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Differentiation of serum and salivary cortisol in holdback lambs after ACTH discharge

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Abstract

The objective of the current study was to compare cortisol concentrations in serum and salivary samples taken concurrently from halter-restrained lambs administered ACTH (100 IU, i.v.) or saline. Although cortisol appeared elevated in all animals, it tended to increase after ACTH administration in both serum (p = 0.085) and saliva (p = 0.078). Additionally, correlation (r = 0.66; p < 0.001) and regression (R^2 = 0.44; p < 0.001) analysis revealed a relationship between cortisol in serum and saliva. Findings indicate that salivary cortisol is indicative of serum cortisol and may provide a viable non-invasive alternative for cortisol quantification.

Keywords: ACTH challenge, saliva collection, salivary cortisol.

INTRODUCTION

Cortisol concentration in blood has long been used as a physiological marker of stress in domestic animals (Cohen et al., 1997). More recently, salivary cortisol has been evaluated as a non-invasive technique of cortisol quantification (Fell et al., 1985; Yates et al., 2010), however, opinions appear to be divided on the ability of salivary cortisol concentration to accurately indicate serum or plasma cortisol concentration. Fell and Shutt (1988) have reported similarities in cortisol concentration patterns measured in both blood plasma and salivary samples taken from sheep in response to introduction of stress. Yates et al. (2010) have found a similar relationship between blood serum cortisol and salivary cortisol concentrations in sheep, even when concentrations were very low. Some concerns with the salivary technique of cortisol quantification have been expressed, however, including inadequate salivary volumes (Blackshaw and Blackshaw, 1989) and greater assay variation (Morméde et al., 2007). An ACTH challenge was conducted to evaluate the methodology of saliva collection in restrained lambs and to compare salivary cortisol concentration to serum cortisol concentration.

MATERIALS AND METHODS

All procedures involving animals were reviewed and approved by the New Mexico State University Institutional Animal Care and Use Committee. Twelve Suffolk x Hampshire ewe lambs (6 months of age, 45 ± 1.2 kg) were used in the study. Animals had little experience with human contact and had not been previously restrained. Feed was withheld from these animals for 24 h before use, and both feed and water were withheld for the duration of the sampling period.

Beginning at 1000 on 16 August, lambs were haltered and tethered to an immovable, outdoor panel fence. Each lamb was given approximately 30 cm of halter slack and was allowed physical contact with adjacent sheep. All lambs were required to stand for the duration of the sampling period. Sampling began approximately 30 min after haltering and restraint. Blood samples were collected via jugular venipuncture into 10 ml vacuum tubes (Corvac serum-separator, Kendall Health Care, St. Louis, MO), kept at room temperature for 30 min, and then centrifuged (1,500 x *g* at 4°C for 15 min) to collect the serum.

For each blood sample, a simultaneous salivary sample was collected via 30 - 45 s oral swab using a 1 × 2 cm cotton strip held in surgical forceps, as previously described (Yates et al., 2010). Serum cortisol was quantified by solid phase RIA using components of a commercial kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) with modifications described by Kiyma et al. (2004). Within and between assay coefficients of variation for serum determinations were 4.3 and 6.5%, respectively. Salivary cortisol concentration was determined using components of the same commercial kit with modifications described by Yates et al. (2010). Within and between coefficients of variation were 11.3 and 3.2%, respectively. Samples were collected

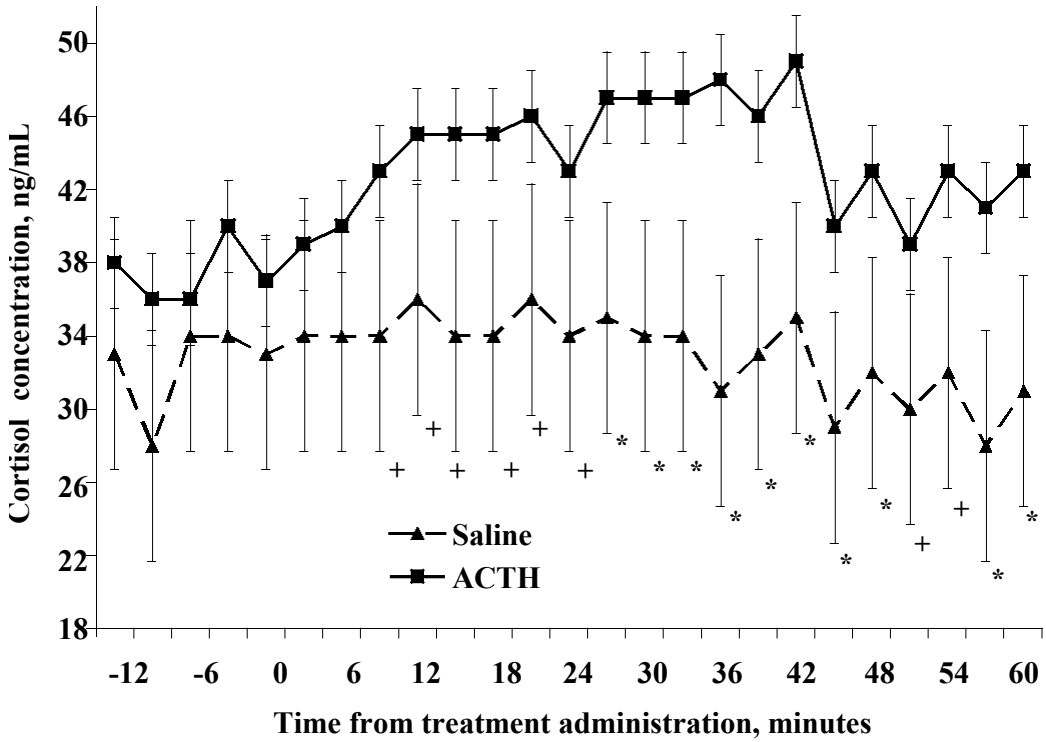

Figure 1. Serum cortisol concentration in restrained ewe lambs administered 100 IU ACTH or saline at time 0. Treatment effect, p = 0.085; time effect, p = 0.058; treatment x time, p = 0.883. Treatment effects at each time are denoted by '+' (p < 0.100) or '*' (p < 0.050).

at 3-min intervals for a total of 12 min just before treatment administration. Immediately after time 0, ACTH (100 IU in 1 ml physiological saline, i.v.; ACTH fragment 1 - 39 from porcine pituitary; 90 IU/mg; Sigma-Aldrich Co., St. Louis, MO, Sigma: A6303) was administered to 6 ewes, while the remaining 6 animals received a placebo injection (1 ml physiological saline, i.v.). After treatment administration, sampling continued at 3-min intervals for 60 min. Experimental design was completely randomized and ewe was the experimental unit. Serum and salivary cortisol concentrations were analyzed using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC) with repeated measures function. The relationship between serum and salivary cortisol concentrations was examined using the correlation and regression procedures of SAS.

RESULTS AND DISCUSSION

A treatment x time interaction was observed ($p < 0.001$) for salivary cortisol concentration, therefore, treatment effects were examined within each time. For purposes of comparison, treatment effects for serum cortisol concentration were also examined within each time, although treatment and time did not interact ($p = 0.883$). As expected, neither serum nor salivary cortisol differed ($p > 0.050$) between treatments (before treatment application). In serum samples (Figure 1), ACTH increased ($p < 0.100$) cortisol concentration beginning 9 min after treatment application. Serum cortisol concentration remained elevated in these lambs throughout the sampling period.

In salivary samples (Figure 2), cortisol concentration was increased ($p < 0.100$) in lambs given ACTH compared to lambs given saline beginning 6 min after treatment administration. As in serum samples, cortisol concentration in saliva remained elevated in lambs receiving ACTH throughout the sampling period. As expected, a time effect was observed ($p < 0.001$) in lambs receiving ACTH, but no such effect was observed ($p = 0.122$) in lambs administered saline. Pearson correlation analysis indicated a strong relationship ($r = 0.66$, $n = 278$, $p < 0.001$) in these lambs, and simple linear regression analysis ($R^2 = 0.44$, $n = 278$, $p < 0.001$) predicted salivary cortisol concentration to be approximately 0.13 that of serum cortisol concentration in these lambs (intercept = -1.012). These findings in restrained lambs that were not preconditioned to human interaction are comparable to previous research by Yates et al. (2010), who used a low-stress ovine model, despite much greater pre-treatment cortisol levels in the current study. Although Blackshaw and Blackshaw (1989) have reported difficulty in obtaining assayable volumes of saliva from swine, no such difficulty was experienced with the young ovine model, even after 72 min of salivary sampling. Additionally, salivary samples were as readily obtained as blood samples at 3-min intervals. Coefficients of variation from the cortisol radioimmunoassay were comparable between serum and salivary samples, despite previous reports of increased variability in cortisol concentrations when

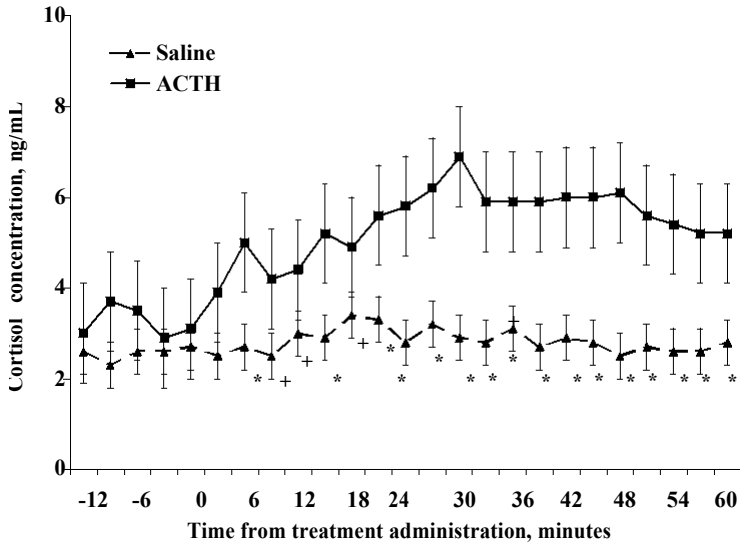

Figure 2. Salivary cortisol concentration in restrained ewe lambs administered 100 IU ACTH or saline at time 0. Treatment effect, $p = 0.078$; time effect, $p < 0.001$; treatment x time, $p < 0.001$. Treatment effects at each time are denoted by '+' ($p < 0.100$) or '*' ($p < 0.050$).

quantified in salivary samples compared to serum samples (Morméde et al., 2007). Data from this study support previous finding that salivary cortisol concentration is indicative of blood cortisol concentration (Fell et al., 1985; Yates et al., 2010), and that salivary sampling provides a viable non-invasive alternative to blood sampling for the quantification of cortisol.

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