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Variation in Wool Cortisol, Progesterone, and Testosterone in Targhee Ewes Across Physiological States and Varying Production Levels[1,2]

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Summary

Determining the pregnancy status of ewes prior to lambing is important for sheep producers to properly manage limited resources and improve profitability. We tested the hypothesis that steroid hormone (progesterone, cortisol, and testosterone) concentrations change through a production cycle and may be used as a pre-lambing pregnancy test. Twenty multiparous (4.5 ± 1.5 y) purebred Targhee ewes were enrolled in this study before the breeding season. Wool samples were collected at four time points beginning with a sample prior to breeding, at 30-d gestation; 110-d gestation; and approximately 40-d postpartum. Wool production data, including fiber diameter and staple length, and lamb birth data, were collected to test linear regression associations between hormone concentration and production. Using a repeated measures ANOVA, we found differences ($P < 0.05$) between time points and a post-hoc analysis showed that the 40 days post-lambing was statistically different (Bonferroni adjusted $P < 0.05$) from the previous three samples points. There were no associations between wool hormone concentrations prior to breeding, at 30-d gestation, or 40-d postpartum with production metrics ($P > 0.05$). An association was observed between progesterone levels at 110 days of gestation and litter size. These findings suggest that wool hormones could serve as valuable tools for researchers assessing animals post-lambing, although their utility as a diagnostic tool for producers may be limited. Nonetheless, further research is necessary to ascertain the potential of wool hormone monitoring in predicting other economically relevant flock performance metrics.

Key Words: Hormones, Pregnancy, Non-invasive Sampling, Ovis Aries

Introduction

Accurate and reliable health and production monitoring is an integral component to a well-managed and sound flock. Most sheep producers currently rely on production measures, veterinary diagnostics, and their own observations to determine the current status and outlook of their flock. Unfortunately, a number of these diagnostics may be expensive, time-consuming, and labor-intensive reducing their usefulness to individual producers. This challenge reflects a need for non-invasive and cost-effective tools to improve monitoring of sheep throughout a production cycle.

Unique to fiber-producing animals, wool is a complex, naturally renewable fiber that continuously grows through a sheep's life and may serve as an important resource for monitoring longitudinal effects on health and production levels. Given that wool grows year-round, wool represents an optimal sample for precise retrospective monitoring without sampling biases that exist with other biological matrices (Palme, 2012; Fürtbauer et al., 2019). Hair and wool are commonly used as tissue specimens for evaluation of chronic stress via cortisol measurements in many livestock species (Stubsjøen et al., 2015; Duran et al., 2017; Heimbürge et al., 2019; Sawyer et al., 2019). This represents a non-invasive sampling technique that producers routinely use for wool trait characterization (Scobie et al., 2015). While measuring wool cortisol is typically restricted to research uses, the potential exists for its application in the commercial industry.

Wool has also been found to incorporate the steroid hormones such as cortisol and progesterone, which are important biomarkers for stress and pregnancy status, respectively (Sawyer et al., 2019). It takes approximately 14 days for glucocorticoids in the circulation to be observed in the wool (Weaver et al., 2021). Elevated testosterone and cortisol levels in wool post-lambing were previously associated with litter size (Alon et al., 2021). Together these hormones could be measured throughout a production cycle to evaluate pregnancy status and chronic stress; however, their association to other aspects of lamb and wool production currently remains unknown.

The objectives of this study were to characterize the wool progesterone, cortisol, and testosterone profiles throughout a production cycle and determine if these measurements could be used as a producer diagnostic tool.

Materials and Methods

All animal procedures were approved by the Montana State University Agricultural Animal Care and Use Committee (Protocol # 2021-AA14).

Animals and Wool Sample Collection

Twenty multiparous purebred Targhee ewes (4.5 ± 1.5 y) from the Montana Agricultural Experiment Station flock were enrolled in the study prior to breeding season. Ewes were estrus synchronized using CIDR devices inserted and kept in place for 10 days prior to exposure to rams. Ewes were naturally exposed to rams for 30 days before rams were removed but conceived on the first cycle due to synchronization. Ewes were confirmed pregnant via trans-abdominal ultrasound on January 31st, 2022. Wool samples used for hormone extraction were collected from the rump using an electric shearing machine (Heiniger, Switzerland) with a 13-tooth comb as close to the skin as possible and were an approximately 5 x 5 cm square. Samples were placed into a Ziplock or brown paper bag and stored in a climate-controlled area at standard room temperature and humidity and out of sunlight until steroid extraction.

Wool samples were collected from each animal at four time points: prior to breeding (taken prior to CIDR insertion), at 30-d gestation, 110-d gestation, and approximately 40 days postpartum, as shown in Table 1. These time points were selected because they coincide with typical management practices occurring on western U.S. range sheep operations when producers would be able to easily collect a wool sample. A sampling schematic and corresponding management events can be found in Figure 1.

Figure 1. Sampling timeline. The dates, physiological stage, and sample collected are represented above the bar. Pregnancy is represented by the blue box. Western U.S. sheep industry management events are noted below the bar and correspond to sampling time points.

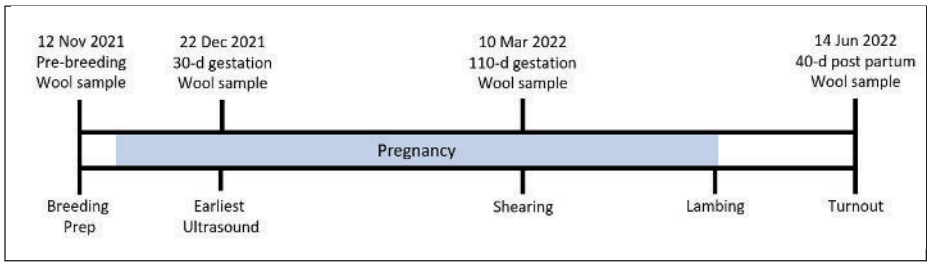

Table 1. Wool sampling dates, characteristics, and mean values ± S.E. for wool hormones

	Sampling date and status at time of sampling			
	12 Nov 2021	22 Dec 2021	10 Mar 2022	14 June 2022
Hormone pg/mg[1]	Pre-breeding	30-d gestation	110-d gestation	40-d post-partum
Cortisol (*n*=14)	1.94 ± 0.27^{ab}	2.78 ± 0.44^{a}	1.22 ± 0.24^{b}	7.97 ± 0.73^{c}
Progesterone (*n*=20)	7.17 ± 1.39^{ab}	12.4 ± 1.88^{a}	5.52 ± 0.67^{b}	33.8 ± 2.62^{c}
Testosterone (*n*=16)	1.36 ± 0.22^{a}	2.55 ± 0.45^{a}	1.44 ± 0.26^{a}	6.40 ± 0.56^{b}

[1] Different superscripts in a row indicate significant differences between sampling times at the Bonferroni adjusted *P*-value of 0.05.

Hormone Extraction and Detection

Cortisol, progesterone, and testosterone were extracted from wool samples as previously described with the following modifications (Sawyer et al., 2019; Alon et al., 2021). 250 mg samples were washed in 50mL conical tubes with 5 mL of 100% 2-propanol overnight and subsequently dried for a minimum of 24 hours. Following drying, a 50 mg sample of the most proximal portion, the approximately 1 cm closest to the skin, of the wool staple was isolated, placed into 1 mL of 100% methanol, and left to soak for 48 hours. The methanol layer was aliquoted and placed in a 0.5 mL microcentrifuge tube and allowed to evaporate in a fume hood at room temperature for a minimum of 24 hours. Once all the methanol was evaporated, the remaining residue was reconstituted in 400 µL of ELISA assay diluent. Hormone concentrations were quantified in duplicate using commercially available ELISA kits following manufacturer's protocols (Salimetrics; Ann Arbor, MI, USA) as previously reported (Fürtbauer et al., 2019; Alon et al., 2021). Extracted wool samples were diluted 1:10 and 1:15 for progesterone and testosterone, respectively. The extractions for evaluating cortisol were not diluted. Plates were read on a BioTek Epoch 2 plate reader at 450 nm and absorbance data captured using the BioTek Gen5 Data Analysis software (Agilent Technologies, Inc; Santa Clara, CA, USA). Intra-assay CV was 3.1%, 3.5%, and 3.5% for cortisol, progesterone, and testosterone respectively.

Production Data

Wool samples from the mid-side of the sheep at 110-d gestation, corresponding with a full-length staple and industry standards in a commercial setting, were used to evaluate fiber characteristics including fiber diameter, fiber diameter coefficient of variation, staple length, and curvature using the Optical Fiber Diameter Analyzer 2000 at the Montana Wool Lab. Wool samples were analyzed along the length of the wool staple. Gross fleece weights were also recorded at 110-d gestation. Lamb birth data collected included litter size and birth weights.

Statistical Analysis

All statistical analyses were performed using R version 4.0.4 (R Core Team, 2021). Data were analyzed for possible outliers defined as values more than three times above or below the interquartile range and assessed for normality by a Shapiro-Wilk test. For each hormone, a repeated measures ANOVA was conducted with sampling date as the independent variable and the hormone measures as the dependent variables. For the repeated measures ANOVA, only complete cases, those with all four time points measured for each hormone were used in the analysis. Post-hoc pairwise paired t-tests were conducted within each hormone to determine which time points were statistically different from one another. A Bonferroni adjusted p-value < 0.05 was considered statistically significant.

For associations with the production traits, a univariate linear regression was performed for each trait and each individual time point hormone value to determine if the level of steroid hormone in wool influences production traits.

Results and Discussion

Cortisol, Progesterone, and Testosterone measures

For each hormone repeated measures ANOVA, a difference was found ($P < 0.05$). Following post-hoc analysis there were no differences detected between the first two time points (Bonferroni adjusted $P > 0.05$) across all three hormones. Cortisol and progesterone concentrations decreased at the 110-d of gestation time point (Bonferroni adjusted $P < 0.05$). All hormone measurements increased at the 40-d postpartum sampling date (Bonferroni adjusted $P < 0.05$). Cortisol, progesterone, and testosterone, had approximately 4.03, 4.05, and 3.60 times the hormone level at 40-d postpartum compared to the average of the first three sampling time points, respectively. These elevated hormone concentrations in the wool at this time point reflect the last approximately 40 days of gestation and first 25 days of lactation. As previously shown in ruminants, concentrations of cortisol, progesterone, and testosterone rapidly increase in matrixes such as serum and milk towards the end of gestation [11-13]. Hormone concentrations for each sampling point can be found in Table 1. It is important to note the lag time between wool and blood hormone measurements and understand that the two measurements will not be identical when comparing to previous literature. As previously reported it takes approximately 14 days for glucocorticoids to incorporate into the wool if the hormones are elevated for sustained periods of time (Weaver et al., 2021).

It was unexpected that the concentrations of cortisol, testosterone, and progesterone were not increased at approximately 110-d in gestation or about 45 days before lambing compared to the prior two time points. This contrasts with results presented from maiden Australian merino ewes which showed progesterone and cortisol concentrations to be higher about two weeks prior to lambing (Sawyer *et al.*, 2019). This difference may be due to sampling time differences. In the present study, samples were collected approximately four weeks earlier than in Sawyer et al. 2019, using wool representing lower circulating concentrations of progesterone, cortisol, and testosterone during mid-gestation as opposed to late gestation (Fylling, 1970; Gaiani et al., 1984; Fowden et al., 1998). All three hormones showed a numerical decrease in concentration for the wool sample collected at 110-d of gestation compared to the 30-d gestation sample indicating there may be a difference from this sample than others. The 110-d samples were collected during the regular shearing process so could possibly have contributed to this unexpected result.

This result likely reduces the utility of wool hormone testing as a possible producer-oriented pregnancy diagnostic tool, given elevated hormone concentrations in the wool are not appearing early enough in gestation to be more valuable to the producer compared to other available tools. Given there are other pregnancy diagnostic tools available, such as blood testing for pregnancy associated glycoproteins and transabdominal ultrasound, which diagnose pregnancy much earlier in gestation, evaluating hormone status using a non-invasive wool sample may not be practical for the U.S. sheep industry. However, this type of testing could be used to determine if a ewe was

pregnant when ewes are lambing without supervision in a range or other extensive setting or ewes are not evaluated by producers shortly after lambing opening doors for monitoring in more extensive settings.

Associations with Production Metrics

The OFDA2000 results showed the ewes had a mean gross fleece weight of 3.22 ± 0.15 kg, mean fiber diameter of 19.9 ± 0.18 microns, mean coefficient of variation of 18.35 ± 0.43 percent, mean staple length of 78.75 ± 1.80 mm, and a mean curvature of 101.1 ± 3.09 degrees/mm.

Ewes lambed began lambing on April 22, 2022 and had a standard error of 1.10 days. Twenty ewes delivered 31 lambs with eleven sets of twins and the remaining lambs being born as singles. Sixteen lambs were male and fifteen were female. Lambs weighed 4.5 ± 0.14 kg at birth.

We did not observe an association between concentrations of post-partum wool testosterone or cortisol and litter size as previously reported (Alon et al., 2021; Zeinstra et al., 2023). This may be due to the fact that the largest litter size in our study population was only two lambs, whereas Alon et al. reported litter sizes of three and four lambs, though Alon et al. reported no statistical difference between those carrying singles and those carrying twins but found a statistical difference between singles and multiples when triplets and quadruplets were included (Alon et al., 2021). However other reports only found this association between large (3-4 lambs per litter) and small (1-2 lambs per litter) comparisons (Zeinstra et al., 2023). It is possible that these differences exist in triplet and quadruplet bearing rangeland type ewes, such as the Targhee breed studied here, but larger litter sizes are less common in a rangeland setting and more data is needed to validate that association in this setting. An association ($P < 0.05$) between wool progesterone level at 110-d gestation and litter size was observed, with ewes carrying singles (6.82 ± 0.90 pg/mg) having a higher level of progesterone than ewes carrying twins (3.96 ± 0.80). This is unexpected given reports in the literature typically show increasing blood progesterone concentrations with increasing litter size in sheep, though not consistent enough to use as a diagnostic for litter size determination (Stabenfeldt et al., 1972; Butler et al., 1981; Karen et al., 2006; Roberts et al., 2017). However, given the wide individual variation reported in progesterone concentrations among pregnant ewes, the relatively small sample size in the present study, and different biological material being tested, additional research is needed to further prove or dispute the present results.

There was no relationship identified between cortisol concentrations and fiber diameter as reported in Australian Merinos (Sawyer et al., 2021). This may be explained by the previous reported study using the topknot of wool for characterizing wool characteristics instead of the mid-side sample in the present study. While the topknot may be a convenient sample, there is variability in fiber diameter and characteristics across the sheep's body making a mid-side sample most appropriate for representing the entire fleece (Scobie et al., 2015). We did not observe any other relationships between wool traits and testosterone, progesterone, or cortisol measured at any other sample point.

This preliminary work shows that wool hormone concentrations do differ at different times over a production cycle. Monitoring wool hormones may be a useful tool for researchers and evaluating animals post-lambing, but may not be beneficial as a producer diagnostic tool prior to lambing given the lack of associations seen in the present study. However, given the small samples size and lack of open ewes in the present study, further work is warranted to determine the utility of wool hormone monitoring for possible predictive ability of long-term performance, longevity, or other economically relevant metrics not measured in this project.

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