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Research paper

Effect of repeated adrenocorticotropic hormone administration on reproductive function and hair cortisol concentration during the estrous cycle in goats

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ABSTRACT

Measurement of the cortisol concentration in hair has been used as an index of chronic stress in several species including humans, wildlife and domestic animals. However, how accurately the cortisol concentration in hair reflects the changes in circulating cortisol concentrations has not been well documented. The objective of the present study was to examine the effect of repeated adrenocorticotropic hormone (ACTH) administration on the reproductive function during the estrous cycle and hair cortisol concentrations in goats. In experiment 1, goats were administered ACTH (0.625 IU/10 kg of body weight, n = 6) or saline (n = 6) intramuscularly once a day for 7 days on Day 11–17 of the estrous cycle (day 0 was the day of ovulation). In experiment 2, goats were administered ACTH (0.625 IU/10 kg of body weight, n = 6) or saline (n = 6) intramuscularly twice a day on Day 11–24 of the estrous cycle. Blood samples were collected 0, 0.5, and 6 h after first administration to determine the circulating cortisol concentrations. Hair was clipped at 0, 1, and 2 months after the start of administration. In both experiments, the plasma cortisol concentration increased at 0.5 h and returned to baseline at 6 h after ACTH administration. During the experiments, estrus was observed in most animals in ACTH and saline groups (6/6 and 4/6 in experiment 1 and 5/6 and 6/6 in experiment 2, respectively) and ovulation was observed in all goats examined. However, the number of ovulatory follicles was significantly different between the ACTH and saline groups, and the maximal diameter of ovulatory follicles tended to be different (P = .07) between the ACTH and saline groups. In experiment 1, the hair cortisol concentration was not influenced by the ACTH administration throughout the sampling period. In experiment 2, the hair cortisol concentration in the ACTH group was greater at 1 month after administration than the pre-administration value, but was not significantly different at 2 months. These results suggest that repeated ACTH administration affects the development and ovulatory process of ovarian follicles and analysis of the hair cortisol concentration can be used for assessing relatively long-term changes in cortisol concentration in the circulation.

1. Introduction

Management of stress is an important component in improving the welfare, health and performance of livestock animals. In modern farms with intensive management, many events can potentially cause stress and impact the reproductive function of animals. Field data has shown that stress caused by clinical disease conditions such as lameness and social stress under group housing conditions can reduce the fertility parameters of dairy cows (Dobson et al., 2000). In goats, there are a few studies investigating the effects of stress on plasma cortisol and other metabolic parameters. Transportation and holding stress before slaughter increased the levels of plasma cortisol and glucose and changed meat quality in goats (Kannan et al., 2003). Increasing the density of housing increased the agonistic behavior, a sign of social stress, but did not affect the plasma cortisol levels and of pregnant goats (Vas et al., 2013). On the other hand, there is still lack of information regarding the effects of stress and cortisol on the reproductive function of goats, and only one study reported that adrenocorticotropic hormone (ACTH) administration increased plasma cortisol concentrations and induced the formation of cystic follicle in goats (Sato et al., 2011). It is difficult to evaluate stress in animals and its association with reproductive disorders for clinical purposes as well as in research studies.

While cortisol concentrations in blood and saliva are used as an index of acute stress, cortisol concentration in hair reflects the

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accumulation of cortisol during a relatively long period (weeks or months). Recently, measurement of the cortisol concentration in hair has been used for assessing chronic hypothalamic-pituitaryadrenal activity and stress in humans as well as in wildlife and livestock animals (Koren et al., 2002; Meyer and Novak, 2012; Burnett et al., 2014). In cattle studies, higher cortisol levels in hair were observed in clinically compromised cows (e.g. metritis, laminitis, mastitis) than in clinically healthy cows (Comin et al., 2013), and in response to the environmental change from indoor winter to summer grazing conditions (Comin et al., 2011). In goats, there is only one study assessing hair cortisol concentrations in association with hair coat condition of goats (Battini et al., 2015). It reported that rough hair goats were in a significantly poor nutritional condition and health status compared with normal hair goats, but no significant difference was observed between rough hair and normal hair goats in the hair cortisol concentrations. Hair is easy to collect noninvasively and can be stored for a long period (>1 year) at room temperature. This advantage may allow use of this parameter as an index of chronic stress in clinical practice and field trials. However, to the best of our knowledge, information is still lacking regarding how accurately the elevations of cortisol in circulating blood can be detected from hair samples during the corresponding period in goat species. To better understand the dynamics of hair cortisol deposition in relation to acute and chronic stress, administration of ACTH is one approach to clarify the temporal profile of cortisol concentration in hair following elevation of the circulating cortisol concentration.

The Shiba goat is a small Japanese native breed that shares common characteristics with full size goats, and is suitable for use as an experimental model of domestic ruminants because details of the basic physiology and endocrine function have been well reported (Mori and Kano, 1984; Orita et al., 2000; Sakurai et al., 2004). In addition, they have white hair over the entire body which eliminates any influence of hair color on measured cortisol values (Burnett et al., 2014). The present study was conducted to determine the temporal changes in hair cortisol concentrations after repeated ACTH administration to female cycling goats. Additionally, in order to clarify the effects of cortisol increase mimicking a stress condition on reproductive function, expression of estrus and ovulation were also examined during the trial.

2. Materials and methods

2.1. Animals

Adult female Shiba goats, aged 4–9 years and weighing 24–35 kg, were used in this study. Shiba goats are annual breeders under natural daylight conditions. The goats were housed in an outside paddock at the density of 5.3 m^2 per animal. Maintenance diets of crushed alfalfa hay cubes were fed twice a day, at 9:00–10:00 and 15:00–16:00. Clean water and mineralized salt were available ad libitum. All procedures were approved by the University Committee for the Use and Care of Animals at Tokyo University of Agriculture and Technology (no. 28-92).

2.2. Experimental protocol

Goats were checked for estrus daily and were confirmed to have normal estrous cycles before starting the experiments. The goats had been synchronized estrus by prostaglandin $F_{2\alpha}$ (2 mg of dinoprost per head, Pfizer, Tokyo, Japan) to minimize the variation in the estrous stages. When goats showed estrus, their ovaries were monitored daily by transrectal ultrasonography with a 7.5 MHz linear probe (HS-1500V; Honda Elect. Aich, Japan) to confirm the day of ovulation (Day 0).

2.2.1. Experiment 1

Experiment 1 was started August 22th, 2016. Goats were administered intramuscularly with ACTH (Cortrosyn: 6.25 μg [0.6 25 IU]/10 kg body weight, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) or 2.0 ml saline (six goats per group) once a day at 8:00–9:00 for 7 days from Day 11 to Day 17. The time of administration was decided to avoid the time of feeding because excitement at feeding time can influence on the circulating cortisol concentrations. Administration was conducted at 8:00–9:00 in order to avoid the interference of The dose of ACTH was based on a study of goats in which significant increases in both cortisol and glucose concentrations were observed at 30 min after administration and persistent follicles were induced in 50% of the goats administered ACTH twice a day for 7 days beginning in the late luteal phase (Sato et al., 2011). We hypothesized that once-a-day administration of ACTH for 7 days has less influence on the reproductive function and result in the smaller increase in hair cortisol concentrations as compared with those of the twice-a-day administration protocol.

All goats were checked for estrus and estrous symptoms after each administration of ACTH. A male goat housed in another paddock was introduced to the female goats' paddock at the time of each observation. Estrous symptoms, including swelling and hyperemia of the vulva, watery mucus discharge from the vulva, tail wagging, and approaching male goats were observed. The onset of estrus was defined as the time of the first acceptance of mounting by the male goat.

Development of follicles and ovulation was monitored by transrectal ultrasonography every other day, starting on Day 18. If goats showed estrus or estrous symptoms, ovarian ultrasonography was performed daily until the detection of ovulation. The diameter of each follicle was recorded at the maximal area. Ovulation was defined as the disappearance of large follicles that were detected on the previous day of examination and by the development of the corpus luteum (Orita et al., 2000).

2.2.2. Experiment 2

Experiment 2 was started October 21th, 2016. Goats were injected ACTH or saline (six goats per group) intramuscularly twice a day at 8:00 and 20:00 for 14 days from Day 11 to Day 24. The doses of ACTH and saline were the same as in experiment 1. Estrus and estrous symptoms were observed at each time of ACTH administration and ovarian ultrasonography was started on Day 17 in the same manner as in experiment 1.

2.3. Blood sampling

In both experiments, blood sampling was performed 0, 0.5, and 6 h after the first administration of ACTH on Day 11 in order to determine plasma cortisol concentrations in response to ACTH stimulation. Three ml of blood was taken by jugular venipuncture with a heparinized syringe and centrifuged at 3000 rpm at 4 °C for 20 min within 20 min after sampling. Plasma was collected and stored at −20 °C until assay.

2.4. Hair sampling and assay

Hair samples were collected at -7-0, 30, and 60 days after the start of ACTH administration (i.e. 0, 1, and 2 months). Hair was collected from the rump region as close to the skin as possible using an electric clipper. In a preliminary experiment, 10 strands of hair per goat were collected from a total of 12 goats, and the mean hair growth rate calculated from them was 1.25 ± 0.2 cm/month. Therefore, 1.5 cm length of hair was considered to reflect the incorporation of cortisol during approximately one month and used for the assay. All hair samples were put in envelopes individually and

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