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Gestational Follow-up in Ewes - Placentome Size and Fecal Progesterone Metabolite Levels

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ABSTRACT

***Background*:** Transabdominal ultrasonography in sheep allows real-time diagnosis and monitoring of pregnancy as well as measurement of the size of placentomes, which are structures that connect the fetal and maternal portions, that increase as pregnancy progresses. Progesterone is involved in the maintenance of gestation, with high levels noted during pregnancy. In this context, it is hypothesized that measurements of placentome and progesterone could help in monitoring pregnancy in sheep. Therefore, the main objectives of this study included evaluating whether placentome size and fecal progesterone levels are associated with gestational age in ewes and whether measurement of the placentome and progesterone could be used as an estimate of gestational age.

***Materials, Methods & Results*:** A total of 63 pregnant adult (between 2 and 5 years of age) Santa Inês ewes were monitored in the city of Boa Vista, Roraima. All ewes were kept in the paddock with *Panicum maximun* cv. tanzania, mineral salt, and water *ad libitum*, supplemented with 200 g/animal/day of soybean residue. Ewes were subjected to ultrasonography every 21 days from day 42 of pregancy until term to monitor pregnancy. Ultrasonography was performed using a linear probe to measure the placentome, including length × width in centimeters. Also every 21 days, fecal samples were collected for measurement of progesterone level. Feces were collected directly from the rectal ampulla, stored individually in plastic bags, and frozen at -20ºC until processing. In the laboratory, these feces samples were freeze-dried and subjected to hormonal extraction with 80% methanol. Then, the levels of fecal progesterone metabolites were measured by enzyme immunoassay. Data was tabulated and submitted to statistical analyses, including descriptive stastistic, correlation, Kruskal-Wallis test and Tukey's test. The levels of fecal progesterone metabolites increased significantly between the 3rd and 4th months of pregnancy, with a decrease close to delivery. Placentome size increased significantly between the 2nd and 3rd months of pregnancy. Placentome measurements did not correlate with progesterone levels and showed a weak correlation with gestational age. Therefore, we concluded that the measurements of placentome size and a single evaluation of fecal progesterone metabolites were not considered efficient methods for estimating gestational age.

***Discussion*:** Measurement of progesterone level in fecal samples is a non-invasive hormone monitoring method that provides values like that of blood levels and is less stressful for the animals at the time of sample collection. The wide physiological variation makes it difficult or even useless to use fecal progesterone, when is measured only once per animal, as an indicator of the presence of pregnancy. Despite this limitation, serial sampling is useful for monitoring of hormones during the pregnancy period and is an important tool for research purposes. Placentome size measurements correlated weakly with gestational age and did not correlate with progesterone level. Thus, the presence of placentomes is indicative of the presence of pregnancy but was considered inefficient for determining gestational age in sheep.

Keywords: hormones, pregnancy, sheep, ultrasound.

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INTRODUCTION

The most common method to diagnose pregnancy is ultrasound, as it is non-invasive, provides results in real time, is practical and fast. In ewes, ultrasonography is indicated from day 30, and it allows visualization of the conceptus, heartbeats, and embryonic/fetal attachments [6].

Progesterone is the main hormone responsible for maintaining pregnancy. In ewes, progesterone is initially produced by the corpus luteum and is secreted by the placenta from day 50 of pregnancy [15]. Plasma progesterone levels remain high throughout pregnancy. During the last 3 weeks, a decrease is noted in progesterone levels [1,5,10,14,17]. Blood progesterone levels are highly correlated with fecal progesterone levels [2,7], and fecal sample collection is a quick, non-invasive, and convenient method that causes minimal stress to animals.

Placentomes allow communication between the fetus and the mother and can be detected in ewes from day 32 of pregnancy [9]. In Santa Inês ewes, placentome was considered moderately reliable to estimate the gestational age between 3rd and 8th week [18]. After this period, placentome growth did not correlate with the gestational age [18]. However, other authors observed a weak correlation between placentome size and gestational age in mixed breed ewes, suggesting that placentome is not a good predictor of gestational age [9]. Factors such as animal breed and methodological differences in the definition of gestational age may have an influence on the results.

In this context, the present study aimed to evaluate: 1) whether placentome size and fecal progesterone levels are associated with gestational age in Santa Inês ewes; and 2) whether measurement of placentome size and fecal progesterone levels can be used as an estimate of gestational age.

MATERIALS AND METHODS

Animals

A total of 63 pregnant adult (between 2 and 5 years of age) Santa Inês ewes were monitored in the city of Boa Vista, Roraima. All ewes were kept in the paddock with *Panicum maximun* cv. tanzania, mineral salt, and water *ad libitum*, supplemented with 200 g/animal/day of soybean cleaning residue.

Ultrasonography examination

Ewes were subjected to ultrasonography every 21 days from day 42 post-mating until term to monitor pregnancy. Transabdominal ultrasonography was performed using the CTS900Neo®[1] device with a linear probe to measure the placentome, including length × width in centimeters. Placentomes that were close to the fetus were examined.

Fecal samples and progesterone measurement

Every 21 days feces samples were collected directly from the rectal ampulla, stored individually in plastic bags, and frozen at -20ºC until processing. In the laboratory, these feces samples were freeze-dried and subjected to hormonal extraction with 80% methanol. Then, the levels of fecal progesterone metabolites were measured by enzyme immunoassay using a CL425 antibody[2]. The applied protocols in sheep have been described previously [2].

Statistical analysis

A prospective observational study with primary data was conducted, collecting 180 data from 63 ewes, with measurements repeated over time. The measured parameters such as fecal metabolic hormone progesterone (MFP4) level and placentome size measurements (length, width, and area) were grouped to obtain descriptive statistics, as well as the response pattern throughout pregnancy (Figure 1).

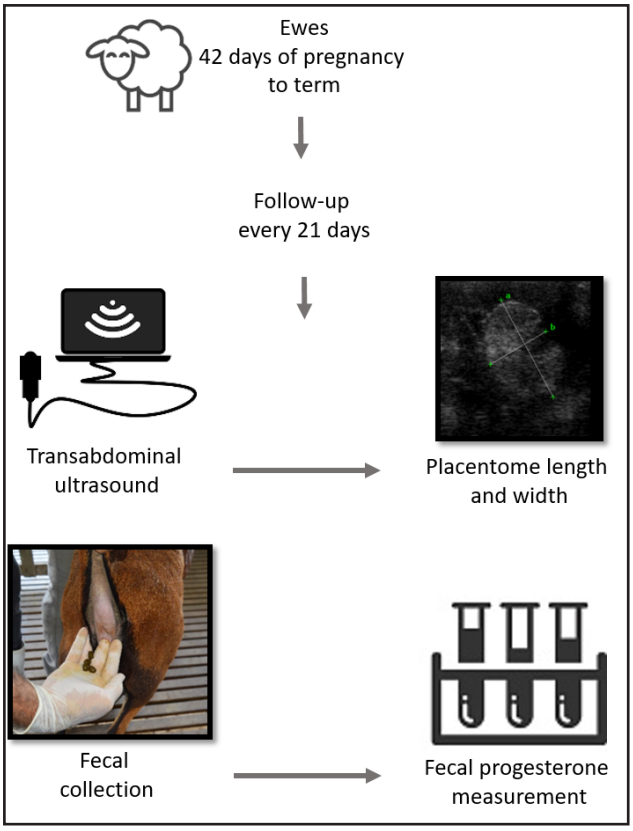

Figure 1. Schematic drawing of the experimental protocol including measurement of placentome size and fecal progesterone in pregnant ewes.

The correlation between the measured parameters and the pregnancy period was calculated. Hormone levels and placentome size measurements between months of gestation showed a non-normal response and were subjected to the Kruskal-Wallis test and Tukey's test (post-hoc test) when comparing median pairs. Significance value of 0.05 (Biostat program, IDSM[3]) was used.

RESULTS

Fecal metabolic progesterone levels and placentome size measurements by gestation month are described in Table 1. Progesterone (P4) levels increased during pregnancy and showed moderate (r = 0.469; $P < 0.01$) and significant correlation with gestation month.

Placentome size measurements such as length (r = 256), width (r = 0.194), and area (r = 0.175) showed a weak correlation with gestation month. Placentome length, width, and area did not correlate with MFP4 level. Placentome size increased significantly only between the 2nd and 3rd months of gestation (Figure 2).

Progesterone level increased significantly only between the 3rd and 4th months of gestation (Figure 3).

Table 1. Concentration (mean ± standard deviation) between fecal progesterone metabolism (MFP4) and placentome measurements by gestation month in Santa Inês ewes (n = 63).

Month of gestation	MFP4 level (ng/g feces)	Placentome size		
		Length (cm)	Width (cm)	Area (cm²)
2	1642.48 ± 363.29	1.36 ± 0.56	0.86 ± 0.13	4.65 ± 2.69
3	2808.3 ± 2008.87	2.82 ± 0.43	2.01 ± 0.38	18.07 ± 5.23
4	4493.76 ± 1938.65	2.67 ± 0.49	1.91 ± 0.32	16.46 ± 5.16
5	5014.48 ± 1993.27	2.68 ± 0.57	1.91 ± 0.40	16.51 ± 6.35

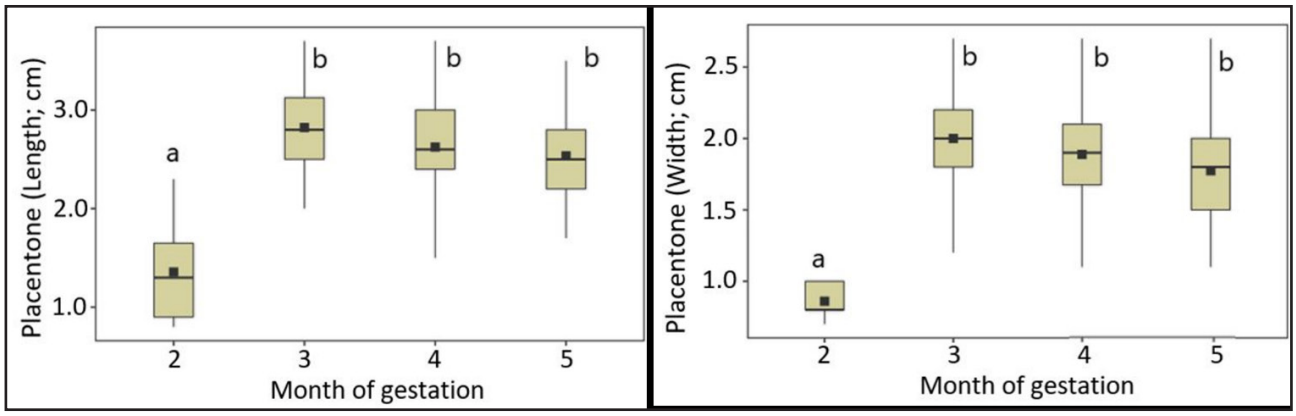

Figure 2. Boxplot of measurements (length and width) of placentomes over months of gestation. [Different letters indicate significant differences ($P < 0.05$) by the Kruskal-Wallis test, followed by the Tukey test (Post-Hoc Test)].

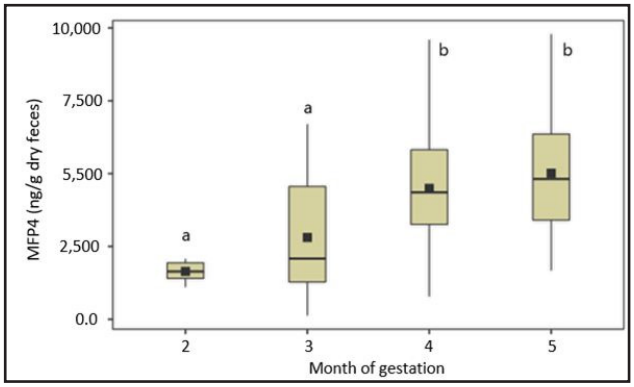

Figure 3. Boxplot of fecal metabolic progesterone (MFP4) levels over months of gestation. [Different letters indicate significant differences ($P < 0.05$) by the Kruskal-Wallis test, followed by Tukey's test (post-hoc test)].

DISCUSSION

In ewes, progesterone is excreted mainly through feces. Metabolism and fecal excretion of this hormone occur in less than 24 h and comprise approximately 76% of progesterone metabolites [16]. Previous studies reported a high correlation between serum levels of progesterone and its fecal metabolites during pregnancy in ewes [7]. Also, serum and fecal progesterone hormone profiles have a significant positive correlation in sheep [2]. Thus, we can assume that the measurement of progesterone level in fecal samples is a non-invasive hormone monitoring method that provides values similar to that of blood levels and is less stressful for the animals at the time of sample collection.

In the initial phase of pregnancy up to day 50, progesterone level follows a trend like that in the luteal phase of the estrous cycle [21]. From day 55 of gestation, serum levels of progesterone gradually increase, which is associated with the beginning of the production of this hormone by the placenta [15]. Previous studies reported that all ewes showed an increasing trend of serum levels and fecal progesterone metabolite levels during pregnancy from the time of mating, with a subsequent decrease to baseline levels after term [2]. These findings are consistent with those of the present study, in which an increase in progesterone levels during pregnancy was also observed.

In ewes, plasmatic progesterone level was reported as remaining high during pregnancy, but with large variation between individuals (ranging from 2.6 to 10.8 ng/mL) [19]. The average P4 level may vary weekly across animals and across pregnancy moment but does not show significant variation according to breeds or number of fetuses [20]. However, this wide physiological variation makes it difficult or even useless to use fecal progesterone, when is measured only once per animal, as an indicator of the presence of pregnancy. Despite this limitation, serial sampling is useful for monitoring of hormones during pregnancy and is an important tool for research purposes.

Plasma P4 level is considered a classic variable for the diagnosis of pregnancy in various mammalian species [5]. However, a high P4 level only confirms the presence of active luteal tissue in the ovaries, which may be due to pregnancy or other conditions that prolong corpus luteum life (example: gestational changes such as hydrometra, mucometra, pyometra, and fetal mummification) [4,5].

Placentome size measurements correlated weakly with gestational age and did not correlate with P4 level. Thus, the presence of placentomes is indicative of the presence of pregnancy but was considered inefficient for determining gestational age in sheep. In pregnancy, placentomes of varying sizes and degrees of development are reported [11], and the variation in placentome size may also be associated with their position in the uterine horns [9]. Even in the face of these variations, studies indicate that the measurement of placentome size can be used to estimate placental growth in sheep [8,12]. The maximum diameter of placentomes in ewes was reported at 11 weeks pregnancy (3.41 ± 0.04 cm) [3] and by at 74 days (3 cm) [9].

In the present study, a significant increase was noted in placentome size from the 2nd to the 3rd month of gestation, followed by stabilization until term. In agreement with our findings, it was reported that placentome size hardly changed during pregnancy in ewes and showed similar values from day 60, indicating early placental maturation that remained unchanged throughout gestation [22]. On the other hand, some authors reported a progressive increase in placentome diameter from day 40 to day 80 of pregnancy and then remained unchanged or even decreased at day 108 [13].

CONCLUSIONS

Placentome size and fecal progesterone levels showed significant variations throughout preg-

nancy. Placentome size measurements did not correlate with progesterone levels. Although placentome size measured by transabdominal ultrasonography is useful to evaluate pregnancy in real time, it was not considered an efficient method for estimating gestational age.

Determination of fecal progesterone levels requires laboratory support and is time-consuming and costly. Collection of only 1 sample per animal was not considered efficient for estimating gestational age. Frequent and serial sampling may be useful for non-invasive hormonal monitoring and for deepening the study of physiological events during pregnancy.

MANUFACTURERS

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***Ethical approval*.** This experiment used animals in the experimental model and had the approval of the Institutional Research Ethics Committee of Universidade Federal de Roraima (UFRR), Boa Vista, RR, Brazil, by the number CEUA 002/2021.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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