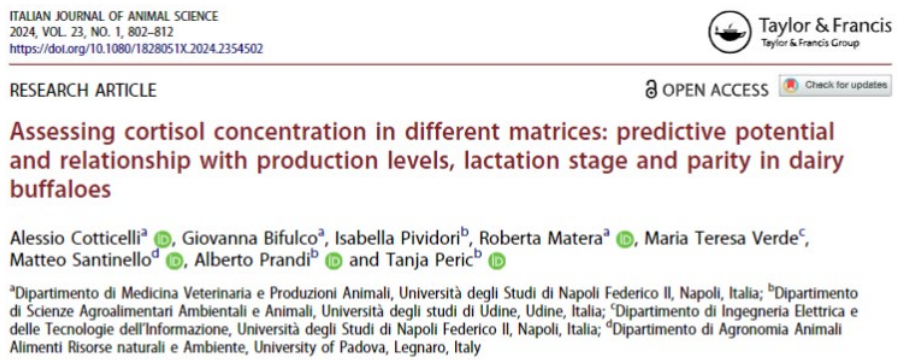
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RESEARCH ARTICLE

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Assessing cortisol concentration in different matrices: predictive potential and relationship with production levels, lactation stage and parity in dairy buffaloes

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Cortisol concentrations in different matrices: predictive potential and relationship with productive level, lactation stage and parity in dairy buffaloes

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Introduction

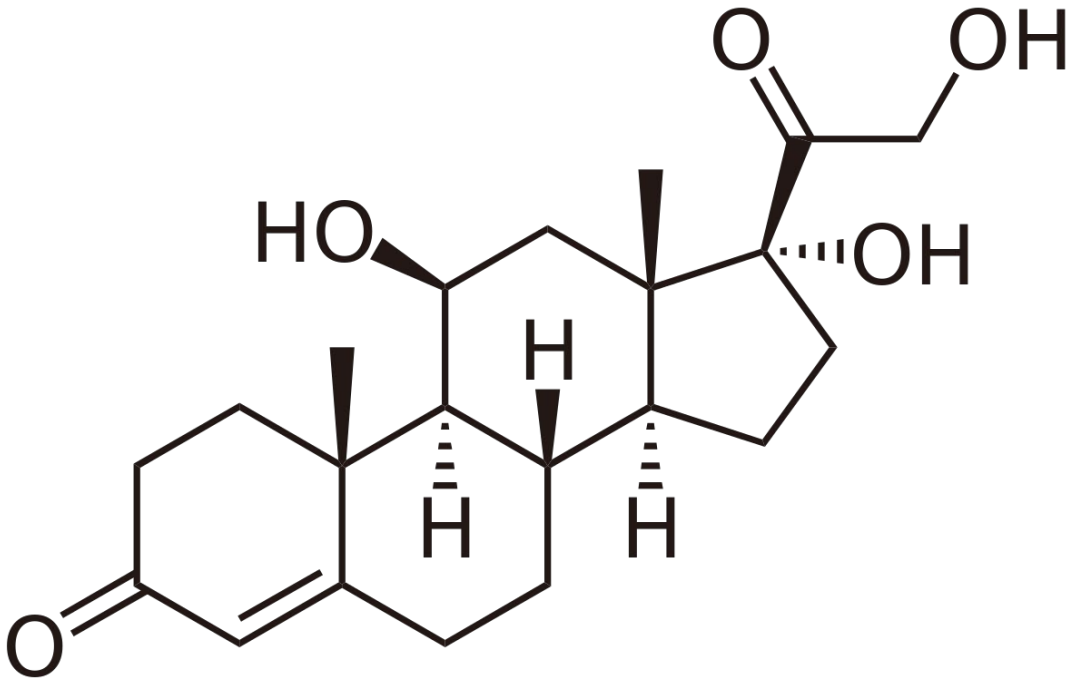
Stress is a condition resulting from the action of one or more stressors of either external or internal origin

Several physiological and pathological conditions can be responsible for an alteration of the homeostasis

Parity and stage of lactation can influence metabolic profile, milk yield, somatic cell count, and acid base balance of dairy buffaloes

Similarly, milk yield may represent a physiological burden and may be associated with the hypothalamic–pituitary–adrenal (HPA) axis activation

Complex adaptation processes take place to enable the maintenance of the homeostasis to meet the requirements for the metabolically prioritized mammary gland in early lactation



Why the cortisol?

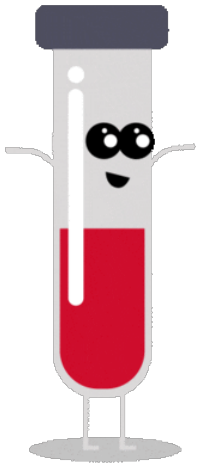
The glucocorticoid hormone cortisol, due to its multifaceted role in the physiological stress response, is the primary physiological biomarker for many phenotypic and metabolic changes in animals associated with activation of the HPA axis

Biological matrices

- Plasma cortisol concentrations evaluate acute responses to stressful stimuli. However, the process of blood sampling is accompanied by additional burden

- Therefore, milk is an alternative to blood for dairy ruminants. Both these biological fluids provide short-term evaluation about the HPA axis activity (in milk with a lag-time)

- On the other hand, hair reflects long-term retrospective concentrations of cortisol, it is non-invasive and has a long lag time

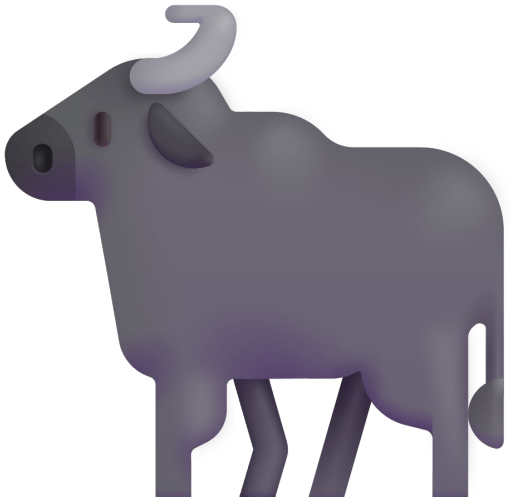


Rationale and aim

In dairy ruminants, conflicting results have been reported about the influence of parity and lactation stage on cortisol concentrations. Cortisol has been previously investigated in buffalo milk[1], whereas the impact of parity and lactation stage on its concentrations was studied on plasma in dairy buffaloes[2]

However, no information is available by using different matrices from the same animals

The aim of this study was to evaluate the influence of parity, stage of lactation and productive levels on cortisol concentrations in biological matrices sampled in Italian Mediterranean buffaloes and to study which one could show a promising predictive potential



[1] Cotticelli A, Verde MT, Matera R, Pividori I, Prandi A, Neglia G, Peric T. 2022. Validation of a radioimmunoassay method for cortisol in buffalo milk whey. A preparatory step for future sensor technology. Ital J Anim Sci. 21(1):1622–1631. doi: 10.1080/1828051X.2022.2147868

[2] Saqib MN, Qureshi MS, Suhail SM, Khan RU, Bozzo G, Ceci E, Laudadio V, Tufarelli V. 2022. Association among metabolic status, oxidative stress, milk yield, body condition score and reproductive cyclicity in dairy buffaloes. Reprod Domest Anim. 57(5):498–504. doi: 10.1111/rda.14086.

Materials and methods

68 Italian Mediterranean dairy buffaloes (*Bubalus bubalis*):

Multiparous (n = 30) and primiparous (n = 38)

Milk quality traits:

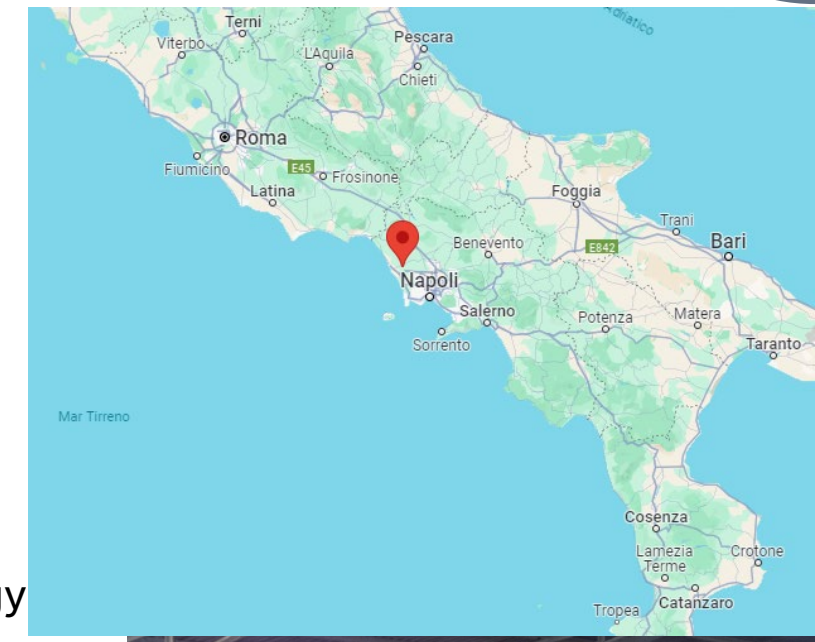
Daily milk yield (kg), fat, protein, lactose content of milk (daily percentage), somatic cells count (SCC), mature equivalent milk yield (EMY), mature equivalent fat content (EFC) and mature equivalent protein content (EPC) (kg/lactation)

Somatic cell score (SCS), energy corrected milk (ECM) and equivalent energy corrected milk (eECM) were calculated as follows:

$$\mathbf{SCS} = \log_2\left(\frac{\mathrm{SCC}}{100}\right) + 3$$

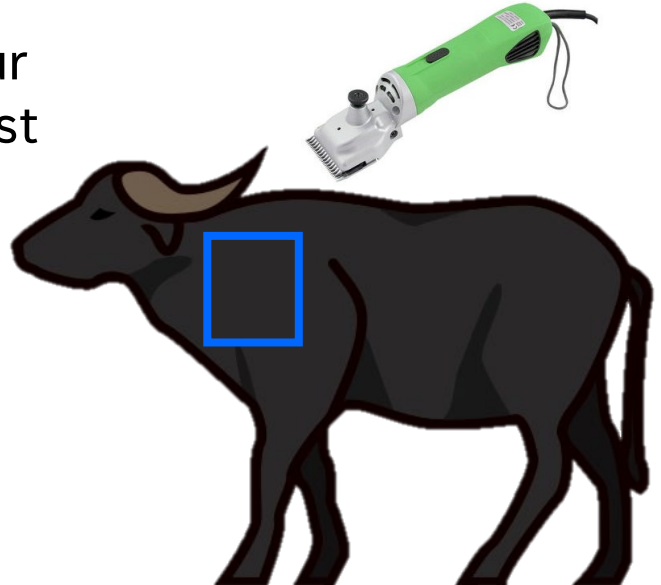
$$\mathbf{ECM} = \text{milk yield} \times \left\{\left[\text{fat}\left(\frac{\text{g}}{\text{kg}}\right) - 40 + \text{protein}\left(\frac{\text{g}}{\text{kg}}\right) - 31\right] \times 0.01155\right\} + 1$$

$$\mathbf{eECM} = \text{dEMY} \times \{[\text{EFC (g/kg)} - 40 + \text{EPC (g/kg)} - 31] \times 0.01155\} + 1$$



Biological samples collection

1. Sterile falcon tubes (Falcon®) were used to collect individual **milk** samples from the at-line sampler (MM15 DeLaval)
2. **Blood** (10 mL) was collected from the mammary vein into vacutainer tubes (lithium heparin anticoagulant). Samples were centrifuged at 1500 × g for 15 min and the plasma was aliquoted into Eppendorf tubes (1mL) and stored at -20 °C
3. For analyses only regrown **hair** was obtained from the scapular region using a razorblade (the animals were shaved for the first time 50 days before the substrates collection)

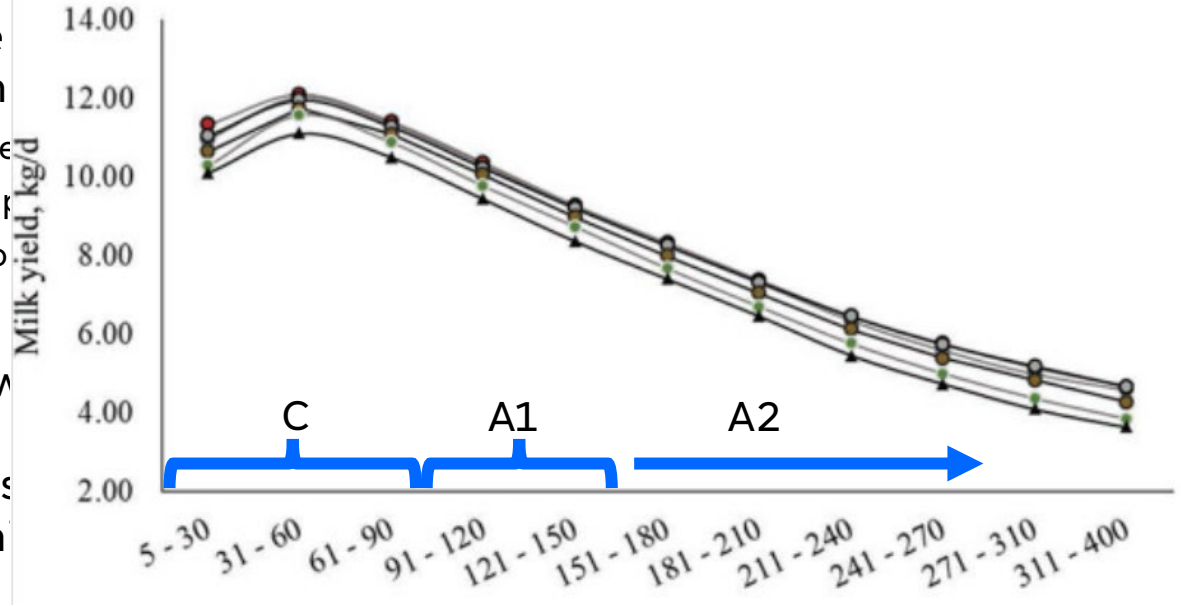


Analysis

The cortisol concentrations were measured in whole extracted milk, whey, hair and plasma using an in-house radioimmunoassay (RIA) method[1]. The sensitivity of the assay was 16.8 pg/mL

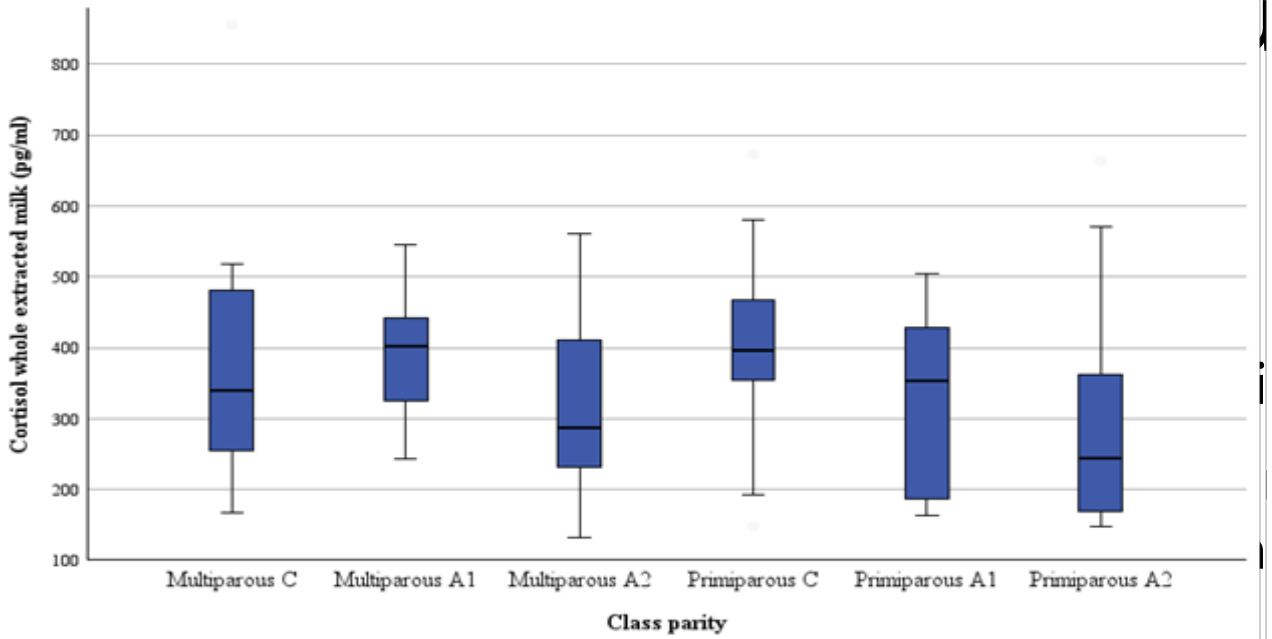
Statistical analyses were S Inc., Chicago, IL). According to the lactation

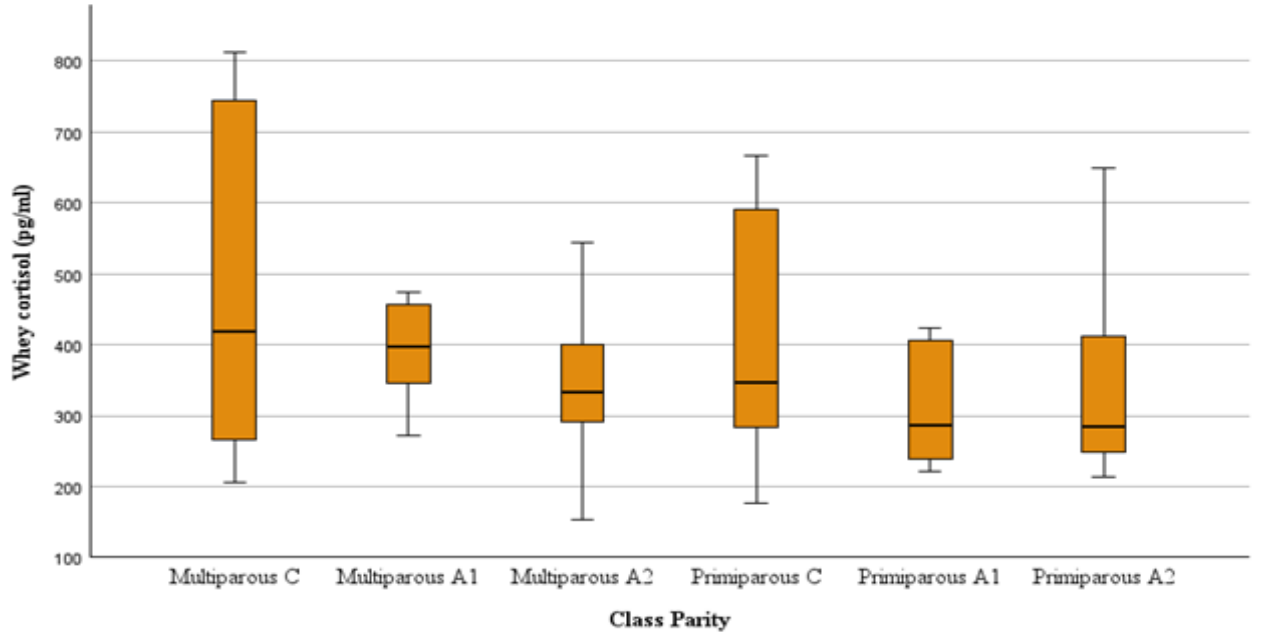
- class C (catabolic phase
- class A1 (first anabolic p
- class A2 (second anabo

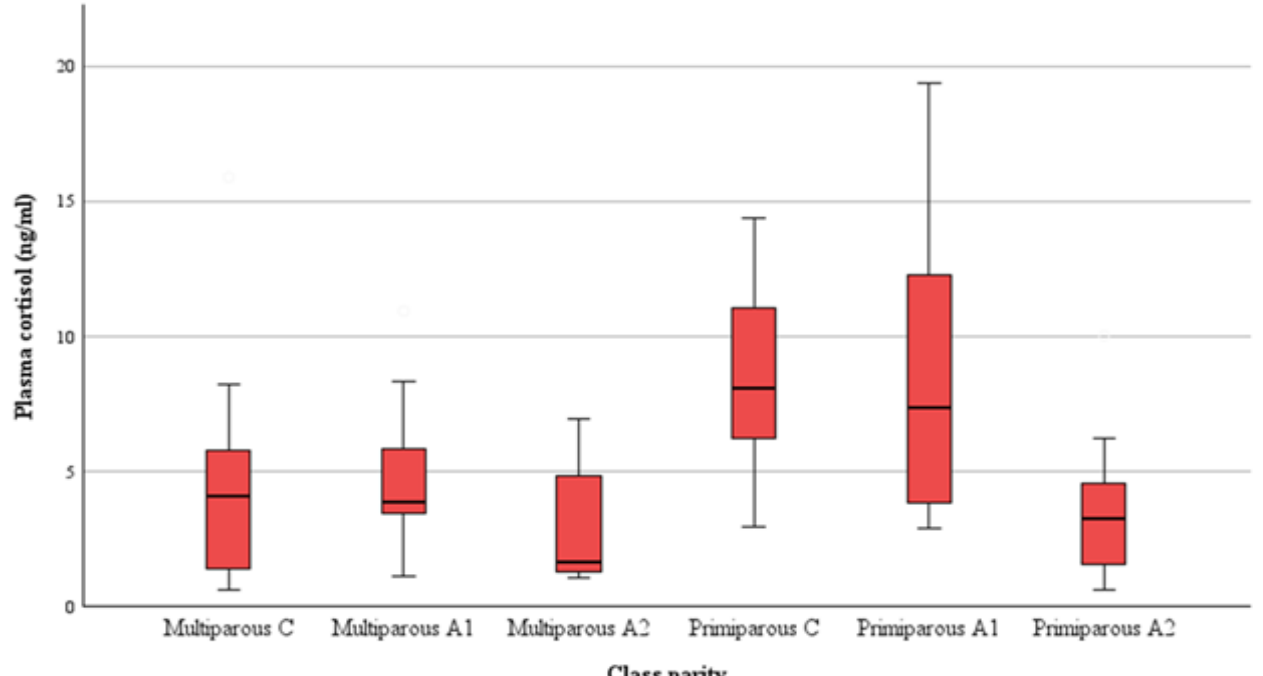


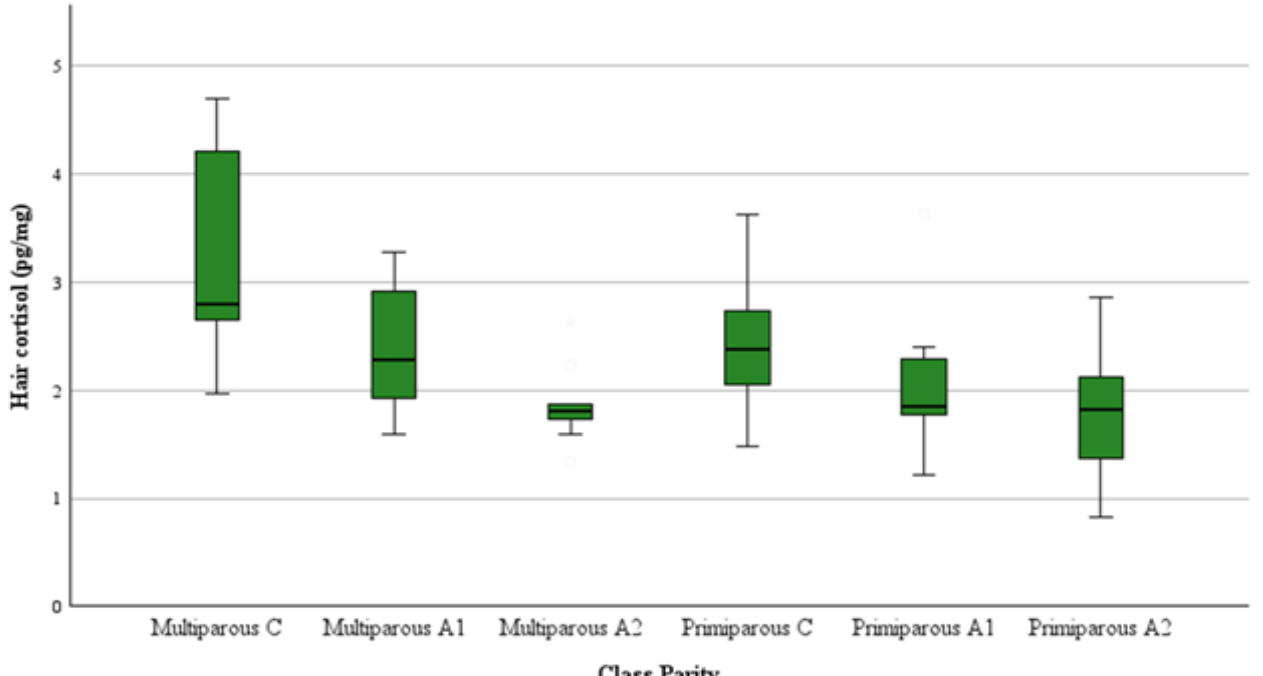
Possible correlations betw ture equivalent milk parameters were studied as used to compare the cortisol concentrations e levels and parities. Whey, whole extracted m n a linear regression model

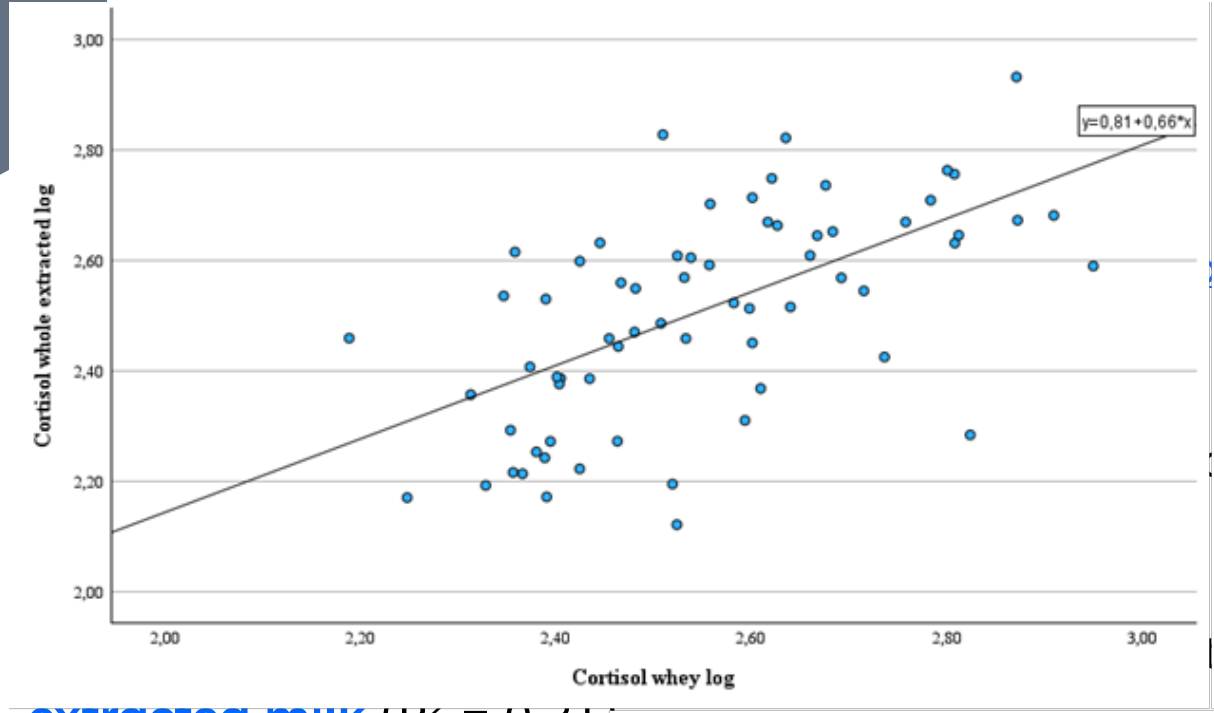
[1] Peric T, Comin A, Montillo M, Spigarelli C, Corazzin M, Cotticelli A, Prandi A. 2022. Postnatal and postweaning endocrine setting in dairy calves through hair cortisol, dehydroepiandrosterone and dehydroepiandrosterone sulphate. Agric Nat Resource. 56(5):867–876

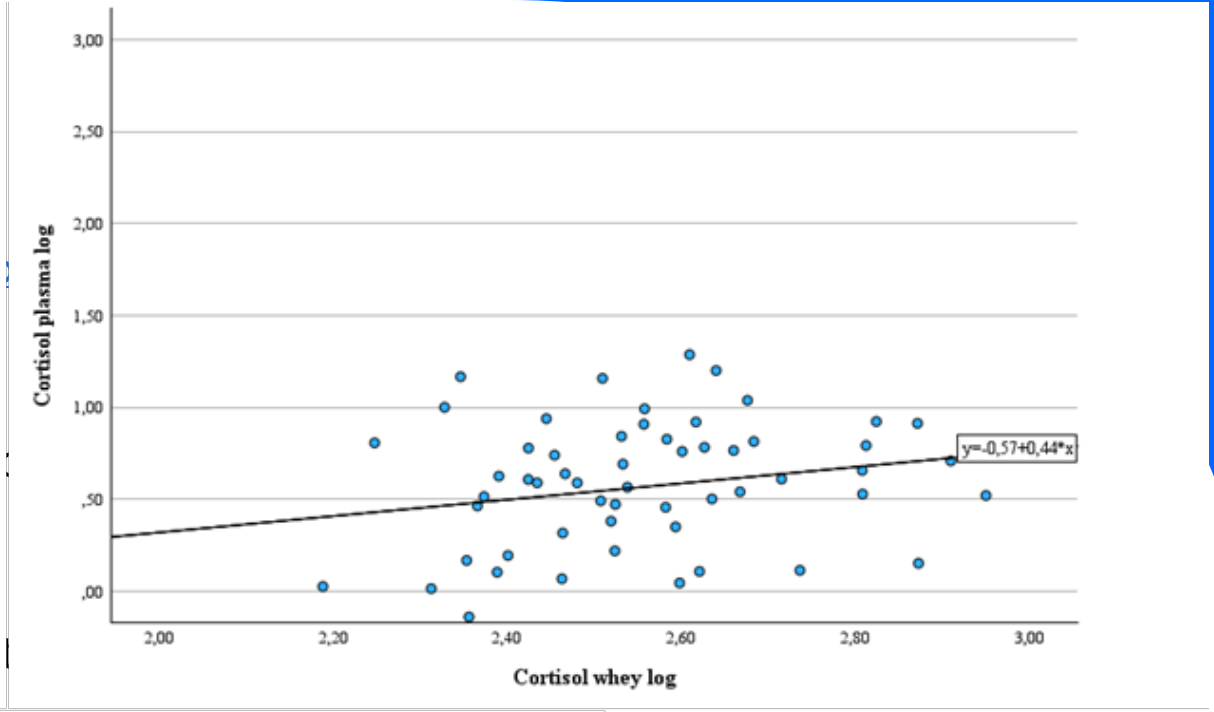

Cortisol whole extracted milk (pg/ml)
800
700
600
500
400
300
200
100
Multiparous C
Multiparous A1
Multiparous A2
Primiparous C
Primiparous A1
Primiparous A2
Class parity


Whey cortisol (pg/ml)
800
700
600
500
400
300
200
100
Multiparous C
Multiparous A1
Multiparous A2
Primiparous C
Primiparous A1
Primiparous A2
Class Parity

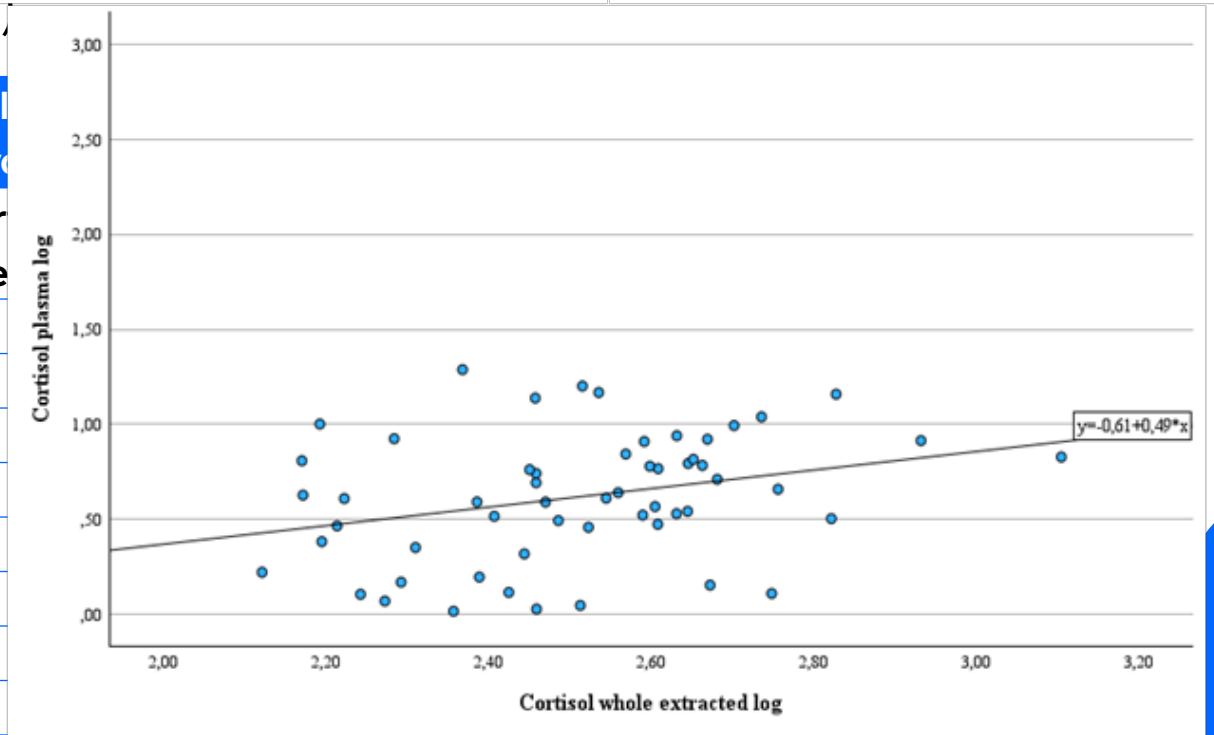

Plasma cortisol (ng/ml)
20
15
10
5
0
Multiparous C
Multiparous A1
Multiparous A2
Primiparous C
Primiparous A1
Primiparous A2
Class parity


Hair cortisol (pg/mg)
5
4
3
2
1
0
Multiparous C
Multiparous A1
Multiparous A2
Primiparous C
Primiparous A1
Primiparous A2
Class Parity





extracted milk (R^2 = 0.31)



Correlations between the cortisol equiv		
Milk parameters	Spearman	Cor e
EMY	rho	
	P	
EFC	rho	
	P	
EPC	rho	
	P	
eECM	rho	
	P	

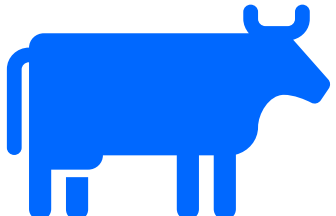
Discussion

Since the dynamics of physiological processes associated with parity are different and they last long timeline, it is likely that they end up being eluted over the time and the biological matrices can't highlight differences between the two primiparous and multiparous buffaloes

As expected, our results showed an increase of cortisol concentration in animals in the first stages of lactation. After calving buffaloes undergo several physiological changes (negative energy balance, uterine involution and ovarian cyclic activity resumption), these conditions represent stressful events for the animals that respond to increasing cortisol concentrations

The negative correlations between the hair cortisol, EMY, EPC and eECM and between whey cortisol and EFC seem to reveal a detrimental effect of the cortisol on both quality and quantity parameters of buffalo milk

Whey and whole extracted milk can be further investigated as potential predictors of the concentrations of cortisol. Predicting the plasma cortisol concentrations by milk could help sparing the animals the blood sampling, that is always invasive and stressful



Conclusions

The lactation stage showed a comparable influence on cortisol concentrations across the four media

Further studies are needed to get conclusions about the effects of parity that seemed to overrun the period retrospectively covered by the biological matrices

The influence of cortisol on productive level was pictured by the hair being a retrospective matrix that include a broader timeframe compared to punctual matrices

Milk seemed to have predictive potential to estimate cortisol concentrations in buffalo and may be used as parameter for stress determination avoiding blood sampling

It is concluded that the development of a real-time automated cortisol measurement in milk could be a profitable tool in Italian Mediterranean Buffalo farming

Thanks for the attention