TÜBİTAK

Research Article

Fecal progesterone analysis for monitoring reproductive status in dairy goats

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Abstract: The present study was undertaken to evaluate the use of fecal progesterone (FP4) concentration for early pregnancy diagnosis, estrus detection, and predicting the litter size and parturition date in dairy goats. Fecal samples were collected from 17 hand-mated goats 3 times a week for 22–23 weeks, beginning on the day of mating. The levels of FP4 were determined by enzyme-linked immunosorbent assay (ELISA). Significant positive correlations were found between the levels of serum P4 and FP4 (r = 0.8787, n = 13). Pregnancy diagnosis was performed at 2 months postmating with transabdominal ultrasonography and confirmed upon birth of the offspring. The results indicated a significant difference in the mean FP4 concentration obtained during days 19 and 20 postmating between the pregnant and nonpregnant does (2492.4 ± 239.1 vs. 577.0 ± 112.9 ng/g, P < 0.05). A significant drop in the FP_4 concentration was noted 3 days prior to the detected day of estrus (P < 0.05). The mean weekly FP_4 profile obtained in this study showed a progressive increase from week 7 to 14 until a plateau was reached between weeks 15 and 21, and then a rapid decline began 5 to 6 days prepartum, with a significant drop 1–2 days prepartum (from 3884.3 ± 576.0 to 1205.0 ± 339.0 ng/g, P < 0.05). No significant correlation was observed between the FP_4 concentration and the number of kids born. In conclusion, the measurement of FP_4 concentration could potentially be an alternative method for early pregnancy diagnosis, prediction of estrus, and parturition in dairy goats.

Key words: Fecal progesterone, pregnancy, estrus, dairy goat

Introduction

Fecal steroid analysis provides a foundation for mapping the endocrine milieu of animals without changing their behavior (1). A previous study reported the feasibility of using fecal progesterone (FP_4) analysis to monitor ovarian function in wild Shiba goats (2). This has spurred the analysis of FP_4 metabolites to emerge as an appropriate method for monitoring reproductive status in domestic ruminants (3–5). Although the time differences between the circulation of steroids in the plasma and their appearance in urine samples is less than 5 h, fecal steroid metabolites have an appreciable lag time (1–2 days), which approximately correlates with the

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time necessary for the intestinal passage of bile to the rectum in ruminants (4,6,7). In small ruminants, fecal extracts were assayed by enzyme immunoassay for progesterone metabolites to accurately determine pregnancy and reproductive function with single or double sampling in bighorn sheep (8,9). Recently, it has also been reported that FP_4 profiles can reflect the serum progesterone concentrations in pregnant goats (10). There is an increasing interest in the application of fecal hormone analysis as an alternative noninvasive approach, primarily owing to the relative ease of sample collection from animals (4,5,8). Therefore, this study aimed to assess the stage of the estrous cycle, pregnancy, onset of parturition, and litter size in dairy goats by measuring the progesterone concentration in the feces (FP_4), in the central region of subtropical Taiwan.

Materials and methods

Animals

This experiment was conducted between October 2008 and April 2010 at the Department of Animal Science Research Station, National Chung Hsing University, Taiwan (25°03′N, 121°30′W). The age and parity of 17 does (Toggenburg, n = 4; La Mancha, n = 6; Saanen, n = 3; Nubian, n = 1; Alpine, n = 3) for this study ranged from 1 to 5 years and 1 to 3 years, respectively. Hand-milking was performed twice a day, at which time feeding was also carried out. The feed consisted of Bermuda grass (9.2% crude protein and 85.4% detergent fiber), alfalfa pellet (19.2% crude protein, 60% detergent fiber, and 2% crude fat), and concentrate (13% crude protein, 27.2% detergent fiber, and <1% crude fat). Water and mineral block were provided ad libitum. The breeding season extends from mid-August to March, peaking in October. The animals were hand-mated. The females were kept in separate stalls, but within close proximity to the males. This study complied with current welfare legislation and all of the management practices followed the animal care guidelines of Chung Hsing University.

Estrus detection

Does were monitored twice a day (0600 and 1800 hours) to detect the onset of estrus. The observed estrus signs included: searching for the male, restlessness, vocalization, frequent urination, tailing, hyperemia and edema of the vulva, vaginal mucous discharge, and immobility on mounting (considered as the onset of estrus). All of the does were monitored during one whole estrous cycle. All of the animals were mated upon detection of each estrus.

Fecal and blood sampling and progesterone analyses

Sampling was initiated on the day after first behavioral estrus, designated as day 0 in this study. Fresh feces were caught in a gloved hand as the animals defecated. This was carried out between 0630 and 0730 hours, 3 times a week, until 1 week after parturition (approximately 23 weeks). The samples were taken immediately to the laboratory and stored at –20 °C until assayed. FP_4 was extracted with a slight modification of the method described by Korndörfer et al. (11). In brief, undried feces (0.5 g ± 10 mg) were weighed and placed in a 15-mL centrifuge vial. Methanol (4.0 mL) and double deionized water (0.5 mL) were added. The mixture was blended, after which the vials were spun at high speed on a vortex for 30 s. The mixture was then rotated for 30 min and centrifuged for 10 min ($1500 \times g$). The supernatant (1 mL) was transferred to a capped vial, diluted 1:2 with double deionized water, and stored at –20 °C until assayed. FP_4 concentrations were determined using a competitive enzyme-linked immunosorbent assay (ELISA) with a slight modification of the method described by Chang et al. (12). We used mouse antiprogesterone-11-BSA monoclonal antibody (MAB 1234, Chemicon Inc., USA) and progesterone-11-horseradish peroxidase (PG-007, aBiox, USA). The standard curve was prepared by adding crescent concentrations of progesterone (P9776, Sigma, USA) diluted in methanol. The standard curve ranged from 0 ng/mL to 1 µg/mL. The curves were tested to verify their parallelism. All of the samples were tested in triplicate on the same ELISA and were compared to the standard curve on the same assay. Concentrations were expressed as nanograms per gram for each fecal sample, indicating the quantity of P_4 (ng) in 1.0 g of feces. The assay sensitivity was 0.57 ng/mL (n = 19). The coefficient of variation for 2 control samples (1 ng/mL and 100 pg/mL) was 0.97%. The inter- and intraassay coefficients of variation were 1.68% and 1.26% for this assay (n = 24), respectively.

Blood samples were collected concurrently with 13 fecal samplings in order to validate the use of FP_4 analysis. Samples were collected from the jugular vein of 13 does into a nonheparinized vacutainer. Serum samples were analyzed using an automated immunoassay analyzer (mini Vidas®, BioMérieux SA, France).

Pregnancy diagnosis

Pregnancy diagnosis was performed by transabdominal ultrasonography during the second month of gestation using a real-time B-mode linear assay ultrasound scanner, equipped with a 3.5-MHz transabdominal probe (Model Scanner 200 Vet, Pie Medical, the Netherlands). The does were scanned while standing, without shaving the ventral abdominal wall. The transducer was applied at the hairless area of the inguinal region of both sides after adding coupling gel. Pregnancy diagnosis was confirmed upon birth of the offspring.

Statistical analysis

For the mean variation of the variables with continuous data, the mean ± standard error (SE) was applied. Estrous cycle lengths were estimated as the interval between successive basal FP_4 concentrations. Similar to the data analysis described by Pereira et al. (13), the baseline for the FP_4 concentration was defined by taking the lowest FP_4 value for each cycle, and the mean value was further obtained as the criterion value. The values above the criterion value were considered indicative of the luteal phase, whereas the values below this criterion were considered indicative of the interluteal phase. In order to align the cycles and calculate the daily hormonal mean values, day 0 of each cycle was defined as the day following the last day of the luteal phase. Analyses were performed using SAS 9.1 (SAS Institute Inc., USA) and SPSS 12.0 (SPSS Inc., USA). Student's t-test was used for the comparison of the mean FP_4 concentrations between the pregnant and nonpregnant goats, and the weekly FP_4 concentrations. Receiver operating characteristic (ROC) curves were used to evaluate the performance of the FP_4 value for pregnancy diagnosis, using area under curve (AUC), and to determine an appropriate cut-off point to calculate sensitivity and specificity. P < 0.05 was accepted as significant unless otherwise specified.

Results

Significant positive correlation was found between the levels of serum P_4 and FP_4. The linear regression formula was as follows: Y = 0.0056x + 0.715 (n = 13, Y = fecal content in ng/g dried feces; x = plasma concentration in ng/mL, and correlation coefficient r = 0.8787). Specimens used for the serum-FP_4 correlation analysis were collected in the luteal phase (n = 5) and the nonluteal phase (n = 8).

A total of 12 complete estrous cycles were observed. Based on FP_4, the average length of the 12 estrous cycles in this study was 20.8 ± 0.6 days. The FP_4 concentration started to increase 2 days after mating, following an initial basal value of 393.4 ± 16.7 ng/g. The highest FP_4 values were reached between days 9 and 11, with concentrations of 3178.1 ± 395.6 and 3535.3 ± 292.0 ng/g. A baseline FP_4 value of 344.3 ng/g was obtained. Figure 1 shows the changes in the FP_4 profile approaching detected estrus (day 0). The FP_4 concentration began decreasing 5 days before estrus from a value of 2957.6 ± 352.0 ng/g, with a significant drop 3 days before (P < 0.05), until reaching a basal value of 396.9 ± 59.8 ng/g.

Of 17 mated goats, 12 were diagnosed as pregnant using ultrasonography at 2 months postmating. Of the does, 11 conceived at the first estrus and 1 conceived at the second estrus. However, 1 doe was suspected of having a late embryonic death. The levels of FP_4 from week 2 postmating onwards started
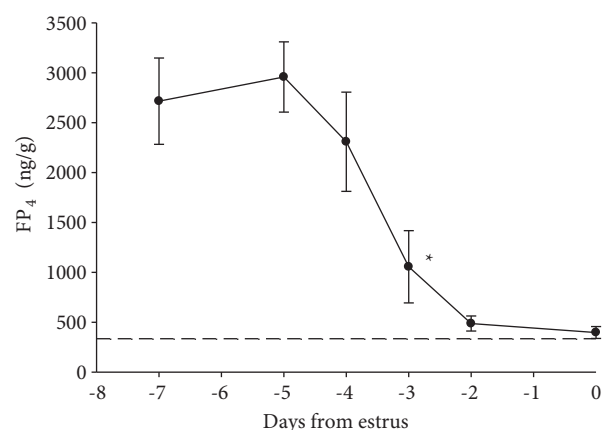

Figure 1. Changes in the mean concentrations of FP_4 (mean ± SE) approaching estrus (day 0) in 12 does. The dashed line represents the baseline FP_4 value. The mean FP_4 concentrations were significantly different between day −3 (*) and day −2 (P < 0.05).

to increase in the pregnant does. However, the mean FP_4 did not differ at 15–16 days (2401.7 ± 330.7 vs. 1662.1 ± 931.1 ng/g) and 17–18 days (2463.6 ± 335.0 vs. 1139.1 ± 538.0 ng/g) between the pregnant (n = 11) and nonpregnant (n = 5) groups. At 19–20 days postmating, the FP_4 concentrations were significantly higher in the pregnant does (2492.4 ± 239.1 ng/g) than in the nonpregnant does (577.0 ± 112.9 ng/g) (Figure 2). The ROC curve calculated an FP_4 concentration of 1044.7 ng/g as the cut-off value for pregnancy diagnosis. This cut-off value had a specificity and sensitivity of 100% and 100%, respectively, at 19–20 days postmating. The AUC was 1.000 and P = 0.002.

The accuracies of the pregnancy and nonpregnancy diagnosis using the FP_4 concentration were 100% and 100%, respectively. FP_4 analysis at 19–20 days postmating diagnosed 12 pregnant does and 5 nonpregnant does.

FP_4 concentration started to decrease at approximately 5–6 days before parturition. There was a drop in FP_4 concentrations from 6361.8 ± 1023.1 ng/g to 5212.8 ± 463.6 ng/g around days 3–4, and then to 3884.3 ± 576.0 ng/g around days 1–2 before parturition. There was a significant difference in the FP_4 concentrations from 1–2 days prepartum to the day of parturition, with values falling from 3884.3 ± 576.0 ng/g to 1205.0 ± 339.0 ng/g on the day of parturition (P < 0.05). By 3–4 days postpartum, the FP_4 concentration had returned to the basal value of 221.1 ± 31.3 ng/g (Figure 3).
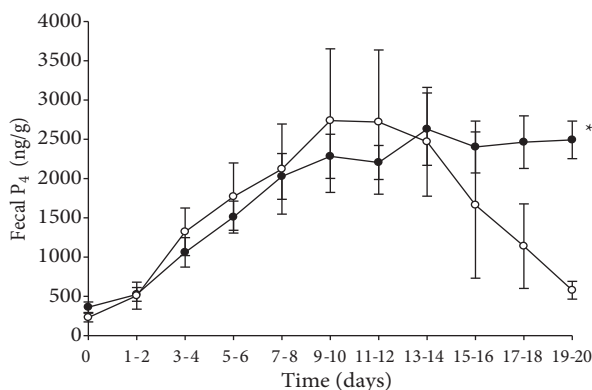
The average litter size was 2.1 kids. Two goats had triplets, 9 had twins, and 1 had a singleton. A total of 12 females and 13 males were born. There was no significant difference in the litter size among the different breeds. There was no significant effect of litter size on the FP_4 concentration throughout pregnancy.

Discussion

The results of the current study showed the correlation between progesterone concentrations in the plasma and feces, suggesting that the noninvasive FP_4 analysis of luteal functions herein could be manipulated for field use in dairy does.

The FP_4 levels obtained at 19–20 days postmating allowed for, in 100% of the cases, an accurate diagnosis of pregnancy to be made. This was confirmed by ultrasonography and the birth of live young in the case of pregnant does and by the absence of births in the predicted nonpregnant does. The results obtained were comparable to 21–24 days postmating for pregnancy diagnosis in does via serum P_4 evaluation (14). Another study also reported early pregnancy diagnosis, via serum P_4 analysis, at 19 and 21 days postmating in ewes and does, respectively (3). The sensitivity and specificity of the FP_4 status at 19–20 days postbreeding as a predictor of positive pregnancy in the present study were 100% and 100%, respectively. These values can be compared to 100%
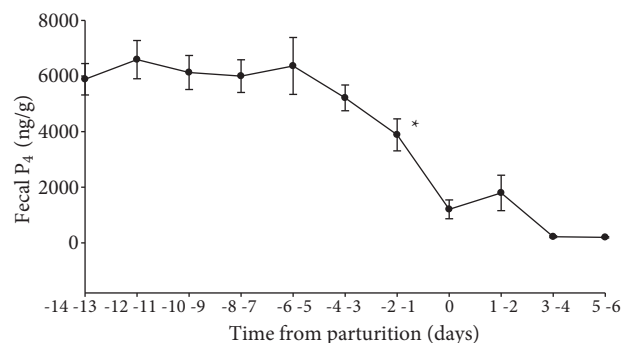

Figure 2. Mean FP_4 concentrations during the first 20 days after mating in the pregnant (–●–; n = 11) and nonpregnant (–■–; n = 5) does. Day of mating = day 0. Error bars represent the standard error of the mean. The means (*) were significantly different between the pregnant and nonpregnant does (P < 0.05).

Figure 3. Changes in the mean concentrations of FP_4 (mean ± SE) with respect to delivery date (day 0) in 11 does. There was a significant difference in the FP_4 concentrations from 1–2 days prepartum (*) to the day of parturition (P < 0.05).

and 65.6%, respectively, using serum P_4 in goats on day 22 postmating (15). In the current study, none of the nonpregnant does had a FP_4 concentration over the cut-off value at 19–20 days postbreeding. Fleming et al. reported that the majority of does had corpus luteum regression by 21 to 24 days postestrus (14). P_4 concentrations in the blood or in the milk decreased sharply following regression of the corpus luteum in the nonpregnant goats. On the contrary, pregnant goats had high P_4 concentrations over the same period, since the corpus luteum does not regress (15). Thus, it is suggested that the reliable early detection of pregnant does by the measurement of FP_4 could be considered for pregnancy diagnosis at 19–20 days postmating. A decrease in the FP_4 concentration in pregnant animals was observed around day 12 postmating. This phenomenon was also reported by Fonseca et al. (16), who found that a decrease in the plasma P_4 concentration of pregnant animals was noted on approximately day 13 after breeding. The factors leading to this decrease in P_4 are still not known.

In the current study, FP_4 began decreasing 6 days prepartum, with a significant drop 2 days before. It was noted that the prepartum decline in the FP_4 levels correlated with the onset of parturition. This finding is consistent with a decrease in maternal plasma P_4, being of primary importance in the induction of parturition in goats (17). The FP_4 drop found in our study resembles those shown in the plasma P_4 profile (18) and serum and FP_4 (10), while Khanum et al. reported a plasma P_4 decrease beginning 19 days prepartum (19) and Khan and Ludri reported the decrease beginning at around 20 days before, with an abrupt drop 1 day prepartum (17). Our results are in agreement with studies reporting that plasma P_4 concentrations remain slightly elevated at the onset of parturition and then decline toward anestrous levels following delivery in the goat (20).

In the present study, the most frequent litter size was twins (75%), followed by triplets (16.7%) and then singletons (8.3%). Amoah et al. (21) also reported a majority of twins being born. A number of studies have reported a significantly higher P_4 concentration in sheep and goats carrying multiple fetuses than in those carrying singletons (10,22,23). Even though sheep and goats are closely related

phylogenetically and have morphologically similar forms of placentation, these 2 species differ in terms of the role of the placenta as a source of progesterone during pregnancy. Since the placenta does not play a critical role in secreting P_4 during late pregnancy in goats, it was suggested that the increased serum P_4 concentrations in does bearing more than one fetus were related to the increased rate of maintained corpus luteum during pregnancy (23). On the other hand, a significant relation between the litter size and P_4 concentration was not reported in some studies (3,24). In this study, a significant difference in the FP_4 concentration with respect to the varying litter sizes was not observed. However, the small sample size obtained may have had a limiting effect on such findings. The FP_4 profile during weeks 11–15 obtained in this study showed a lower concentration in the doe that delivered a singleton when compared to those that gave birth to multiple offspring. Additionally, some studies have also reported positive correlations between the litter size and P_4 concentration at certain points during gestation in sheep (22,25,26).

The length of the interestrus intervals observed in this study was in agreement with that reported in Dwarf goats (27). The duration of the luteal phase was also similar to that of other reports based on assays of FP_4 levels (28–30). The rise in the P_4 level from day 2 to day 10 could be attributed to the growth and development of the corpus luteum. It was also noted that the FP_4 concentration began declining 5 days before the estimated time of estrus, with a drastic reduction 4 days before.

In conclusion, the measurement of FP_4 concentration could potentially be used as a noninvasive alternative method for monitoring reproductive status in dairy goats. It was determined that pregnant animals can be correctly diagnosed within 19–20 days after mating. Impending estrus can be predicted up to 4 days before. This is of particular importance and profitability in large herds where heat detection is made based upon observation by a stockman. Additionally, FP_4 analysis has shown its versatility in its application for estimating the time of parturition. Impending parturition can be predicted 2 days before, allowing for the provision of emergency personnel or additional housing facilities when and where necessary. Based on the results of this study,

FP_4 analysis allows the veterinarian and farm owner convenient monitoring of the reproductive status, especially in cases where ultrasound is unavailable or not feasible.

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