Biomarkers of Stress in Different Animal Species

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Contributor: Jalil Ghassemi Nejad , Morteza Hosseini Ghaffari , Mohammad Ataallahi , Jang-Hoon Jo , Hong-Gu Lee

When studying stress in animals, it is important to understand the types of stress and their classification, and how to assess the stress levels in different animal species using different matrices accurately and precisely. Among biomarkers, it is crucial to always identify the reliable ones depending on the applicability of the sample, as the markers must be highly correlated with the specific pathophysiological aspects of the particular stress.

stress hormones biomarkers of stress

1. Biomarkers of Stress

Among biomarkers, it is crucial to always identify the reliable ones depending on the applicability of the sample, as the markers must be highly correlated with the specific pathophysiological aspects of the particular stress [1][2]. In general, biomarkers for stress may include proteins, enzymes, hormones, chemicals, metabolites, genes, or byproducts [1]. Under stressful conditions, two major endocrine systems are activated [1][3], resulting in the release of various hormones, including epinephrine, norepinephrine, and cortisol [2][4][5]. Cortisol and corticosterone are the primary glucocorticoids and have been used as classic biomarkers of stress in animals [1][6][7][8]. Although cortisol and corticosterone are both detectable in many animal species, cortisol is the primary endogenous adrenal steroid in most mammals, including humans, many larger mammals, vertebrates, and fish, while corticosterone is the primary adrenal corticosteroid in few rodents, and birds to understand biological systems [1]. In research experiments or medical diagnostics, the majority of cortisol assays have been performed on biomatrices such as serum, saliva, urine, milk, and other biological fluids. However, most of these liquid biomatrices are suitable for measuring cortisol concentration at a single time point and represent the acute stress state in the physiological diurnal fluctuations.

Over the past decade, the use of hair as a biomarker of stress has been well-documented and established [1]. Several non-liquid biomatrices including hair [6][7], feather [1][9][10][11], fin [8], wool [7], turtle claws [12], dog nails [13], cat nails [14], feces [15][16][17][18], nails [19], tooth [20] are applied for measuring overall long-term systemic glucocorticoids (cortisol, corticosterone) exposure. These biomatrices can be used to monitor chronic stress levels, and noninvasive, stress-free sampling is particularly beneficial to animal well-being. For example, in healthy animals, blood cortisol levels can fluctuate (blood cortisol levels peak in the early morning and gradually decline thereafter) [21], and in addition, factors such as low or hot temperatures, humidity, and wind can affect blood cortisol levels. In addition, cortisol or corticosterone levels in biomatrices such as serum and saliva reflect hypothalamic-pituitary-adrenal (HPA) axis function shortly after its activation, and in biomatrices such as urine and feces reflect HPA activity ranging from a few hours to a few days before measurement. Feces can be used for monitoring both acute and chronic stress. That is not a matter of the material, but the frequency of sample collection. Capturing the acute response requires more frequent samples (in order not to miss the peak excretion of fecal cortisol metabolites), while chronic stress (or baseline HPA activity) can be assessed by a few samples (especially because levels are smoothened in the feces) [1][15][17]. However, cortisol or corticosterone levels in biomatrices such as hair, feathers, fins, scales, nails, and teeth reflect HPA activity over longer periods (weeks or even months), making non-liquid biomatrices useful for estimating chronic stress in animals and humans [1]. However, the use of alternative biomatrices in different animal species depends on the objectives of the study [8][9][19]. Therefore, all established alternative matrices as well as the exploration of new alternative stress indicators should be considered when studying stress in animals.

There is a potential relationship between cortisol levels in hair and those in saliva, urine, and feces, but each of these has its limitations [1][22]. Furthermore, correlations between different samples of materials have not proven useful, especially those reflecting different time windows. Nonetheless, the trend (of increased or decreased) stress hormones in various matrices provide insights into interpreting the results of studies which they can be studied by comparing the level of hormones in different matrices (e.g., saliva vs. blood, hair, etc.) [1]. Given the variety of the research analyzed, meta-analyses of correlation coefficients showed significant variability between studies [23]. There are a number of factors that tend to bias the result of blood cortisol and its metabolites rather than the treatment effect, which can also be used in the same manner to alter saliva values. It should be noted that while the use of urine and feces for hormone measurement is promising and associated with hormone production over an extended period, collecting urine and feces samples from each individual and the difficulty of storing these samples pose some difficulty [1]. It seems that the use of hair, wool, and feathers from pets to better identify hormonal changes over time is a better approach to overcoming the above difficulties [1]. Earwax has been recently presented as a promising matrix that can be reliably used to measure stress by extracting cortisol. Earwax has the same advantages of hormone measurement and sampling in hair/wool/feathers over traditional biological fluids (blood, plasma, serum, and saliva) and eliminates ethical concerns because sampling is non-invasive [24]. The recent indicator of cortisol in earwax as a non-stressful sampling method in both humans [25] and animals [no published data] is also considered to provide cumulative retrospective measurements of stress hormones (up to a few weeks) and sampling is simple and not painful. Earwax is thought to represent an accumulation of cortisol output over weeks or months [1]. In addition, the collection and storage of earwax and hair are simple, which may facilitate their use in chronic stress research. In addition, the scales, fins, and jawbones of aquatic animals appear to be promising matrices to show stress levels by measuring cortisol levels [1][8]. However, further studies are needed to validate these matrices as a reliable indicator of stress. A comparison of the hair matrix with other biomatrices that have been used to study chronic stress in animals is shown in **Table 1** [24][26]. In addition, a classical biomarker should have the following characteristics: (1) ease of collection and processing of the appropriate biological sample; (2) stability and durability of the marker throughout the storage and evaluation period; (3) availability of assays with sufficient specificity and sensitivity for the marker in question. Additionally, the identification of non-invasive methods for biomarker assessment has the potential to provide accurate data concerning induced stress stimuli to ensure a standard measurement in response to stress.

Table 1. Properties of hair matrix in comparison to other biological matrices where cortisol/corticosterone can be analyzed [7][24][26].

Properties	Biomatrices									
	Hair/wool	Feather	Nail/ Teeth/Scale [1]	Earwax	Feces	Urine	Sweat	Saliva	Milk	Blood
Stressful-sampling procedure	Low [1]	Low	No	Low	Low	Low	Low	Low [2]	Low	High
Sampling effects on results	No	Low	No	No	No	No	Yes	Yes	No	Yes
Painful-sampling procedure	Low	Low	No	Low	Low	Low	Low	Low	Low	High
Liability to external contamination	High	High	Low	Low	High	High	High	High	Low	Low
Application acute stress	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Application chronic stress	Yes	Yes	Yes	Yes	Yes	Yes	No [3]	No [4]	No [5]	No [6]
Possibility of repeated sampling	No	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes
Liability to blood contamination of samples	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	No
Effect of pH on composition	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Affected by location of sample collection	Yes	No	No	No	Yes	Yes	Yes	No	No	No

2. Evaluation of Stress in Domestic Animals Including Ruminants, Birds, and Aquatic Organisms

Properties	Biomatrices									
	Hair/wool	**Feather**	**Nail/ Teeth/Scale [1]**	**Earwax**	**Feces**	**Urine**	**Sweat**	**Saliva**	**Milk**	**Blood**
Need for vet personnel for sample collection	No	No	No	Yes	No	Yes	No	No	No	Yes
Time periods of cortisol represented	Weeks, Months, Years	Weeks, Months, Years	Weeks, Months, Years	Weeks, Months	Two to four days	One to two days	Single point, hour	Single point, hour	4–10 h	Single point, hour
Measuring cortisol forms	Unbound	Unbound	Unbound	Unbound	Unbound	Unbound	Unbound	Unbound	Bound and unbound	Bound and unbound
Storage condition	Room temperature	Room temperature	Refrigeration, freezing	Refrigeration, freezing	Refrigeration, freezing	Refrigeration, freezing	Refrigeration, freezing	Refrigeration, freezing	Refrigeration, freezing	Refrigeration, freezing
Analytical costs	High	High	High	Medium	Medium	Medium	High	Medium	Medium	Medium

[27][28][29][30][31][32][33] [34][35] [29][30][36] [37] [29][36][38] [39][40] [38] [38][41] [42][43]

ded and hms [30]; [39][40], other [38][41]. The straining ults. The cies [42], ge of the eas free cortisol is the active fraction only in saliva [42][43]. In aquatic animals, recent studies focused on the measurement of cortisol in golden fish [44] and sturgeon [8] and indicated the usability of fins and scales as matrices to extract and show cortisol levels. Recently, Ghassemi Nejad et al. [8] took a step forward and successfully extracted cortisol from the jawbones of sturgeons. They also identified the type of washing solvent and its effect on cortisol detection and found that there were no differences in cortisol levels in fins of three sturgeon species or cortisol levels in the fins and jawbones between washing solvents. Overall, they suggested that the sturgeon jawbone matrix is a promising alternative stress indicator to non-liquid matrices in dead fish.

[1] Depending on the species. [2] In mice and rats that may be stressful. [3,4,5,6] Series of measurements could provide information about chronic stress.

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