tr>
<tr>
<td>Luteal area and progesterone</td>
<td>0.43</td>
<td>0.001</td>
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<tr>
<td>Luteal diameter and luteal area</td>
<td>0.69</td>
<td>&lt;0.001</td>
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### TABLE 2. Correlations between luteal diameter, luteal area, and plasma progesterone concentration in pregnant Indian crossbred cows (n=29)

<table>
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<tr>
<th>Parameters</th>
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<td>&lt;0.001</td>
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<tr>
<td>Luteal area and progesterone</td>
<td>0.48</td>
<td>&lt;0.001</td>
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<tr>
<td>Luteal diameter and luteal area</td>
<td>0.88</td>
<td>&lt;0.001</td>
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