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Original Research Article

Postpartum hair cortisol, dehydroepiandrosterone sulfate and their ratio in beef cows: Exploring association with parity and conception outcome

T. Peric [a], M.C. Veronesi [b], A. Prandi [a], J. Fusi [b,*], M. Faustini [b], M. Probo [b]

[a] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via Sondrio, 2/a, 33100, Udine, Italy
[b] Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Via dell'Università 6, 26900, Lodi, Italy

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ABSTRACT

Hair steroid measurement has received increasing attention for monitoring hypothalamic-pituitary-adrenal axis function, as it offers the advantages of being noninvasive, fast, and able to indicate steroid concentrations over long periods. The objects of the study were to evaluate cortisol (C) and dehydroepiandrosterone sulfate (DHEA-S) hair concentrations and their ratio (C/DHEA-S) in beef cows from calving to 100 days (d) postpartum (pp) and to assess possible differences related to parity (primiparous vs multiparous) and conception outcome (pregnant vs not pregnant). Hair samples were collected from 6 primiparous and 5 multiparous pregnant beef cows by clipping the coat at calving (T0) and every 20 d for 5 times (T1-T5), collecting only the regrown hair. Starting from the 6th-week pp, cows were submitted to artificial insemination at spontaneous estrus; by 100 d pp, 7 cows were pregnant and 4 were not pregnant. Statistical analysis showed higher hair C concentrations in the 11 cows at calving (T0) compared to all the subsequent samplings except for T1, and higher C concentrations at T1 compared to T3, T4, and T5. These results indicate that hair C concentrations in beef cows are affected by sampling time, with a decrease from calving, as reported in other matrices. When exploring changes within parity groups, no differences were found in the multiparous among sampling times, while hair C concentrations at T0 and T1 tended to be higher than at T2 ($0.01 \leq p < 0.05$) and were higher ($p < 0.01$) than in all the subsequent samplings (T3, T4 and T5) within the primiparous group. Higher hair C concentrations were found at T0 and T1 in the primiparous compared to multiparous ($p < 0.01$), suggesting that primiparous cows undergo a greater stress level before and around parturition compared to multiparous, probably due to the novelty of the calving experience. No differences were detected in C hair concentrations according to conception outcome (pregnant versus not pregnant) in each sampling time. Hair DHEA-S concentrations were neither affected by time nor by parity or conception outcome. Differences in the C/DHEA-S ratio were found at T1, with higher C/DHEA-S in the multiparous compared to primiparous cows ($p < 0.001$), and a tendency for higher ratio in the not pregnant compared to the pregnant ($0.01 \leq p < 0.05$). These results support the choice of hair as a valuable biological matrix when investigating long-time periods such as postpartum in cows and suggest an enhanced immunoprotective effect of DHEA-S in the postpartum of primiparous cows, and in cows that get pregnant within 100 d postpartum.

1. Introduction

Stress has been defined as the result of an external event or condition (stressor) that places a strain on a biological system [1]. Stressors can affect reproductive efficiency through several mechanisms, such as slowing the pulsatile release of LH [2], decreasing follicular estradiol production and the responsiveness of ovarian follicles to LH [3], and blocking the LH surge [4]. In some instances, stress can act directly on the pregnancy itself, for example when heat stress affects the ovarian function, the developmental capacity of the oocyte, or the early pregnancy development, or when postpartum hormonal and metabolic imbalances cause immune dysfunction that leads to uterine disease and infertility [5].

While stress conditions can be quantified and applied equally across animals, the stress response can vary among individuals. Allostatic load and resilience capacity can be evaluated through the measurement of biological markers, such as cortisol (C), dehydroepiandrosterone (DHEA) [6–8], DHEA sulfate (DHEA-S) [9], and their ratios [10,11].

* Corresponding author. Via dell'Università, 6, 26900, Lodi, Italy.
E-mail address: jasmine.fusi@unimi.it (J. Fusi).

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Cortisol and DHEA are the main hormones secreted by the adrenal cortex during multiple conditions and after stress stimulation. As the primary endpoint of the hypothalamic-pituitary-adrenal (HPA) axis, C is an appropriate hormone for the investigation of HPA axis function, being used to monitor stress and welfare conditions in farm animals [12]. DHEA is the primary precursor of natural estrogens and represents a metabolic intermediate in ovarian follicular steroidogenesis [13]. Moreover, this steroid hormone acts at multiple levels, playing a role in immune system activation, with anti-glucocorticoid qualities presumably as competitive inhibitors of C [14] and exhibiting anti-inflammatory effects and antioxidant properties [8]. During pregnancy, C concentrations result from both fetal and maternal HPA axis activity [15], while DHEA and its sulfate are produced mainly by the fetal/placental compartment [16]; cow placental 3β-hydroxysteroid dehydrogenase converts DHEA to androstenedione [17], which is used as a precursor for estrogen by trophoblastic cells [18–20]. Postnatally, DHEA and DHEA-S are primarily secreted by the zona reticularis of the adrenal glands and act as prohormones for sexual steroids in both males and females [21]. As C and DHEA mediate largely opposing biological, neurologic, and immunologic functions, measuring their concentrations simultaneously may be an important indicator of net glucocorticoid activity [22], and the glucocorticoid:DHEA-S ratios may be helpful in identifying HPA axis dysfunctions.

Literature on steroids measurement in bovine species is dotted with investigations on blood, saliva, feces, and milk [12,23,24], but none of these matrices can display long-term retrospective steroid accumulation [25,26]. Research about the analysis of hair steroids began about two decades ago in humans [27–29] and expanded two years later to animal species [30]. More recently, hair steroid measurement has received increasing attention for assessing chronic stress in cattle, as hair seems to embed circulating steroids throughout all growth periods, providing an integrative value [26]. The advantages of this matrix are also the ease of collection and storage, the absence of a circadian rhythm influence, and the minimum restraint requested for collection, having no impact on the well-being of animals. Cortisol measurement in hair samples has been already reported as a validated method in cows, through investigations on its association with health status [31,32], reproduction [31–33], breed [34], environmental conditions [8,35], stocking density [36], and synchronization protocols [37]. Regarding hair DHEA, its assay has been validated in guinea pigs [38], pigs [39], cows [8,40,41], and recently also in calves [42], while hair DHEA-S has been investigated in pigs [43], dogs [44], calves [21] and mares and foals [45]. Recently, the cortisol:DHEA ratio has been proposed as a resilience factor that may prevent the potential negative effects of stress [46]. In humans, higher DHEA or a lower cortisol/DHEA ratio mitigate possible deleterious effects of high cortisol concentrations, while in cattle it has been reported an increased cortisol/DHEA ratio in lame dairy cows [40] and following transportation of young bulls [47].

To the authors' knowledge, no reports about the assessment of both C and DHEA-S and their ratio in the postpartum period with the use of a noninvasive matrix like hair was reported. However, assessing C and DHEA-S with this method could provide information about the activity of the HPA axis during the delicate postpartum period which can be useful from a scientific and economic point of view, also because hair allows investigation of hormonal variations in a non-invasive way and with a lower number of samplings compared to other matrices. In particular, different HPA axis activity may be detected in cows according to parity [48], and this might play a role in the establishment of the pregnancy in the postpartum period. Investigations on beef cows are not available in literature and would allow to avoid the influence of the milking system, which is instead known to deeply interfere with postpartum adaptation and health in dairy cows. Therefore, the objectives of this study were to assess hair C and DHEA-S concentrations and their ratio in beef cows from calving to 100 d postpartum (pp) and to verify differences according to parity and conception outcome.

2. Materials and methods

Although hair sampling is a non-invasive procedure, the trial was carried out in accordance with EU Directive 2010/63/EU, and it was approved by the Ethical Committee of the University of Milan (OPBA_146_2019).

2.1. Animals

A sample size test was performed before the beginning of the study, and the G*Power test (ver. 3.1.9.6, Kiel University, Germany – type of power analysis "Compute required sample size – given alpha, power, and effect size) set the minimum sample size to 10 subjects (assuring a test power >80 %) to obtain valid and sound results. Therefore, a total of 12 late pregnant crossbreed beef cows, 6 primiparous and 6 multiparous, were enrolled in the study and surveilled starting from 2 weeks before the estimated calving date. All animals belonged to a single beef herd in northern Italy. The animals were loose-housed with straw bedding (3.5 m^2/head) and were offered a hay-based diet containing 6.15 MJ of NE_L/kg of dry matter (DM) during the prepartum period, and 7.28 MJ of NE_L/kg of DM during the postpartum period. Fresh water was available ad libitum; general health conditions and the body condition score (BCS, according to Edmonson et al. [49]) were monitored during the study period.

Calvings occurred spontaneously at term and were all singletons, except for one multiparous cow that delivered twins and was thus excluded from the study. The clinical monitoring of cows did not record diseases or abnormalities during the whole period of observation; the calves were allowed to suckle and stay with their mother throughout the period.

Simultaneously to the second hair sampling collection (T2, see 2.2 section), a rectal palpation and ultrasound examination (real-time B-mode linear array scanner with a 7.5 MHz transducer; Sigma I-AC, Kontron Instruments, Milan, Italy) of the genital tract was performed to ensure the normal postpartum course and to rule out the presence of ovarian and/or uterine abnormalities. Starting from the 6th-week pp, the cows showing estrus, with a pre-ovulatory follicle and normal conditions of the genital tract (uterine tone, cervical mucus) were submitted to artificial insemination (AI), which was performed 12 h after the beginning of estrus with semen of proven fertility, as scheduled by the herd management system. Pregnancy was diagnosed by rectal palpation and ultrasound examination around 25–30 d after AI and confirmed by rectal palpation and ultrasound examination at about 40 d of pregnancy. Cows found to be not pregnant were submitted again to AI following the same procedures above described; the last AI attempts were performed around 75 d pp, in order to allow a pregnancy diagnosis before the 100-d pp. Those cows found empty at the last diagnosis at around 100 d pp were included in the not pregnant group.

2.2. Hair samples collection

At calving, immediately after the calf was born, a first hair sample was taken with clippers from an area of about 10 cm^2 on the cows' forehead (T0). At this time, each single collected hair was at a different physiological phase (anagen, catagen, telogen), and because of this, T0 samples reflect the hormone concentrations of at least one month before parturition. Next, samples of newly re-grown hair were taken from the same area every 20 d for a further 5 times. Thus, hair was collected 20 (T1), 40 (T2), 60 (T3), 80 (T4), and 100 (T5) d after calving. Each of these samples reflects the hormone accumulations that occurred in between the two subsequent samplings. At each collection time, individual hair samples were coded and stored in envelopes at room temperature and in the dark until C and DHEA-S analysis.

2.3. Hormone analysis

C and DHEA-S concentrations in hair were assessed using solid-phase microtiter RIA as described by Peric et al. [34] and by Probo et al. [42], respectively. Both hormonal concentrations were expressed as pg/mg of hair.

2.4. Statistical analysis

Firstly, data were checked for normal distribution by the Shapiro-Wilk test and then analyzed by a three-way ANOVA and posthoc Tukey test, with time after calving, parity, and conception outcome considered as fixed factors. Differences in hair C concentrations, hair DHEA-S concentrations, and their ratio (C/DHEA-S, expressed as C/DHEA-S*100) were assessed among sampling times in the whole group (11 cows) and within each group of primiparous, multiparous, pregnant and not pregnant cows; for each sampling time between primiparous and multiparous cows, and between pregnant and not pregnant cows. Due to the small sample size, and considering a 99 % simultaneous CI, significance was set at $p < 0.01$, while $0.01 \leq p < 0.05$ was considered as tendency to significance (JASP, ver. 9 for Windows platform).

3. Results

All cows enrolled in the study were not affected by postpartum disorders or general health problems. Seven out of the 11 cows became pregnant with a calving to conception interval (mean SD) of 70.7 ± 6.3 d and 1.6 ± 0.5 AI attempts/pregnancy, while 4 cows were not pregnant within 100 d pp. The BCS was always within a range of 3.5–4.5.

Mean (±SD) C and DHEA-S hair concentrations and hair C/DHEA-S*100 ratio in the 11 cows, are reported in Fig. 1; mean (±SD) C and DHEA-S hair concentrations and hair C/DHEA-S*100 ratio in the primiparous versus multiparous cows are reported in Fig. 2; mean (±SD) C and DHEA-S hair concentrations and hair C/DHEA-S*100 ratio in the pregnant versus not pregnant cows are reported in Fig. 3.

Statistical analysis showed differences in hair C concentrations in the 11 cows among sampling times. Specifically, higher hair C concentrations were detected in the 11 cows at calving (T0) compared to T2 ($p = 0.039$), T3 ($p = 0.012$), T4 ($p = 0.018$), and T5 ($p = 0.001$), and higher hair C concentrations at T1 compared to T3 ($p = 0.036$), T4 ($p = 0.039$), and T5 ($p = 0.033$). Hair DHEA-S concentrations and the C/DHEA-S*100 ratio were not affected by sampling time.

When exploring changes within parity, no differences were found in the C hair concentrations of the multiparous cows in the different sampling times, while within the primiparous group, hair C concentrations at T0 and T1 tended to be higher than those at T2 ($p = 0.016$ and $p = 0.015$, respectively), and were higher compared to T3 (both with $p = 0.006$), T4 (both with $p = 0.007$) and T5 ($p = 0.005$ and $p = 0.004$, respectively). When compared to multiparous cows, primiparae showed higher hair C concentrations at T0 ($p = 0.006$) and T1 ($p = 0.005$). A higher C/DHEA-S*100 ratio was registered in the multiparous cows compared to primiparous cows at T1 ($p < 0.001$), while DHEA-S did not show changes according to parity.

Hair C concentrations in the pregnant cows tended to be higher at T1 than at T3 ($p = 0.024$), T4 ($p = 0.016$), and T5 ($p = 0.021$), while within the not pregnant group, a tendency for higher C concentrations was detected at T0 compared to T3 ($p = 0.032$) and T5 ($p = 0.031$). When comparing DHEA-S hair concentrations in pregnant and not pregnant cows, no differences were detected between groups in each sampling time. A tendency to difference was found in C/DHEA-S*100 at T1, with a higher ratio in the not pregnant group ($p = 0.025$).

4. Discussion

The present investigation showed an effect of time after calving on C hair concentrations in beef cows. The hair C pattern, showing a peak at parturition (reflected by the high C concentrations at T1) and a subsequent decrease, appears to be typical of healthy postpartum cows [31, 50–53] and it agrees with results obtained using other biological samples such as milk [54,55] and plasma [56]. It must be underlined that the sampling at calving was performed on an unshaved area, while the subsequent samplings were performed only on the regrown hair. It has been reported that sampling performed on regrown hair more accurately reflects the circulating hormones concentration of the past 30 days; the anagen phase, which is predominant in actively growing hair, cam capture more circulating hormones compared to the catagen and telogen phase, which are predominant in hair sampled from previously unshaved area [51]. According to these findings, the hair hormonal concentrations found at calving could therefore be even higher than

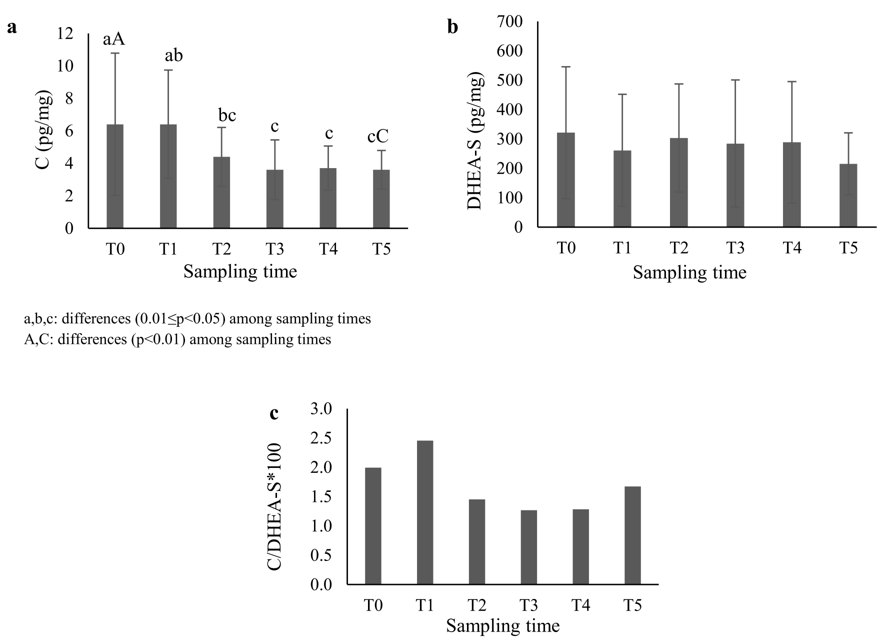

Fig. 1. Mean (±SD) C (**a**) and DHEA-S (**b**) hair concentrations and hair C/DHEA-S*100 (**c**) in the 11 beef cows.

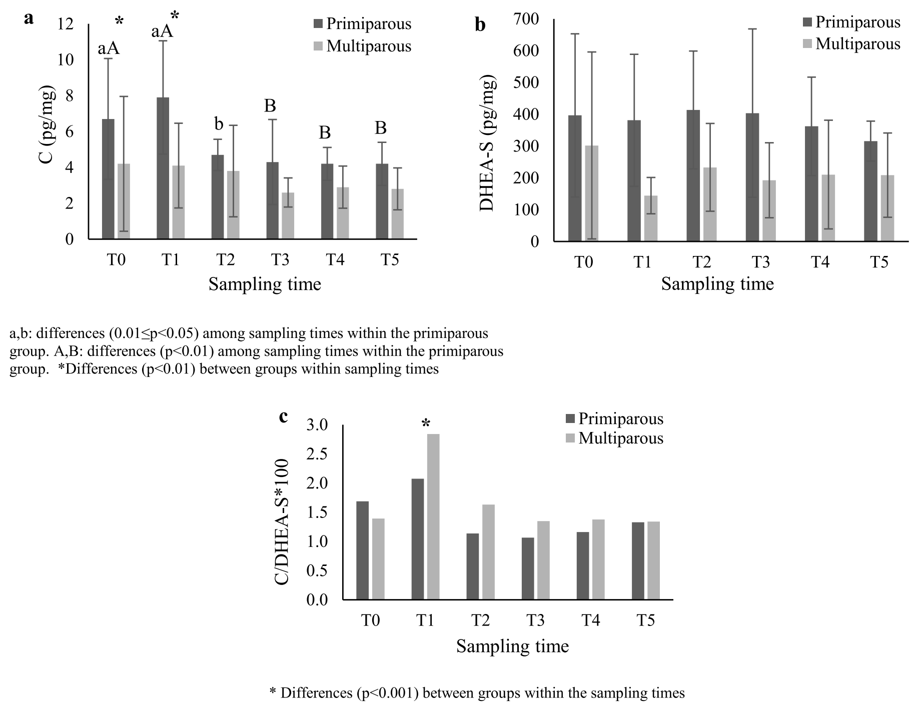

Fig. 2. Mean (±SD) C (**a**) and DHEA-S (**b**) hair concentrations and hair C/DHEA-S*100 (**c**) in primiparous and multiparous cows.

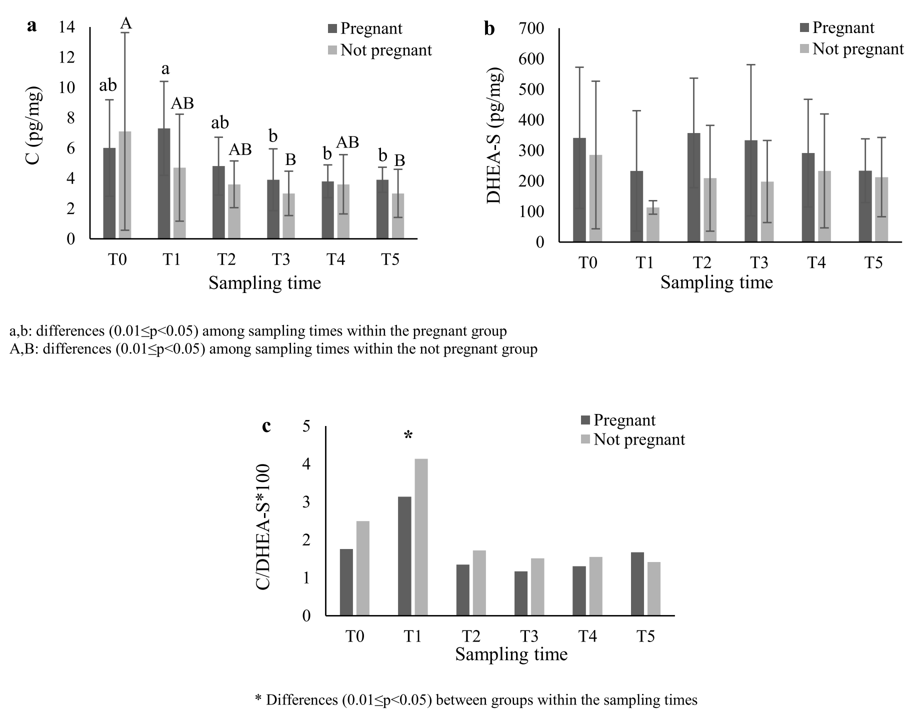

Fig. 3. Mean (±SD) C (**a**) and DHEA-S (**b**) hair concentrations and hair C/DHEA-S*100 (**c**) in pregnant and not pregnant cows.

what registered in the present study.

Increased hair C concentrations during late pregnancy and at the onset of lactation were also reported in monkeys [57] and humans [58]. In addition to the physiological C increase around parturition, other multiple stress factors can increase C secretion during the lactation period in cows, and these might explain the still high C hair concentrations found at T2, although not significantly different from the subsequent; for example, the onset of lactation in dairy cows causes acute metabolic changes and loss of body condition score caused by milk production. Furthermore, clinical and subclinical disorders such as mastitis and metritis/endometritis occur mainly in the lactation period. However, cows enrolled in this study showed no clinical symptoms of periparturient disorders at any sampling point, so the hair C concentrations reflect physiological changes typical of the periparturient period. Moreover, when dealing with beef cows, stress induced by lactation represents a minor issue compared to the high-producing dairy cows. When examining separately primiparous and multiparous cows, the sampling time effect on C hair concentrations remained evident only in the primiparous group; when comparing the two groups, hair C concentrations at birth and 20 d pp in primiparous cows were higher than C concentrations in multiparous cows in all the sampling times, suggesting the need for specific attention in the management of primiparous beef cows at calving. Recent results provided convincing evidence that C release in parturient cows reflects the stress response during calving [59], and it has been demonstrated that primiparous dairy cows behave differently during the transition period, being more likely to experience negative health outcomes compared to multiparous cows [60]. According to the present study, stress levels seem higher in cows giving birth for the first time (primiparous), because of the novelty of the calving event and the changes associated with it, i.e., regrouping, diet changes, and the onset of lactation.

In this regard, conflicting results can be extracted from the literature. Using milk samples, Fukasawa et al. [54] observed no difference in C concentrations between primiparous and multiparous cows, but samples were taken only four times a year (one sample per season), and therefore peripartum period was not specifically investigated. The findings of the present study, instead, do agree with the results reported in plasma by Galvao et al. [61] and in hair by Grelet et al. [62], with higher C concentrations in primiparous cows. These results support the hypothesis that primiparous cows could respond differently at calving, experiencing a combination of stressors associated with novelty, loss of control, or fear [48]. As an alternative, C concentrations may tend to decline with age, as hypothesized by Heimbürge et al. [63]; since differences in hair C between primiparous and multiparous cows disappeared from 40 d after parturition (T2), they most likely reflect a different stress response in the peripartum period according to parity. However, the parity effect may be useful in the potential large-scale use of hair C to monitor stress, expressing results as differences from expected parity values in specific moments. Contrarily, Burnett et al. [30] found higher hair C concentrations in multiparous dairy cows in the postpartum period until 126 d, but not at calving, suggesting an association between parity and lactation rather than between parity and parturition. In a recent study from Endo et al. [64], higher hair C concentrations were found in multiparous dairy cows (parity 5–8) compared to primiparous cows in a prepartum sample performed about 19 ($\pm$ 11) d before calving; afterward, the same authors found no differences in hair C concentrations according to parity in other sampling times performed at 44.8 $\pm$ 11.9, 103.0 $\pm$ 9.9 and 168.0 $\pm$ 9.7 dd pp. The present study did not include a prepartum sample, but consistently with the Endo et al. [64], no differences according to parity were found from the second month after calving (T2) and forward, and this could be suggestive of the resilience in the primiparous cows, that are able to be minimally affected by disturbances and rapidly return to the state pertained before exposure to disturbances.

It should, however, be kept in mind that the different results obtained in studies employing plasma, milk, or hair samples as sources for stress response measurement are a clear example of how different methodologies and experimental designs can play a vital role in the interpretation of results themselves, preventing direct comparisons among results obtained using different specimens. Moreover, when comparing dairy and beef breeds, the different attitudes should be considered a potential bias.

In the present study, reproductive data were like other studies reporting AI conception rates in beef cows [65,66], as the conception rate was 63.6 % with 1.6 AI/pregnancy and 70.7 $\pm$ 6.3 d open in the pregnant cows. According to the present results, there are no differences in the C hair concentrations from pregnant and not pregnant cows in the first 100 d pp. In contrast, Burnett et al. [31] reported that multiparous dairy cows that were not pregnant by 100 d pp had higher hair C concentrations at 42 and 84 d in milk compared to pregnant ones. However, in the latter study, cows that were not pregnant by 100 d pp also showed a higher prevalence of clinical disease, so the higher hair C concentrations may have been indicative of chronic stress induced by diseases. Besides, as before mentioned, inter-individual variability should also be considered.

Regarding DHEA, Marinelli et al. [67] suggested that the fetoplacental unit represents its most important source of production; the placenta mainly uses the Δ5 steroidogenic pathway to produce estrogen [68]. Previous works [67,69] indicate that DHEA placental secretion increases in late pregnancy, probably depending upon the tissue mass [68], and suddenly decreases after parturition [70]. Since in the present study, the first sample collection was performed at calving, thus reflecting events of the last months of pregnancy, differences in DHEA-S concentrations between T0 and subsequent samples could be expected. Conversely, no significant differences were detected in hair DHEA-S concentrations according to sampling times. Differently from the blood samples, employed in the abovementioned studies, the use of a cumulative matrix as hair for DHEA-S measurement avoids the influence of acute events or diurnal fluctuation, thus possibly masking differences due to rapid changes. The fact that DHEA hair concentrations continue to be relevant also in the postpartum period, suggests that the fetoplacental unit is not the only important source of secretion of this steroid in the cow. Adrenal glands and ovaries also secrete DHEA and DHEA-S as sexual steroid precursors, and the lactating mammary gland can affect the circulating concentrations of DHEA by converting it into a metabolite with immunoenhancing activity, the androstene-3β,17β-diol [71,72]. Moreover, quite variable DHEA concentrations between individuals in both female [67] and male [73] animals were found in the bovine species. The present study results are consistent with these previous observations on other species, as the analysis of raw data highlights a great inter-individual variability. In women, DHEA is known to be involved in conception and fertilization, and treatments with DHEA supplementation can increase the probability of conception [74] and reduce miscarriages [75]. No information is available regarding the same mechanisms in other mammals; scientific works exploring DHEA secretion in domestic mammals, in fact, rarely evaluate factors that could affect this phenomenon, such as inflammatory and reproductive status, and the time-interval (chronicity) of exposure to stressors. To our knowledge, this is the first study investigating DHEA-S hair concentrations in beef cows according to parity and pregnancy, and results regarding DHEA-S hair concentrations can hardly be discussed with previous literature.

Last, significances in hair C/DHEA-S were detected only in the first sample after parturition (T1); while T1 C hair concentrations were higher in primiparous than multiparous cows, the C/DHEA-S ratio was the opposite, being lower in the primiparous group. Some authors stated that the glucocorticoid:DHEA(S) ratio may serve as a diagnostic or prognostic tool in terms of physical health [40,76] and also as a marker for resilience and allostatic load [8,39,77]. According to these authors, it can be hypothesized that the lower C/DHEA-S ratio found at T1 in the primiparous cows is due to an enhanced immunoprotective effect of DHEA-S in this group, although concentrations were numerically but not significantly different from the multiparous group, possibly due to the high standard deviations. A similar situation occurred when comparing cows according to pregnancy status, as C hair concentrations were not different between pregnant and not pregnant cows, while C/DHEA-S tended to be lower in the pregnant group at T1. Once more, looking at the T1 DHEA-S concentrations in the pregnant group, numerically but not significantly higher DHEA-S concentrations can be observed, and thus a decrease in the C/DHEA-S ratio consequently takes place. As previously stated by some authors [78,79], the glucocorticoid:DHEA(S) ratio may be helpful in identifying HPA axis dysfunction that cannot be assessed by examining glucocorticoid concentrations alone; from this perspective, the present results suggest that the C/DHEA-S ratio may display a hormonal counter-regulation to maintain a balance between the opposing effects of C and DHEA, and may possibly better define stress response compared to C or DHEA-S measurements alone. Since the T1 sampling time reflects the hormonal accumulation during the first 20 d after calving, it can be hypothesized that a lower C/DHEA-S ratio is favorable for the subsequent establishment of pregnancy.

5. Conclusions

The overall results of the present study further support the choice of hair as a valuable biological matrix when investigating long-time periods such as postpartum in cows. The current investigation showed higher C hair concentrations around calving and in the immediate postpartum compared to the following weeks, as previously reported using other biological matrices. The higher hair C concentrations in primiparous cows compared to multiparous cows suggest that first-calving cows undergo greater stress around parturition, probably due to the novelty of the calving experience, and underline the need for specific attention in the management of the primiparous cows at calving. Understanding differences in stress responses between primiparous and multiparous animals can provide insight into how they cope with these challenges, suggesting management recommendations and future directions for research that may ultimately help to create better environments for the animals. DHEA-S hair concentrations were not affected by sampling time, parity, or conception outcome, but the C/DHEA-S ratio suggests an enhanced immunoprotective effect of DHEA-S in the primiparous group after calving, and the same seems to occur in cows that get pregnant within the first 100 d postpartum. Therefore, special attention should be addressed also to the multiparous cows, whose C/DEHA-S*100 ratio showed a lower resilience capacity when bearing the allostatic load of the postpartum period.

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CRediT authorship contribution statement

T. Peric: Data curation, Methodology, Formal analysis. **M.C. Veronesi:** Conceptualization, Data curation, Methodology. **A. Prandi:** Data curation, Methodology, Formal analysis. **J. Fusi:** Investigation, Data curation, Writing – review & editing. **M. Faustini:** Formal analysis, Methodology. **M. Probo:** Investigation, Writing – original draft, Visualization, Project administration, Supervision, Writing – review & editing, All the authors contributed to the final approval of the version to be submitted.

Declarations of competing interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

[1] Collier RJ, Renquist BJ, Xiao Y. A 100-Year Review: stress physiology including heat stress. J Dairy Sci 2017;100:10367–80. https://doi.org/10.3168/jds.2017-13676.
[2] Karsch FJ, Battaglia DF, Breen KM, De Nathalie H, Thomas G. Mechanisms for ovarian cycle disruption by immune/inflammatory stress. Stress 2002;5(2):101–12. https://doi.org/10.1080/10253890290027868.
[3] Dobson H, Smith RF. What is stress, and how does it affect reproduction? Anim Reprod Sci 2000;60–61:743–52. https://doi.org/10.1016/s0378-4320(00)00080-4.
[4] Breen KM, Karsch FJ. New insights regarding glucocorticoids, stress and gonadotropin suppression. Front Neuroendocrinol 2006;27(2):233–45. https://doi.org/10.1016/j.yfrne.2006.03.335.
[5] Lucy MC. Stress, strain, and pregnancy outcome in postpartum cows. Anim Reprod 2019;16:455–64. https://doi.org/10.21451/1984-3143-AR2019-0063.
[6] McEwen BS. Interacting mediators of allostasis and allostatic load: towards an understanding of resilience in aging. Metabolism 2003;52:10–6. https://doi.org/10.1016/S0026-0495(03)00295-6.
[7] Charney DS. Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptations to extreme stress. Am J Psychiatr 2004;161:195–216. https://doi.org/10.1176/appi.ajp.161.2.195.
[8] Peric T, Corazzin M, Romanzin A, Bovolenta S, Prandi A, Montillo M, Comin A. Cortisol and DHEA concentrations in the hair of dairy cows managed indoor or on pasture. Livest Sci 2017;202:39–43. https://doi.org/10.1016/j.livsci.2017.05.020.
[9] Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Front Neuroendocrinol 2009;30:65–91. https://doi.org/10.1016/j.yfrne.2008.11.002.
[10] Logan JG, Barksdale DJ. Allostasis and allostatic load: expanding the discourse on stress and cardiovascular disease. J Clin Nurs 2008;17:201–8. https://doi.org/10.1111/j.1365-2702.2008.02347.x.
[11] Saczawa ME, Graber JA, Brooks-Gunn J, Warren MP. Methodological considerations in use of the cortisol/DHEA(S) ratio in adolescent populations. Psychoneuroendocrinology 2013;38:2815–9. https://doi.org/10.1016/j.psyneuen.2013.06.024.
[12] Mormède P, Andanson SS, Aupérin B, Beerda B, Guémené D, Malmkvist J, et al. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiol Behav 2007;92:317–39. https://doi.org/10.1016/j.physbeh.2006.12.003.
[13] Mo Q, Lu S, Simon NG. Dehydroepiandrosterone and its metabolites: differential effects on androgen receptor trafficking and transcriptional activity. J Steroid Biochem Mol Biol 2006;99:50–8. https://doi.org/10.1016/j.jsbmb.2005.11.011.
[14] Hazeldine J, Wiebke A, Lord JA. Dehydroepiandrosterone as a regulator of immune cell function. J Steroid Biochem Mol Biol 2010;120:127–36. https://doi.org/10.1016/j.jsbmb.2009.12.016.
[15] Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. Endocr Rev 1997;18(3):378–403. https://doi.org/10.1210/edrv.18.3.0304.
[16] Geisert RD, Conley AJ. Secretion and metabolism of steroids in subprimate mammals during pregnancy. In: Bazer FW, editor. Endocrinology of pregnancy. Contemporary endocrinology, vol. 9. Totowa (NJ): Humana Press; 1998. p. 291–318. https://doi.org/10.1007/978-1-4612-1804-3_10.
[17] Sousa LMMC, Campos DB, Jjr Buratini, Binelli M, Papa PC. Growth factors and steroidogenesis in the bovine placenta. Anim Reprod 2008;5:3–15.
[18] Albrecht ED, Pepe GJ. Placental steroid hormone biosynthesis in primate pregnancy. Endocr Rev 1990;11:124–50. https://doi.org/10.1210/edrv-11-1-124.
[19] Schuler G, Hartung F, Hoffmann B. Investigations on the use of C-21-steroids as precursors for placental oestrogen synthesis in the cow. Exp Clin Endocrinol 1994;102:169–74. https://doi.org/10.1055/s-0029-1211278.
[20] Albrecht ED, Pepe GJ. Secretion and metabolism of steroids in primate mammals during pregnancy. In: Bazer FW, editor. The endocrinology of pregnancy. Contemporary endocrinology, vol. 9. Totowa (NJ): Humana Press; 1998. p. 319–51. https://doi.org/10.1007/978-1-4612-1804-3_10.
[21] Peric T, Comin A, Montillo M, Spigarelli C, Corazzin M, Cotticelli A, Prandi A. Postnatal and postweaning endocrine setting in dairy calves through hair cortisol, dehydroepiandrosterone and dehydroepiandrosterone sulphate. Agr Nat Resour 2022;56:867–76.
[22] Kamin HS, Kertes DA. Cortisol and DHEA in development and psychopathology. Horm Behav 2017;89:69–85. https://doi.org/10.1016/j.yhbeh.2016.11.018.
[23] Duncan IJH. Science-based assessment of animal welfare: farm animals. Rev Sci Tech 2005;24:483–92. PMID:16358502.
[24] Palme R. Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. Anim Welf 2012;21:331–7.
[25] Russell E, Koren G, Rieder M, Van Uum S. Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. Psychoneuroendocrinology 2012;37:589–601. https://doi.org/10.1016/j.psyneuen.2011.09.009.
[26] Stalder T, Kirschbaum C. Analysis of cortisol in hair – state of the art and future directions. Brain Behav Immun 2012;26:1019–29. https://doi.org/10.1016/j.bbi.2012.02.002.
[27] Bévalot F, Gaillard Y, Lhermitte MA, Pépin G. Analysis of corticosteroids in hair by liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr B Biomed Sci Appl 2000;740(2):227–36. https://doi.org/10.1016/s0378-4347(00)00085-2.
[28] Cirimele V, Kintz P, Dumestre V, Goullé JP, Ludes B. Identification of ten corticosteroids in human hair by liquid chromatography-ionspray mass spectrometry. Forensic Sci Int 2000;107(1–3):381–8. https://doi.org/10.1016/s0379-0738(99)00180-2.
[29] Gaillard Y, Vayssette F, Pépin G. Compared interest between hair analysis and urinalysis in doping controls: results for amphetamines, corticosteroids and anabolic steroids in racing cyclists. Sci Int 2000;107:361–79. https://doi.org/10.1016/S0379-0738(99)00179-6.
[30] Koren L, Mokady O, Karaskov T, Klein J, Koren G, Geffen E. A novel method using hair for determining hormonal levels in wildlife. Anim Behav 2002;63:403–6. https://doi.org/10.1006/anbe.2001.1907.
[31] Burnett TA, Madureira AML, Silper BF, Tahmasbi A, Nadalin A, Veira DM, Cerri RLA. Relationship of concentrations of cortisol in hair with health, biomarkers in blood, and reproductive status in dairy cows. J Dairy Sci 2015;98:1–13. https://doi.org/10.3168/jds.2014-8871.
[32] Comin A, Peric T, Corazzin M, Veronesi MCC, Meloni T, Zufferli V, Cornacchia G, Prandi A. Hair cortisol as a marker of hypothalamic-pituitary-adrenal axis

activation in Friesian dairy cows clinically or physiologically compromised. Livest Sci 2013;152:36–41. https://doi.org/10.1016/j.livsci.2012.11.021.

[33] Comin A, Tidu L, Cornacchia G, Cappa A, Renaville B, Prandi A. Neonatal period and hair cortisol in cattle as a marker of stress. XVI FeMeSPRum-Mediterranean Federation Congress; 2008. p. 221–5.

[34] Peric T, Comin A, Corazzin M, Montillo M, Cappa A, Campanile G, Prandi A. Short communication: hair cortisol concentrations in Holstein-Friesian and crossbreed F1 heifers. J Dairy Sci 2013;96:3023–7. https://doi.org/10.3168/jds.2012-6151.

[35] Comin A, Prandi A, Peric T, Corazzin M, Dovier S, Bovolenta S. Hair cortisol levels in dairy cows from winter housing to summer highland grazing. Livest Sci 2011; 138:69–73. https://doi.org/10.1016/j.livsci.2010.12.009.

[36] Silva PRB, Lobeck-Luchterhand KM, Cerri RLA, Haines DM, Ballou MA, Endres MI, Chebel RC. Effects of prepartum stocking density on innate and adaptive leukocyte responses and serum and hair cortisol concentrations. Vet Immunol Immunopathol 2016;169:39–46. https://doi.org/10.1016/j.vetimm.2015.11.007.

[37] Biancucci A, Sbaragli T, Comin A, Sylla L, Monaci M, Peric T, Stradaioli G. Reducing treatments in cattle superovulation protocols by combining a pituitary extract with a 5% hyaluronan solution: is it able to diminish activation of the hypothalamic pituitary adrenal axis compared to the traditional protocol? Theriogenology 2016;85:914–21. https://doi.org/10.1016/j.theriogenology.2015.10.041.

[38] Shen M, Xiang P, Yan H, Shen B, Wang M. Analysis of anabolic steroids in hair: time courses in Guinea pigs. Steroids 2009;74:773–8. https://doi.org/10.1016/j.steroids.2009.04.008.

[39] Bergamin C, Comin A, Corazzin M, Faustini M, Peric T, Scollo A, Gottardo F, Montillo M, Prandi A. Cortisol, DHEA, and sexual steroid concentrations in fattening pigs' hair. Animals 2019;9:345. https://doi.org/10.3390/ani9060345.

[40] Almeida PE, Weber PSD, Burton JL, Zanella AJ. Depressed DHEA and increased sickness response behaviors in lame dairy cows with inflammatory foot lesions. Domest Anim Endocrinol 2008;34:89–99. https://doi.org/10.1016/j.domaniend.2006.11.006.

[41] Ghassemi Nejad J, Lee BH, Kim JY, Chemere B, Sung KI, Lee HG. Effect of alpine grazing on plasma and hair cortisol, serotonin, and DHEA in dairy cows and its welfare impact. Domest Anim Endocrinol 2021;75:106581. https://doi.org/10.1016/j.domaniend.2020.106581.

[42] Probo M, Peric T, Fusi J, Prandi A, Faustini M, Veronesi MC. Hair cortisol and dehydroepiandrosterone sulfate concentrations in healthy beef calves from birth to 6 months of age. Theriogenology 2021;175:89–94. https://doi.org/10.1016/j.theriogenology.2021.08.037.

[43] Montillo M, Rota Nodari S, Peric T, Polloni A, Corazzin M, Bergamin C, Balestrieri A, Prandi A, Comin A. Steroids in pig hair and welfare evaluation systems: combined approaches to improve management in pig breeding? Vet Ital 2020;56(3):177–84. https://doi.org/10.12834/VetIt.1974.11885.1.

[44] Fusi J, Peric T, Probo M, Cotticelli A, Faustini M, Veronesi MC. How stressful is maternity? Study about cortisol and dehydroepiandrosterone-sulfate coat and claws concentrations in female dogs from mating to 60 days post-partum. Animals 2021;11(6):1632. https://doi.org/10.3390/ani11061632.

[45] Lanci A, Mariella J, Ellero N, Faoro A, Peric T, Prandi A, Freccero F, Castagnetti C. Hair Cortisol and DHEA-S in foals and mares as a retrospective picture of feto-maternal relationship under physiological and pathological conditions. Animals 2022;12:1266. https://doi.org/10.3390/ani12101266.

[46] Russo SJ, Murrough JW, Han MH, Charney DS, Nestler EJ. Neurobiology of resilience. Nat Neurosci 2012;15:1475–84. https://doi.org/10.1038/nn.3234.

[47] Buckham Sporer KR, Weber PSD, Burton JL, Earley B, Crowe MA. Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress. J Anim Sci 2008;86:1325–34. https://doi.org/10.2527/jas.2007-0762.

[48] Proudfoot KL, Huzzey JM. A first time for everything: the influence of parity on the behavior of transition dairy cows. JDS Commun 2022;3(6):467–71. https://doi.org/10.3168/jdsc.2022-0290.

[49] Edmonson AJ, Lean IN, Weaver LD, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. J Dairy Sci 1989;72:68. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

[50] Burnett TA, Madureira AML, Silper BF, Nadalin A, Tahmasbi A, Veira DM, Cerri RLA. Factors affecting hair cortisol concentrations in lactating dairy cows. J Dairy Sci 2014;97:7685–90. https://doi.org/10.3168/jds.2014-8444.

[51] Braun U, Michel N, Baumgartner MR, Hassig M, Binz TM. Cortisol concentration of regrown hair and hair from a previously unshorn area in dairy cows. Res Vet Sci 2017;114:412–5. https://doi.org/10.1016/j.rvsc.2017.07.005.

[52] Endo N, Kuroki R, Tanaka T. Comparison of productive and reproductive performance and hair cortisol levels between Brown Swiss cross-bred and Holstein cows housed in the same barn. Anim Sci J 2017;88(10):1506–12. https://doi.org/10.1111/asj.12828.

[53] Hayashi H, Arai C, Ikeuchi Y, Yamanaka M, Hirayama T. Effect of growth and parturition on hair cortisol in Holstein cattle. Anim Sci J 2021;92(1):e13518. https://doi.org/10.1111/asj.13518.

[54] Fukasaw M, Tsukada H, Kosako T, Yamada A. Effect of lactation stage, season and parity on milk cortisol concentration in Holstein cows. Livest Sci 2008;113(2–3): 280–4. https://doi.org/10.1016/j.livsci.2007.05.020.

[55] Gellrich K, Sigl T, Meyer HHD, Wiedemann S. Cortisol levels in skimmed milk during the first 22 weeks of lactation and response to short-term metabolic stress and lameness in dairy cows. J Anim Sci Biotechnol 2015;6:31. https://doi.org/10.1186/s40104-015-0035-y.

[56] Hudson S, Mullford M, Whittlestone WG, Payne E. Bovine plasma corticoids during parturition. J Dairy Sci 1976;59(4):744–6. https://doi.org/10.3168/jds.S0022-0302(76)84267-1.

[57] Dettmer AM, Rosenberg KL, Suomi SJ, Meyer JS, Novak MA. Associations between Parity, hair hormone profiles during pregnancy and lactation, and infant development in Rhesus Monkeys (Macaca mulatta). PLoS One 2015;10(7): e0131692. https://doi.org/10.1371/journal.pone.0131692.

[58] D'Anna-Hernandez KL, Ross RG, Natvig CL, Laudenslager ML. Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. Physiol Behav 2011;104:348–53. https://doi.org/10.1016/j.physbeh.2011.02.041.

[59] Nagel C, Trenk L, Aurich C, Ille N, Pichler M, Drillich M, Pohl W, Aurich J. Sympathoadrenal balance and physiological stress response in cattle at spontaneous and PGF2α-induced calving. Theriogenology 2016;85:979–85. https://doi.org/10.1016/j.theriogenology.2015.11.009.

[60] Neave HW, Lomb J, von Keyserlingk MAG, Behnam-Shabahang A, Weary DM. Parity differences in the behavior of transition dairy cows. J Dairy Sci 2017;100: 548–61. https://doi.org/10.3168/jds.2016-10987.

[61] Galvão KN, Flaminio MJ, Brittin SB, Sper R, Fraga M, Caixeta L, Ricci A, Guard CL, Butler WR, Gilbert RO. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. J Dairy Sci 2010; 93:2926–37. https://doi.org/10.3168/jds.2009-2551.

[62] Grelet C, Vanden Dries V, Leblois J, Wavreille J, Mirabito L, Soyeurt H, Franceschini S, Gengler N, Brostaux Y, Consortium HappyMoo, Dehareng F. Identification of chronic stress biomarkers in dairy cows. Animal 2022;16:100502. https://doi.org/10.1016/j.animal.2022.100502.

[63] Heimbürge S, Kanitz E, Otten W. The use of hair cortisol for the assessment of stress in animals. Gen Comp Endocrinol 2019;270:10–7. https://doi.org/10.1016/j.ygcen.2018.09.016.

[64] Endo N, Kitamura T, Okubo M, Tanaka T. Hair cortisol concentration in pre- and postpartum dairy cows, and its association with body condition, hock health, and reproductive status. Anim Sci J 2019;90(8):924–31. https://doi.org/10.1111/asj.13247.

[65] McFadden AM, Heuer C, Jackson R, West DM, Parkinson TJ. Reproductive performance of beef cow herds in New Zealand. N Z Vet J 2005;53(1):39–44. https://doi.org/10.1080/00480169.2005.36467.

[66] Crites BR, Vishwanath R, Arnett AM, Bridges PJ, Burris WR, McLeod KR, Anderson LH. Conception risk of beef cattle after fixed-time artificial insemination using either SexedUltra™ 4M sex-sorted semen or conventional semen. Theriogenology 2018;118:126–9. https://doi.org/10.1016/j.theriogenology.2018.05.003.

[67] Marinelli L, Trevisi E, Da Dalt L, Merlo M, Bertoni G, Gabai G. Dehydroepiandrosterone secretion in dairy cattle is episodic and unaffected by ACTH stimulation. J Endocrinol 2007;194:627–35. https://doi.org/10.1677/JOE-07-0226.

[68] Geisert RD, Conley AJ. Secretion and metabolism of steroids in subprimate mammals during pregnancy. In: Bazer FW, editor. The endocrinology of pregnancy. Totowa, NJ: Humana Press Inc.; 1998. p. 291–318.

[69] Gabai G, Marinelli L, Simontacchi C, Bono G. The increase of plasma C195steroids in subcutaneous abdominal and jugular veins of dairy cattle during pregnancy is unrelated to estrogenic activity. Steroids 2004;69:121–7. https://doi.org/10.1016/j.steroids.2003.12.001.

[70] Fustini M, Galeati G, Gabai G, Mammi LE, Bucci D, Baratta M, Accorsi PA, Formigoni A. Overstocking dairy cows during the dry period affects dehydroepiandrosterone and cortisol secretion. J Dairy Sci 2017;100(1):620–8. https://doi.org/10.3168/jds.2016-11293.

[71] Belvedere P, Gabai G, Dalla Valle L, Accorsi PA, Trivoletti M, Colombo L, Bono G. Occurrence of steroidogenic enzymes in the bovine mammary gland at different functional stages. J Steroid Biochem Mol Biol 1996;59:339–47. https://doi.org/10.1016/s0960-0760(96)00131-8.

[72] Loria RM, Padgett DA, Nuynh PN. Regulation of the immune response by dehydroepiandrosterone and its metabolites. J Endocrinol 1996;150:S209–20. PMID: 8943803.

[73] Simontacchi C, Perez de Altamirano T, Marinelli L, Angeletti R, Gabai G. Plasma steroid variations in bull calves repeatedly treated with testosterone, nortestosterone and oestradiol administered alone or in combination. Vet Res Commun 2004;28:467–77. https://doi.org/10.1023/b:verc.0000040244.27933.f1.

[74] Fusi FM, Ferrario M, Bosisio C, Arnoldi M, Zanga L. DHEA supplementation postively affects spontaneous pregnancies in women with diminished ovarian funtion. Gynecol Endocrinol 2013;29:940–3. https://doi.org/10.3109/09513590.2013.819087.

[75] Gleicher N, Ryan E, Weghofer A, Blanco-Mejia S, Barad DH. Miscarriage rates after dehydroepiandrosterone (DHEA) supplementation in women with diminished ovarian reserve: a case control study. Reprod Biol Endocrinol 2009;7:108. https://doi.org/10.1186/1477-7827-7-108.

[76] Gundlach NH, Schmicke M, Ludes-Wehrmeister E, Ulrich SA, Araujo MG, Siebert U. New approach to stress research in phocids—potential of dehydroepiandrosterone and cortisol/dehydroepiandrosterone ratio as markers for stress in harbor seals (Phoca vitulina) and gray seals (Halichoerus grypus). J Zoo Wildl Med 2018;49: 556–63. https://doi.org/10.1638/2017-0191.1.

[77] Trevisan C, Montillo M, Prandi A, Mkupasi EM, Ngowi HA, Johansen MV. Hair cortisol and dehydroepiandrosterone concentrations in naturally Taenia solium infected pigs in Tanzania. Gen Comp Endocrinol 2017;246:23–8. https://doi.org/10.1016/j.ygcen.2017.03.007.

[78] Guilliams TG, Edwards L. Chronic stress and the HPA axis: clinical assessment and therapeutic considerations. Standard 2010;9:1–12.

[79] Wang LJ, Huang YS, Hsiao CC, Chiang YL, Wu CC, Shang ZY, Chen CK. Salivary dehydroepiandrosterone, but not cortisol, is associated with attention deficit

hyperactivity disorder. World J Biol Psychiatr 2011;12:99–109. https://doi.org/10.3109/15622975.2010.512090.