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Hair testosterone and cortisol concentrations and their relationships to physiological and social status in feral horses (*Equus ferus caballus*)

Sarah A. Medill[1], David M. Janz[2], Philip D. McLoughlin[3]
[1]Department of Biology, University of Saskatchewan, Saskatoon, SK. Phone: (306) 966-4254, Fax: (306) 966-4461, E-mail: sarah.
[2]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK. E-mail: david.janz@usask.ca
[3]Department of Biology, University of Saskatchewan, Saskatoon, SK. E-mail: philip.mcloughlin@usask.ca

Determining steroid hormone concentrations in hair has been frequently performed in humans, and increasingly in wildlife and domestic animals. Hair hormone concentrations may provide insight on how individuals are responding to their physiological condition or social situation. Cortisol is most frequently measured in hair as a biomarker of long-term stress, while testosterone may be linked with reproductive status in males. These hormones are commonly measured in substances that reflect either current (e.g. blood) or very recent (e.g. saliva, urine, feces) circulating levels. However, these hormones are also incorporated into hair during hair growth and provide a chronological record of circulating hormone levels. Thus, analysis of steroid hormones in hair provides a much longer representation of an animal's endocrine status than other tissues frequently targeted for non-invasive monitoring.

The feral horse (*Equus ferus caballus*) population on Sable Island, Nova Scotia, Canada, has been annually censused during the mid-late summer since 2008 to track individual life histories and population dynamics. We collected tail hair (n = 144 females, n = 162 males) from known individuals either opportunistically or from natural or artificial snags to investigate how hair cortisol and testosterone might be associated with physiological state (e.g. lactating vs. non-lactating, body condition, age), as well as their social situation (e.g. dominant band stallion, subordinate band stallion, or bachelor) and measures of sociality. The proximal 5 cm of hair (excluding first 4mm or root region) were ground to a fine powder and hormones extracted with methanol and analyzed by using enzyme-linked immunoassay.

Preliminary analyses of the data showed a general sex based difference in hair cortisol concentrations (females lower than males; t = 3.16, df = 317, P=0.002). Among females, the presence of nursing foals was accompanied by an increase in hair cortisol (z = 2.93, df =140, P = 0.003); however, no significant difference was found in hair cortisol concentrations based on sex of the foal (t = -0.06, df = 82, P = 0.95). Horses in poor body condition tended to have higher hair cortisol than those in good or excellent condition (slope= -0.203, df = 312, P = 0.003). We also observed an increased concentration of hair cortisol as horses increased in age from 3-6 or entered into reproductive maturity. Adult male dominant band stallions did not have significantly less cortisol than bachelors or subordinate stallions but these three groups were significantly greater than young males (aged 3 and 4) who generally do not challenge the older males for reproductive opportunities.

Additionally, we looked at hair testosterone concentrations for n=46 males. Testosterone is known to influence traits and behaviours that enhance sexual selection. Often there is an inverse relationship between cortisol levels and testosterone; in particular, being able to maintain high testosterone and not have elevated cortisol related to the metabolic costs of sexual trait production ensures that traits or behaviours honestly signal the quality of the individual. For this reason we'd expect to see band stallions (those males in a position to mate) have a lower value in the ratio Cortisol: Testosterone. Early indications suggest we see this phenomenon in feral horses.

As a relatively new approach in wildlife research, the use of hair hormone analysis shows promise in contributing to our understanding of physiological aspects of sexual selection and other processes. Additionally, hair hormone analysis may have applications in advancing knowledge of animal husbandry and in particular, welfare.

Corresponding author: Sarah A. Medill1
Email: medill@usask.ca