



Hair cortisol as a biomarker of chronic stress in periparturient dairy cattle: associations with health status, lactation, and metabolic profiles

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Abstract

Dairy cows experience long-term stress during the pre- and postpartum period, as during this period they face extreme challenges related to their health, productivity and overall welfare. Recently, hair cortisol has gained attention as one of the most promising and non-invasive biomarkers for evaluating stress. However, the exact mechanism of its deposition in the hair remains unknown. This review combines the utility of hair cortisol as a tool for evaluating chronic stress and its effect on health status, lactation, parity, body condition score (BCS), and metabolic parameters, specifically glucose, β -hydroxybutyrate (BHBA), and non-esterified fatty acids in dairy cows. Emphasis is given to the transition period around parturition, where hormonal fluctuation, negative energy balance, and immunosuppression increase the animal's susceptibility to stress. Methodologically, tail hair exhibits a higher cortisol concentration than other regions due to a faster growth rate, making it an ideal site for maintaining a proper sampling interval during the transition period. Furthermore, regrown hair contains higher cortisol levels than native hair during parturition. In addition, hair cortisol level significantly elevated during peak lactation in multiparous cows and in cows with oversized or twin calves. Notably, newborn calves have higher cortisol than their dam during birth. Cows diagnosed with a disease condition display higher cortisol levels than healthy cows during the postpartum period. During the transition period, Glucose was found to be positively correlated with hair cortisol level, whereas β -hydroxybutyrate (BHBA) was negatively correlated with hair cortisol in multiparous cows. Ultimately, evaluating hair cortisol concentration alongside associated production and metabolic factors during the periparturient period can provide a proper framework for improving and monitoring dairy cattle welfare. However, further research is required on hair cortisol concentration and its connection with various stressors of the transition period, sampling and analytical methods, and a standardized reference range so that it can have practical use at the farm level.

Keywords Hair cortisol · Stress · Transition period · Lactation · Dairy cows

Introduction

During the transition period, dairy cows undergo profound physiological, metabolic, and hormonal challenges spanning from late gestation to early lactation (Yogeshpriya et al. 2024), marked by negative energy balance. This altered physiological status can be observed through hair cortisol concentration, which elevates from a pre-partum baseline level of 0.4 pg/mg to 0.8 pg/mg post-partum (Tekin et al. 2023). A stress reaction occurs when an animal's body initiates adaptive mechanisms in response to significant challenges, known as stressors, thereby activating the hypothalamic-pituitary-adrenal (HPA) axis and a subsequent rise in cortisol levels (Tiwari et al. 2015). Chronic

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stress affects not only immune function but also reproductive performance in animals due to prolonged activation and overproduction of stress-related mediators (Matteri et al. 2000). Stress during the pre- and postpartum periods is usually assessed using cortisol concentrations in blood, saliva, urine, or feces. However, cortisol concentrations in these matrices only provide information about stress exposure within a short time frame, ranging from several hours to days (Ghassemi Nejad et al. 2022). To measure long-term stress during the peri-parturient period, measurement of cortisol levels in hair has gained attention from researchers as a non-invasive biomarker (Peric et al. 2024; Heimbürge et al. 2019). The exact process by which cortisol is incorporated into the hair shaft remains unclear due to the complex structure of hair. One possible explanation is that cortisol may enter the hair follicle via the blood circulation (Heimbürge et al. 2019) or surrounding tissues (Meyer and Novak 2012) as depicted in Fig. 1. However, since most circulating cortisol is attached to cortisol-binding globulin, it must first be released from this protein before it can move into tissues (Ralph and Tilbrook 2016). Moreover, cortisol can

be incorporated through sweat and sebum in hair or from external sources (Pragst and Balikova 2006), or it can be locally produced in small quantities by hair follicles (Ito et al. 2005). Once inside the follicle, cortisol likely binds to receptor proteins and becomes involved in the keratinization process. As the follicular bulb cells carrying receptor-bound cortisol undergo terminal differentiation into keratinocytes, the cortisol becomes embedded within the hardening hair cuticle (Burnard et al. 2017). In dairy cows, during prepartum and partum phases, hair cortisol concentration (HCC) fluctuates due to the animal's changing energy demands and expected milk production, with significant elevation occurring around parturition (Tekin et al. 2023). Besides, elevated cortisol levels are associated with pregnancy and calving (Braun et al. 2017), disease conditions, and multiparity in cows (Burnett et al. 2015). Additionally, HCC serves as a robust indicator of long-term health issues and welfare quality metrics, correlating with issues like lameness or skin damage (Lavrijsen-Kromwijk et al. 2025). Despite growing interest in using hair cortisol concentration as a biomarker of chronic stress, its practical implementation at the herd

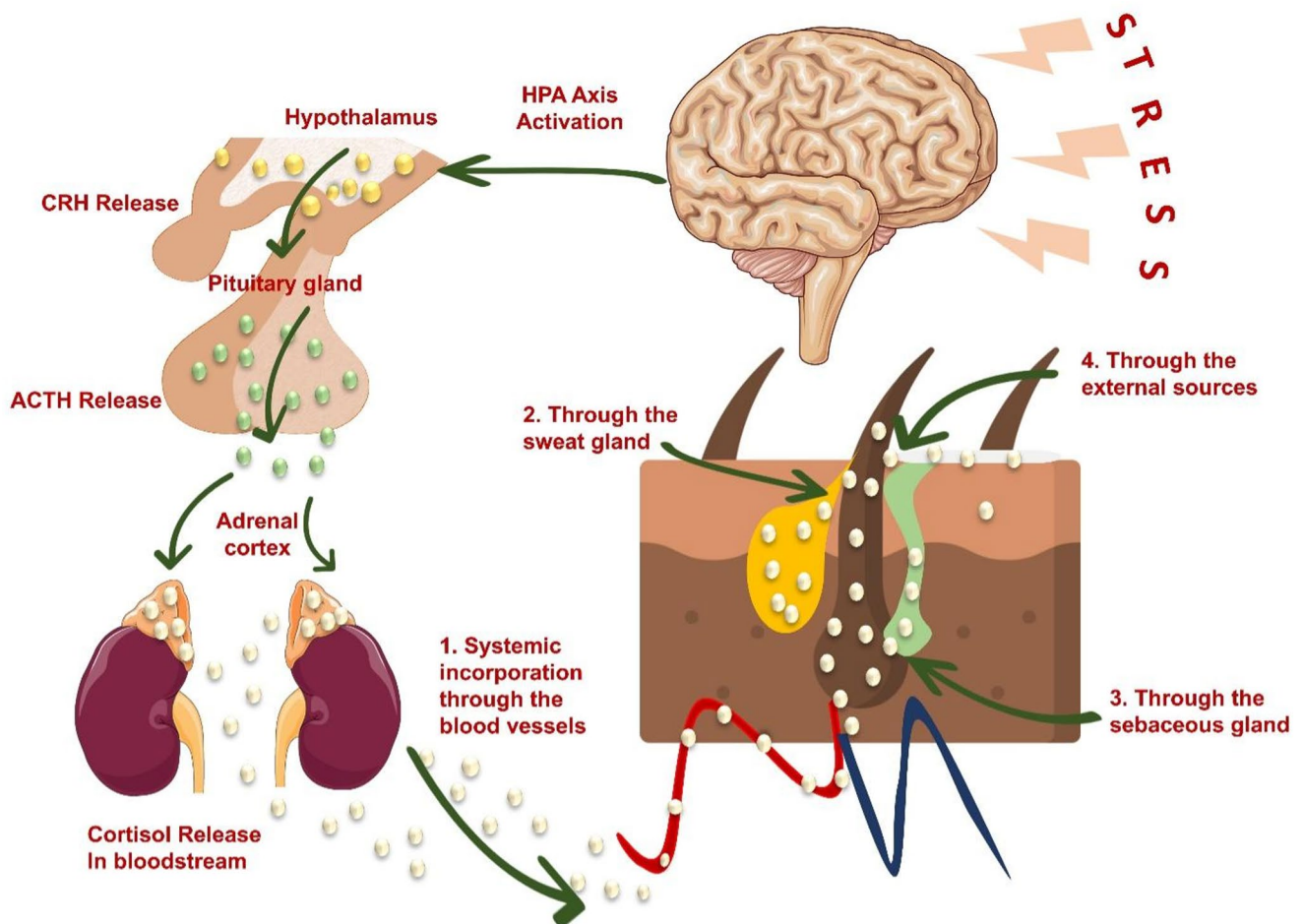


Fig. 1 The mechanism of cortisol secretion in response to stress and incorporation in hair shaft via circulation; via sweat or sebum; via external sources

level is still limited. This is due to inconsistencies in sampling techniques, result interpretation, and lack of standardized reference values. Moreover, the association between chronic stress reflected via hair cortisol levels and complex physiological and biochemical changes around the periparturient period is still not so clear, despite it being the most crucial phase for dairy cows. Therefore, a comprehensive review is needed to combine the current knowledge with existing knowledge gaps and future directions to highlight the potential of hair cortisol measurement in herd health monitoring and management strategies. By evaluating existing literature, this review solely aims to explore the use of hair cortisol to measure chronic stress levels, its association with health status, the lactation stage of dairy cows, and its effect on the metabolic profile of dairy cows, particularly during the transition period.

Methodology

Literature search strategy

This review aimed to collect, evaluate, and summarize existing scientific literature on the application of hair cortisol concentration (HCC) as an indicator of chronic stress in dairy cattle during the pre- and post-partum periods and its association with health status, stages of lactation, and related biochemical parameters. A comprehensive search of peer-reviewed articles was conducted across major electronic databases such as PubMed, ScienceDirect, Google Scholar, ResearchGate, Web of Science, and Scopus. Studies published between 1996 and 2025 were considered and evaluated. Older studies were included to capture the foundational studies establishing hair analysis methodologies and cortisol incorporation. These relevant benchmark studies were included alongside the recent ones to ensure the inclusion of both fundamental and recent findings on hair cortisol as a chronic stress indicator, analytical methodologies, and how various factors affect cortisol levels during the transition period.

The electronic search was executed using a combination of words connected through Boolean operators (“AND” and “OR”). The main search words include: Pre and post-partum period, hair cortisol concentration or hair cortisol or HCC or biomarker of chronic stress, Transition period or Periparturient period or calving, Dairy cows or lactating cows, Health condition or Calving condition or calving environment, Lactation status or milk parameter, Biochemical profile or metabolic profile, Disease condition and hair cortisol concentration, Body condition score and Hair cortisol concentration, Parity and hair cortisol concentration, hair cortisol analysis protocol.

Study selection and eligibility criteria

For review, articles retrieved through the initial search were first screened by title and abstract, then the full text of articles was evaluated through predefined selection criteria. The inclusion criteria included literature published in English from 1996 to 2025. Studies focused on dairy cattle, especially on the periparturient period, but other animals and humans were also considered in some cases to validate analytical methodologies or fundamental physiology. Besides, Studies focused primarily on hair cortisol as a biomarker of chronic stress were included. However, in certain cases, studies used other biomarkers, such as blood, saliva, and feces, which were also considered if they were evaluated side by side with hair in dairy cows. Moreover, studies investigating the association of hair cortisol concentrations with production and health factors such as parity, lactation, health status, or metabolic and biochemical indicators were also given emphasis. The conference papers, book chapters, and non-English articles were not included. Besides, Studies that solely assessed cortisol levels using biomarkers such as blood, saliva, or feces of animals were not considered, as they indicate acute stress.

Factors related to sampling method and analysis

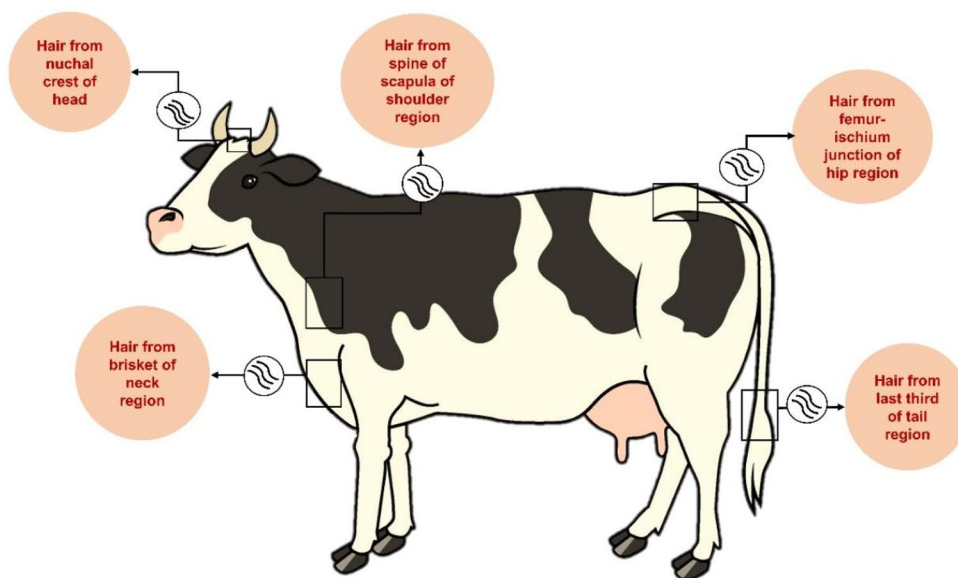
Sampling site, hair color & time of sampling

Factors like hair collection site, hair color, or growth phase of hair can affect the cortisol concentration, as shown in Table 1. According to Bennett and Hayssen (2010) cortisol levels varied with different hair colors and site of collection. Hayashi et al. (2021) found a lower concentration of cortisol in black hair compared with white hair across various phases of the transition period (dry period, 2 months prepartum, 1month prepartum, etc.) in Holstein cows. Burnett et al. (2014) also found higher cortisol in white hair than black hair (7.8 ± 1.1 vs. 3.8 ± 1.1 pg/mg) in dairy cows. But Brahmi et al. (2025) and Tallo-Parra et al. (2018) found greater cortisol levels in black hair compared to white hair. While the exact reasons for this variation remain unclear, it can be associated with the development and differentiation of hair melanocytes (Slominski et al. 2004; Roulin et al. 2008).

There are several anatomical sites for collecting hair samples, including the head (Comin et al. 2011), neck (Tallo-Parra et al. 2015), shoulder (Braun et al. 2017), back (Heimbürge et al. 2020a), thoracic region (González-de-la-Vara et al. 2011), and tail switch (Endo et al. 2017) as depicted in Fig. 2. The site of sampling can have a significant effect on cortisol concentration. For example, Macbeth

Table 1 Biological and methodological factors affecting Hair cortisol concentration

Factors	Hair cortisol concentration	Reference
Color of hair	Variable	(Hayashi et al. 2021) (Burnett et al. 2014) (Brahmi et al. 2025)
Sampling site	Higher in tail than shoulder (11 ± 1.2 vs. 6.2 ± 1.2 pg/mg) Higher in the tail (1.99 ± 0.189 pg/mg) than shoulder, head, neck, hip (0.82 ± 0.189 , 1.14 ± 0.189 , 1.50 ± 0.189 and 1.59 ± 0.189 pg/mg)	(Burnett et al. 2014) (Moya et al. 2013)
Sampling method	LCMS (higher sensitivity with a limit of quantification down to 0.5 pg/mg)	(Binz et al. 2016)
Type of hair	Maximum in regrown hair (1.40 ± 1.08 pg/mg) during parturition	(Braun et al. 2017)

Fig. 2 Site of hair collection in cow. Head region; neck region; shoulder region; hip region; tail region

et al. (2010) reported that hair collected from the neck region had comparatively higher cortisol concentration than hair collected from the rump, shoulder, or abdomen. Moya et al. (2013) found higher cortisol concentration in the hair of the tail region than the head, shoulder, neck, and hip in beef cattle. The hair from the tail switch grows more than 10 times faster (0.51 ± 0.05 mm/d) than the hair on the hips and shoulders (0.04 ± 0.05 and 0.03 ± 0.05 mm/d). As a result, biologically significant resampling intervals in dairy cows can only be accurately achieved by measuring the hair growth rate of the tail switch, particularly during the transition period (Burnett et al. 2014). Growing hair needs additional blood supply (Vesel et al. 2020), leading to greater exposure to cortisol. So, the tail switch can sense greater cortisol change within a short interval of 3 weeks; hence, a more suitable hair collection site for cortisol analysis. The growth rate of hair also affects cortisol concentration across different hair colors and collection sites (Moya et al. 2013). Timing of sample collection is also crucial to detect cortisol levels in hair induced by various stressors like calving or disease conditions (Tallo-Parra et al. 2018). Specifically, utilizing the shave-re-shave protocol requires an approximate interval of 2–3 months from initial clipping

to complete hair regrowth, as bovine hair grows 0.6–1 cm/month with complete moulting in 3 months (Schwertl et al. 2003). According to Braun et al. (2017), stress during the parturition period can be well observed from the regrown hair area compared to hair collected from the unshorn area, as the actively growing anagen phase can incorporate more cortisol than the catagen and telogen phases of unshorn hair. So, it can be a cause of variation in hair cortisol concentration at different periods of sampling.

Sample processing and analysis

Hair samples should be cut as close to the skin surface as possible with scissors from the tail region and should be kept in dry paper envelopes at room temperature, away from light, until further processing (Burnett et al. 2014). In a study conducted by Vesel et al. (2020), hair samples required manual cleaning due to contamination with dirt and feces, in order to prevent the potential measurement of cortisol originating from feces. The sample should be weighed out, washed with 5 ml isopropanol for 3 min, and dried for 5 days (Burnett et al. 2014). But Nejad et al. (2019) repeated the washing protocol for 2 times and then dried for 7 days

at room temperature. Then the sample can either be cut with surgical scissors or minced with a mixer mill (Burnett et al. 2014). Mincing can be done either with a ball mill (Nedić et al. 2017) or a pestle and mortar (Vesel et al. 2020). Hair cortisol levels were found to be higher in samples cut with scissors than in minced samples (Slominski et al. 2015). However, Burnett et al. (2014) indicated that milling samples can yield up to 3.5 times more cortisol than using surgical scissors while providing more precise and less variable data. Grinding of hair samples creates more homogenized and ultra-small particles, which optimizes the potential for higher cortisol extraction (Nejad et al. 2019). Extraction of hair cortisol can be done by adding methanol to the sample in a glass vial, sonicating, incubating it overnight, pipetting the methanol out into a glass tube, and evaporating it (Koren et al. 2002). After evaporation of methanol, hair sample should be reconstituted with PBS medium. But Slominski et al. (2015) performed a two-step double extraction with methanol and acetone, which resulted in higher cortisol extraction (98%–100%) than single methanol extraction, yielding only 46.1% cortisol on average. The sample can be analyzed with ELISA, Radioimmunoassay, HPLC-Mass Spectrometry, HPLC-fluorescence detection, or chemiluminescent immunoassay (Meyer and Novak 2012). Hair cortisol levels were found to be higher by ELISA (172.2 ng/ml) than LCMS (38.7 ng/ml) (Slominski et al. 2015). Russell et al. (2015) also found 2.5 to 10 times higher cortisol levels by ELISA than LCMS due to cross-reactivity with other steroid hormones such as progesterone (7.2%) and cortisone (6.2%). Tallo-Parra et al. (2015) found both intra-assay and inter-assay coefficients of variation (CV) less than 15% ($4.9 \pm 2.39\%$ and $10.6 \pm 1.77\%$, respectively) with recovery of $89.0 \pm 23.52\%$ for Enzyme Immunoassay (EIA) validation, which indicates its accuracy for measuring hair cortisol levels in cattle. On the other hand, LCMS is capable of detecting minute quantities of cortisol down to 0.03 ng/mg of hair (Gupta et al. 2023). That's why LCMS is regarded as the gold standard test for detecting hair cortisol levels due to its high specificity and sensitivity, with a limit of detection (LOD) and a limit of quantification (LOQ) less than 0.5 pg/mg and 85% recovery (Binz et al. 2016). The specific type of assay used isn't critically important, as long as it can accurately detect cortisol and is sensitive enough for the sample size being tested. Therefore, hair cortisol concentration varies with collection site, color, sample processing (washing, cutting, extraction), and chosen analytical method, which further makes setting a standard reference range impossible; thus, comparisons remain relative.

Physiological and environmental factors affecting hair cortisol concentration during the transition period

Parity

Studies revealed that the influence of parity on hair cortisol concentration varies between pre- and postpartum phases of the transition period. Both Sano et al. (2023) and Burnett et al. (2015) found no significant difference in hair cortisol concentration in primiparous and multiparous animals in the prepartum period. This consistent baseline suggests that the initial physiological preparation for parturition creates a similar stress response across all heifers and cows regardless of parity. However, Burnett et al. (2015) observed a significant change in HCC in terms of parity, as the days in milk (DIM) increase after calving. According to that study, multiparous cows have higher cortisol concentrations than primiparous cows at day 21 after calving (postpartum period), which gradually decrease as the days in milk (DIM) increase. This observation completely contradicts the general belief that primiparous cows suffer from more stress than multiparous cows during the transition period, as primiparous cows are not adapted to this transition. While a recent study by Botia et al. (2025) similarly found higher HCC levels with increased parity but no significant association with DIM, as observed by Burnett et al. (2015). So, more studies should be conducted to clarify the inconsistent relationship between DIM and HCC. Overall, parity significantly affects hair cortisol concentration, as multiparous cows face drastically higher metabolic demands due to higher milk yields with the additional challenge of repeated calving and rearing offspring, which leads to elevated levels of HCC.

Calving environment and difficulty

Cortisol concentration in hair significantly elevates during the calving period (Braun et al. 2017). So, the provision of an optimal calving environment during this time is crucial for proper calving. Calving environment involves the type of area in which a cow gives birth. The cow should be shifted to the specific calving area before calving. The calving area should be clean to ensure the safety and comfort of the animals (Villetaz Robichaud et al. 2016). Specifically, replacing old bedding in calving areas has been shown to decrease the significant rates of neonatal calf diarrhea and Johne's disease transmission (McKenna et al. 2006). According to Sano et al. (2023) there was no significant difference in hair cortisol concentration (HCC) of cows kept in different calving environments (calving pens, tie stall and paddocks, respectively). It can be due to exposure to similar

managerial practices, such as having a similar feeding and milking schedule and exposure to the same weather conditions, despite having different calving environments.

According to Sano et al. (2023) Higher HCC was found in cows having twin calves or oversized calves than having normal calves during calving. Cows having oversized fetuses or twins need more nutritional support and have higher metabolic requirements for fetal development, which can act as a stressor for the cows. Besides this, these cows are at higher risk of dystocia (difficult calving) or other complications, leading to a higher level of hair cortisol concentration during the transition period.

Cortisol concentration in newborn calves

Stress during the transition period in the dam can affect the cortisol concentration of neonates born. Braun et al. (2023) found higher HCC in newborn calves on the day of birth than in their dam in both healthy and sick conditions of the dams, as shown in Table 2. Heimbürge et al. (2020b) also found increased HCC in newborn calves than in adult cattle. Fetal plasma corticosteroid levels rise 10–15 days before birth in most mammals for fetal development and initiation of parturition (Fowden et al. 1998). Another possible explanation is that cows exhibit higher HCC in late pregnancy, which can be transferred to the fetus and elevate fetal HCC levels.

Health condition

Cows can have various health issues or diseases during the transition period, such as metritis, clinical or subclinical endometritis, retained placenta, ovarian cysts, mastitis, or other metabolic diseases. These can act as stressors during the pre- and postpartum periods. According to the study of Burnett et al. (2015) multiparous cows with at least one clinical disease (Metritis, mastitis, laminitis, etc.) have higher HCC levels than healthy cows during the postpartum period. But no change was detected in cows with only

subclinical endometritis. This study also has similarities with the study of Sano et al. (2023). These findings suggest that while subclinical conditions might not trigger the prolonged activation of the HPA axis, clinical illnesses can serve as an indicator of chronic stress by elevating the HCC level. But Tekin et al. (2023) found no difference in HCC between healthy and diseased cows, which can be due to management variation. Braun et al. (2023) Also found no significant difference in hair cortisol concentration in ill cows due to immediate treatment. In another study by Endo et al. (2019), older cows have more HCC before parturition, indicating more subclinical disorders they face after parturition. This suggests that cows with increased parity and age have to bear long-term physiological stress, which increases their susceptibility to different diseases. High metabolic and hormonal demand and physiological stress during the pregnancy and lactation period increase cortisol levels during parturition (Burton and Kehrlri 1996) which in turn decreases the functional capacity of peripheral blood mononuclear cells and causes immunosuppression (Preisler et al. 2000). Ultimately, the inherent stress suppresses the immune system during calving, making the cow susceptible to transition diseases, which then drive chronic HPA axis activation and further elevate postpartum HCC.

Reproductive performance

The relationship between hair cortisol concentration and reproductive performance stems from a direct inhibitory effect of stress hormones on the HPA axis. According to the study of Dobson and Smith (2000) cortisol level decline the reproductive performance of cows. This decline occurs as cortisol suppresses the Luteinizing hormone (LH) surge by lowering the sensitivity of the pituitary gland to Gonadotropin-releasing hormone (GnRH), which ultimately delays ovulation (Gupta et al. 2023). Furthermore, Higher levels of cortisol reduce the release of estradiol and suppress estrous behavior (Hansen and Aréchiga 1997) and lead to silent ovulation and ovulation failure in dairy cows. Endo et

Table 2 Factors associated with hair cortisol concentration at the transition period

Factor	Hair cortisol concentration	Reference
Parity of cow	Higher in multiparous cows than primiparous cows (9.2 vs. 7.8pg/mg)	(Burnett et al. 2015; Botia et al. 2025)
Calving difficulty	Higher in cows having oversized or twin calves than normal or single calves (4.2 ± 2.2 pg/mg vs. 2.1 ± 1.5 pg/mg)	(Sano et al. 2023)
Newborn calf	Higher than the dam (31 pg/mg vs. 0.6 pg/mg)	(Braun et al. 2023)
Disease	Higher in diseased cows (3.8 ± 1.1 pg/mg vs. 2.3 ± 1.9 pg/mg) than in healthy cows.	(Sano et al. 2023)
Fertility	Higher in sub-fertile cows ($p < 0.05$) than in fertile cows	(Endo et al. 2019)
Lactation	Higher in peak lactation (4.1 ± 0.5 pg/mg)	(Endo et al. 2017)
BCS	Variable	(Burnett et al. 2015; Endo et al. 2019)
Breed	Higher in Holstein Friesian (1.75 pg/mg) than other breeds Higher in Holstein than Busha cows ($p < 0.05$)	(Braun et al. 2022) (Nedić et al. 2017)

al. (2019) stated that cows show a different pattern of hair cortisol concentration at the postpartum period according to the time of fertility and artificial insemination. A significant change was found in HCC in normal and delayed first Artificial insemination (AI) cows during the postpartum period. Cows with delayed AI had higher HCC than those with normal AI at different sampling times of the postpartum period. In addition, sub-fertile cows had more HCC than fertile cows in the postpartum period. Similarly, Burnett et al. (2015) also reported that multiparous cows that became pregnant 100 days postpartum had lower HCC than those that were not pregnant by 100 days postpartum. Sub-fertile cows often suffer from negative energy balance, reproductive diseases, and other metabolic disorders. These physical strains serve as chronic stressors, which ultimately affect the HPA axis, elevate cortisol levels, and in turn suppress the hormonal cascade necessary for fertility, successful ovulation, and conception rate.

Lactation and milk yield

According to Fukasawa and Tsukada (2010), cortisol concentration was higher in the early lactation stage than in other stages. Similarly, Endo et al. (2017) found elevated cortisol levels (4.1 ± 0.5 pg/mg) during peak lactation of 60–90 days postpartum. According to Otten et al. (2023), there is a positive correlation between milk yield and hair cortisol (HCC) concentration from parturition to 300 days postpartum. It can be due to the higher metabolic demand of the body for higher milk production during the lactation period. But Burnett et al. (2015) found higher HCC in cows with low milk yield. Tallo-Parra et al. (2018) also found a negative effect of HCC on milk yield. These findings may stem from the stress-induced activation of the HPA axis, which triggers cortisol release that subsequently interferes with milk letdown and the energy mobilization process (Tallo-Parra et al. 2018). Otten et al. (2023) found a positive correlation between HCC and somatic cell count in milk, which is similar to the study of Tallo-Parra et al. (2018), indicating that cows with high milk yield are prone to disease conditions due to elevated cortisol levels (Burnett et al. 2015). Season and climatic region had a great influence on hair cortisol concentration in lactating cows (Uetake et al. 2018). So, hair cortisol levels can vary during the lactation period due to the presence of stressors, adverse environmental conditions, seasonal influence, or metabolic demand of the cows.

Body Condition Score (BCS)

The relationship between hair cortisol concentration and body condition score (BCS) is highly dynamic, depending

on both the cow's parity and whether it is evaluated during the prepartum or postpartum period. According to Burnett et al. (2015), thin multiparous cows had lower HCC than average and moderate cows at the prepartum period, while BCS did not affect HCC of primiparous cows. This can be due to higher metabolic and physiological burden that is carried out by the multiparous cows with average and moderate body condition scores. But Endo et al. (2019) found a negative correlation between BCS and HCC, as cows with low BCS have higher HCC than cows with high BCS, which has similarity with the study of Vesel et al. (2020). Cows with low BCS go through prolonged undernourishment or sub-clinical complexes, which can be a cause of increased hair cortisol concentration. Ultimately, these conflicting patterns show that BCS is not a static indicator of stress. Rather, BCS can be a non-invasive factor to assess how different nutritional or metabolic status affects the cortisol concentration under varying environmental conditions, sampling time or method, BCS scoring system or genetic or physiological differences.

Breed

Hair cortisol concentration may vary among different dairy breeds depending on their production stage, whether they are in lactation or in the dry period. Braun et al. (2022) found significantly higher HCC in Holstein Friesian (1.75pg/mg) than in other dairy breeds, Brown Swiss, Swiss Fleckvieh, and water buffalo cows. Similarly, Endo et al. (2017) also found higher HCC in Holstein cows than in Brown Swiss crossbreds at the early lactation period (60–90 days postpartum). Nedić et al. (2017) reported similar trends, noting higher HCC in Holstein cows than in Busha cows. Because Holstein cows are genetically selected for their higher milk yield, metabolic stress of peak lactation likely causes prolonged activation of the HPA axis, therefore elevating HCC. In contrast, Endo et al. (2017) found no significant variation in HCC between Holstein and Brown Swiss during the dry period, indicating that without higher demand for milk production, different dairy breeds maintain uniform cortisol levels. So, HCC variation among breeds can be due to their milk yield and the metabolic stress associated with it rather than inherent genetic differences.

Correlation of biochemical parameters with hair cortisol concentration

Several biochemical parameters, such as glucose, β -hydroxybutyrate, and non-esterified fatty acids, affect the hair cortisol concentration as shown in Table 3, either by influencing the hypothalamic-pituitary-adrenal axis or by

Table 3 Biochemical parameters related to hair cortisol concentration

Parameter	Correlation with HCC	Reference
Glucose	Positive ($r=0.26$) in multiparous cows	(Burnett et al. 2015)
Fructosamine	Positive ($p=0.035$)	(Grelet et al. 2022)
β -hydroxybutyrate	Negative ($r = -0.10$) in multiparous cows.	(Burnett et al. 2015)
Non-esterified fatty acids	Positive ($p<0.05$) in sub-fertile cows	(Endo et al. 2019)

reflecting the body's metabolic and physiological response to stress.

According to Burnett et al. (2015), during the transition period, glucose had a positive correlation with hair cortisol concentration in both primiparous and multiparous cows. Ghanem et al. (2018) also found higher glucose level at pre-partum period. But according to Grelet et al. (2022), chronic stress does not affect glucose levels, as they rapidly fluctuate over time. Jensen et al. (1993) indicate it to be a more suitable biomarker for acute stress in dairy cows. Grelet et al. (2022) also found higher levels of fructosamine, which is formed by the reaction of glucose and protein, and it has a relatively long half-life (approximately 16 days) in cattle. It indicates the plasma glucose level over 1–3 weeks without rapid fluctuation. So, a higher glucose level in relation to higher cortisol concentration indicates the effect of chronic stress on metabolism. Mechanistically, elevated cortisol levels during the transition period are thought to stimulate hepatic gluconeogenesis by cAMP-dependent protein kinase, thereby raising glucose levels in circulation. Beta-hydroxybutyrate (BHBA) had a negative correlation with hair cortisol concentration in multiparous cows, whereas no effect of BHBA on hair cortisol concentration has been observed in primiparous cows (Burnett et al. 2015). This decline in BHBA during chronic stress may occur as elevated cortisol levels direct the metabolic pathway toward ketogenesis, thus potentially shifting flux away from ketogenesis. Moreover, chronic stress can suppress hepatic mitochondrial activity, where ketogenesis occurs, and decrease the production of BHBA. Endo et al. (2019) found elevated non-esterified fatty acids (NEFA) around parturition in sub-fertile cows, which decrease after parturition as the cows may mobilize the fats to meet higher metabolic demand during this period. Alkaline phosphatase (ALP) has been found to be decreased in heat stress in primiparous cows by Abeni et al. (2007). It also decreases significantly with stressors like high stocking density or changing groups in dairy cows (Calamari et al. 2003). But no further study has been found on its correlation with hair cortisol concentration, though it shows promise as a potential significant biomarker for estimating chronic stress.

Critical evaluation of contradictory findings

Despite the growing use of HCC as a retrospective biomarker of chronic stress during the periparturient period, significant inconsistencies and conflicting findings persist across published studies. For instance, Burnett et al. (2015) and Comin et al. (2013) found higher HCC in cows suffering from peripartum health disorders, whereas others observe no significant variation in cortisol accumulation during similar clinical conditions (Tekin et al. 2023). These conflicting outcomes mainly stem from methodological and biological variations. Methodologically, mincing hair, either with scissors or with a ball mill, makes a difference in extraction efficiency. Furthermore, the choice of extraction solvent (ethanol or methanol) and analytical method (ELISA versus LC-MS/MS) impact the cross-reactivity and quantitative accuracy (Slominski et al. 2015). Biologically, hair coat color, hair segment length, and anatomical site of hair collection introduce variation in cortisol levels; for example, tail hair exhibits consistently higher HCC than shoulder and hip (Burnett et al. 2014). Additionally, season exerts a great effect on cortisol levels, with higher concentrations typically observed in winter than in summer (Heimbürge et al. 2020b). This can be due to sunlight or UV radiation degrading the cortisol in hair. Therefore, standardization of collection site, maintaining uniform analytical methodologies, and accounting for season, hair segment, and coat color are crucial prerequisites for establishing hair cortisol as a clinical diagnostic tool in dairy production.

Conclusion and future direction

Hair cortisol is a highly promising, non-invasive biomarker for assessing chronic stress during the crucial periparturient period, as it effectively reflects prolonged activation of the hypothalamic-pituitary-adrenal axis associated with factors such as parity, lactation, calving, and health status. However, methodological variations hinder its routine application in monitoring herd health. To make it a practical diagnostic tool for monitoring herd welfare, further research should prioritize standardized sampling, validation of analytical procedures, and the establishment of reference ranges across distinct physiological stages to reduce data variability. Moreover, researchers should place more emphasis on

significant factors related to the transition period to make welfare assessment much easier during the pre and postpartum period of dairy cows.

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