

Fecal Cortisol and Progesterone Concentrations in Post Partus of Etawah Crossbreed Goat

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Abstract. Progesterone (P4) is a dominant hormone during pregnancy. In the later stage of pregnancy, the stress hormone particularly cortisol (C) may increase for initiating the parturition process as a consequence of fetal stress. This study was a preliminary study to compare the concentration of P4 and C in feces of Etawah Crossbreed Goat during their last stage of pregnancy and post partus. This study used 5 pregnant Etawah Crossbreed Goats (\pm 20th weeks) of pregnancy. Fecal samples were collected in the 20th week of pregnancy to 2 weeks of postpartum. All fecal samples were then dried using a freeze dryer (Labfreez FD-10-MR) for 7 days at -80°C . Afterward, dried feces were pulverized and extracted by using 3ml of methanol 80%. The fecal extract was then analyzed the P4 and C concentrations using the enzyme-linked immunosorbent assay method. Concentrations of P4 and C metabolites in the last stage of pregnancy were $5,506.18 \pm 3,396.72$ ng/g dry feces and $136,625.83 \pm 42,479.22$ ng/g feces, respectively. Concentrations of P4 and C metabolites in the 2 weeks postpartum decreased at 669.38 ± 643.9 ng/g feces and $110,295 \pm 14,378, 8$ ng/g feces, respectively. It can be concluded that there was a difference in the fecal progesterone and cortisol concentrations between the last phase of pregnancy and the postpartum phase.

Keywords: crossbreed, feces, metabolite hormone, cortisol, progesterone.

1 Introduction

Progesterone is a hormone that has an important role in the process of implantation [1]. Progesterone has strong evidence that shows their role in modulating the immune response [2]. Many studies have shown that suppressive activity against Th1 increases the secretion of Th2 cytokines, inhibits the cytotoxic activity of T cells, and increases cell differentiation to Th0. Modulation activity is also seen in NK cells, antibodies (increasing inefficient asymmetric antibodies), and dendritic cells, where these cells recruit more cells with immuno-tolerant (protolerogenic) properties [3]. Progesterone causes a decrease in proinflammatory cytokine production by macrophages in response to infection stimuli [4]. Cortisol (cortisol, hydrocortisone, 11 beta, 17alpha, 21-trihydroxy-4-pregnene-3, 20-dione) is a glucocorticoid steroid hormone group that is generally produced by cells of the adrenal gland fasciculate zones [5].

Normal conditions will release stress hormones in small amounts throughout the day, but when faced with stress, levels of these hormones increase dramatically [6]. Every type of body's response to stress, both physical stress, and psychological stress, can increase the secretion of the adrenocorticotrophin (ACTH) hormone which can ultimately increase cortisol levels. Pregnancy is a physiological stressor. Maternal hypothalamic pituitary adrenal axis changes dramatically and increases cortisol levels [7]. According to Lisdiana [8] stress can cause

cortisol secretion to increase up to 20 times. The metabolic process, in addition to turning steroids into inactivity, can change the nature of steroids into water-soluble through the process of conjugation with glucuronide. This conjugation process requires the enzyme glucuroniltransferase and uridine diphosphoglucuronic acid (UDPGA). Glucuride acid binds to the OH group of steroid molecules. The conjugated steroid will enter the bile and then enter the enterohepatic circulation to return to the liver or intestine which will then be excreted through feces.

The aim of the study was to compare the concentrations of fecal progesterone and cortisol at the end of pregnancy (20 weeks) and 2 weeks after parturition in crossbreed goat.

2 Materials and Methods

2.1 Samples

The sample used in this study was Etawah crossbreed goat feces samples at the end of pregnancy (20 weeks) and 2 weeks after parturition.

2.2 Fecal extraction

Before the fecal extraction, fecal samples were transferred into a centrifuge tube for lyophilization using a freeze dryer. Dried fecal samples were then pulverized to get fecal powder. The fecal powder was then extracted

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as described by Gholib *et al.* [9-10]. ~50 mg fecal powder was extracted using 3 ml of 80% methanol in a 15 ml polypropylene tube. The solution was then vortexed for 10 minutes. After that, it was centrifuged at 500x g for 10 minutes. The supernatant was decanted into 1.5 ml microtube and stored in the freezer at -20°C until hormone measurement using Enzyme-Linked Immunosorbent Assay (ELISA) technique.

2.3 Assay Validation

The validation test was carried out as described by Gholib *et al.* [11-12] by performed with the parallelism test on the commercial progesterone (Calbiotech, USA) and cortisol (Arbor Assay®) ELISA kits. The parallelism test was performed by comparing the curve of cortisol and progesterone standard with the curve of selected serial dilution of fecal extract. The serial dilution of fecal extract used was 1:5 to 1:40. The commercial progesterone ELISA kit had cross-reactivity to other steroid hormones <1%.

2.4 Hormone Measurements

Measurement of cortisol concentrations used the EIA cortisol (Arbor Assay®) kit. A sample of 25 µL was put into a well then 50µl of Biotin detection antibody was added and incubated for 45 minutes at 37 °C. Next, the microplate was washed 3 times and then added 100 µl of SABC working solution, then incubated for 30 minutes at 37° C. In the next step, the microplate was washed 5 times and then added 90 µl TMB substrate and incubated for 15 minutes. The addition of a stop solution was 100 microns and then measured with a wavelength of 450 nm. The results of testing cortisol hormone levels are expressed in the unit of ng/ml of blood, but because the sample used is feces the calculation will be multiplied by the volume of extraction and divided by the weight of feces so that the feces cortisol levels are in the unit of ng/gr of feces.

Measurement of progesterone concentrations used a commercial EIA Progesterone KIT produced by of Calbiotech®, USA. The procedure for carrying out hormone testing followed the procedure written in the manual kit procedure. The EIA KIT used had a sensitivity of 0.22 ng/ml with a coefficient of intra-assay variation of 1.62% -5.36% and inter-assay of 6.3% - 9.68%. Standard progesterone levels used were 2.5 ng/ml to 40 ng/ml. Before the assay, the conjugate enzyme was diluted using a 1:1 buffer assay. The procedure for checking feces samples that had been extracted was as follows: all cortisol samples and kits were placed at room temperature (25 °C). A total of 10 ul standard and samples were put in a well, then added with 200 ul conjugate enzyme conjugate into each well. The next process was incubation for 60 minutes at temperature. After incubation, it was washed three times using ELISA washer then given 100 ul TMB substrate and incubated for 15 minutes. The next step was to add the stop solution of 100 ul. The well was read with an ELISA reader at a wavelength of 450 nm. The results of data acquisition in the form of optical density (OD) were computerized interpolated using the general formula: $y = -a \ln(x) + b$.

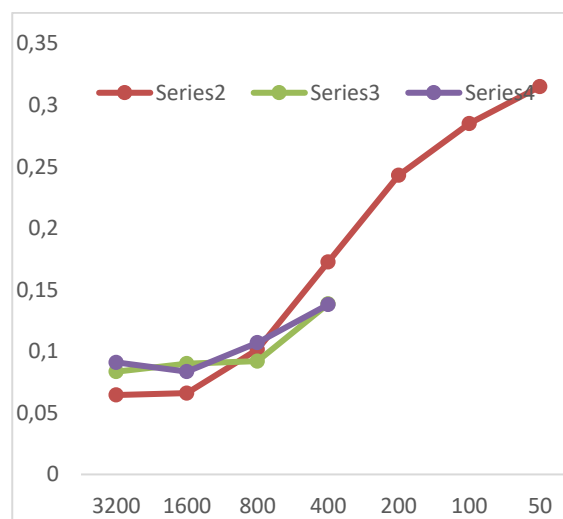
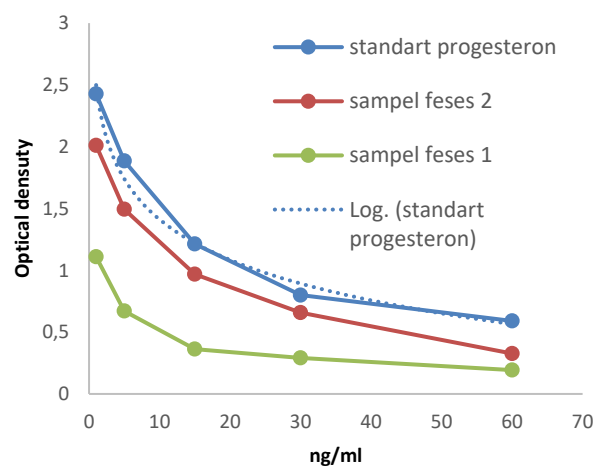
3 Results and Discussion

The concentration of fecal cortisol and fecal progesterone were presented in Table 1. Based on the results, concentrations of fecal cortisol and fecal progesterone showed a difference between the end of pregnancy and 2 weeks after parturition.

Table 1. Concentrations of fecal cortisol and fecal progesterone at the end of pregnancy and 2 weeks after parturition of goat

Timing	Cortisol (ng/gr dry feces)	Progesterone (ng/gr dry feces)
End of pregnancy	136,625.23 ± 42,479.22	5,506.18 ± 3,396.72
2 week after parturition	110,295 ± 14,378.8	669,38 ± 643.9

The parallelism test results showed that there was a decrease in optical density along with sample dilution, as shown in Figure 1.



According to Gholib, *et al.* [11], the parallelism test is one of the analytical validation tests conducted to determine what the actual testing should be measured. In addition, this test can also be used as a reference to determine the optimal dilution of the fecal extract [11].

In this study, fecal progesterone and fecal cortisol concentration levels decreased 2 weeks after parturition compared to the last pregnancy period. Feces hormone levels can indicate of hormone levels in the blood. There was a positive correlation between plasma progesterone and fecal progesterone in dairy goat [13] (). In addition, there is a positive correlation between plasma progesterone and fecal progesterone in cattle [14]. According to Karadaev [15] (14), fecal progesterone concentrations will decrease before birth with insurance measurement of fecal progesterone between pregnant and non-pregnant goats reaching 100%.

Cortisol can be detected through blood, serum, feces, sweat, urine, hair and saliva samples [16,9]. In this study, fecal cortisol concentrations decreased at 2 weeks after parturition. Besides functioning as stress indicators, carbohydrate metabolism, and electrolyte homeostasis, cortisol is also playing role in several physiological functions such as carbohydrate metabolism, electrolyte homeostasis, and can be used as indicators of animal and human health being [1,17]. According to Kumar et al. [1], blood cortisol levels on the day before birth will decrease at 2 weeks after parturition in goats that are kept at various temperatures.

The amount of decrease in progesterone levels was also influenced by the number of fetuses. In twin births, the decline in progesterone reached 56%, whereas in single births it only reached 46% [19]. Decreased levels of progesterone, increased estradiol and prostaglandins play an important role in stimulating birth and recovery after parturition [20]. In addition to decreasing progesterone, during parturition, there will be a decrease in plasma insulin levels due to the mobilization of nutrients and fats to be synthesized [19,20]. In this study, cortisol levels were 1-2 times higher than they were after parturition along with progesterone levels. This shows that during pregnancy, cortisol also plays an important role besides the hormone progesterone. According to Norman and Henry [20] and Vaughan, *et al.* [21], throughout the process of pregnancy the basal plasma cortisol levels must increase to accommodate the nutritional needs of developing embryos and help in the development of fetal organs. The concentration of maternal plasma cortisol at the end of pregnancy can be tripled compared to non-pregnant sheep [22]. According to Esposito and Bianchi [23], this is due to the activation of the HPA-placental axis which affects cortisol levels in the pregnancy phase

4 Conclusion

Based on the results of the study, it can be concluded that the metabolite levels of cortisol and progesterone have decreased significantly in 2 weeks after parturition.

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