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Hair cortisol and testosterone concentrations and semen production of *Bos taurus* bulls

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ABSTRACT

The experiment was aimed to evaluate the usefulness of hair cortisol concentrations (HCC) in revealing the activation of the hypothalamic-pituitary-adrenal (HPA) axis due to the passage of young bulls from genetic rearing stations to artificial insemination (AI) centre and the influence of this passage on hair testosterone concentrations (HTC). Hair samples on 33 yearling bulls were collected at the time of arrival (ST1) to the AI centre and 3 more hair samples (ST2, ST3, ST4) were collected at monthly intervals. After the end of quarantine each animal was submitted to the standard procedures for collection and production of semen. Overall HCC were significantly affected by period of collection ($p < .01$). In particular, HCC was higher in samples obtained after finishing the quarantine period (ST2) compared with those obtained on ST1. After ST2, the HCC decreased reaching at ST4 a value similar to that recorded at ST1. An effect of sampling time on HTC was not found ($p > .05$). The effect of breed was observed only for HTC that was higher in PRI than BS ($p < .01$). The HCC group have not influenced the semen variables ($p > .05$). On the contrary, the effect of breed was evident. The results of this study replicated the effects of a major environmental stressor on mean cortisol concentrations assessed in hair, and add to the growing body of evidence that HCC is an effective and simply collected marker for long-term activity of the HPA system in response to persistent environmental challenge.

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Introduction

Bulls contribute more to the overall reproductive success of the herd than any other individual animal; therefore, male subfertility has significant economic consequences. Evaluation of semen motility and morphology are an integral part of the Breeding Soundness Evaluation, and both components are correlated to fertility (Saacke et al. 1994). Attention nowadays is directed also towards the assessment of new aspects of semen quality as predictors of the fertility of a given sperm sample (Barros et al. 2007).

Glucocorticoids inhibited reproductive functions in most domestic species studied (Calogero et al. 1999). Dexamethasone (DXO), a synthetic glucocorticoid, reduced LH secretion and consequently testosterone concentrations (Thiebier & Roland 1976); this reduction in testosterone may impair both testicular and epididymal function (Barth & Bowman 1994). Additionally, DXO

may act directly on the testes, as Leydig cells have glucocorticoid receptors (Tsantarliotou et al. 2002). In bulls, the administration of DXO induced a spermogram similar to that of bulls experiencing heat stress (Barth & Bowman 1994). In humans (Miesel et al. 1997), bulls (Barth 1993), dogs (Hatamoto et al. 2006) and rats (Retana-Márquez et al. 2003) stress induces a significant reduction in semen quality, especially due to a decrease in sperm concentration and motility and an increase in sperm defects.

It was already shown that concentrations of cortisol in semen were elevated 20 min after the systemic injection of cortisol, indicating that significant concentrations of cortisol as well as oestrogen (Graves & Eiler 1979) can be rapidly transferred into semen from blood. The effect on fertility is yet to be tested. It has also been shown that incubation of spermatozoa *in vitro* in media containing cortisol decreased fertilising ability of the spermatozoa (Briggs 1973). Different clinical

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situation may result in bulls suffering from normally occurring or iatrogenic adrenal hypercorticism, where sperm may be subject to abnormal concentrations of cortisol for a long period of time. Moreover, persistently high cortisol concentrations due to a situation of chronic stress can have detrimental effects on reproductive processes in animals (Rivier et al. 1986).

The blood cortisol concentrations has been used to investigate the role of the adrenocortical system in regard to the stress-mediated influence on the hypothalamic-pituitary-gonadal (HPG) axis (Rasooli et al. 2010). Cortisol concentrations can be routinely determined from blood but these methods provide a measurement of the cortisol concentration at a single point in time or within 12 h (Palme et al. 1996). Hair cortisol concentration (HCC) analysis is a complementary means of monitoring the hypothalamic-pituitary-adrenal (HPA) axis, as it reflects cortisol secretion over longer periods of time as it has been showed also by Comin et al. (2012b) and by Montillo et al. (2014). In this way, it is possible to detect long-term cortisol concentrations that can be associated with chronic stress (Kalra et al. 2007). Unlike previous methods, the analysis of cortisol in hair are unaffected by circadian variations in the hormone or by factors inducing short-term variations. Information about testosterone secretions can be also stored in hair.

The collection of hair is simple and non-invasive, and the sample does not decompose like other body fluids or tissues (Balíková 2005). Hair is a relatively stable medium known to incorporate blood-borne hormones through passive diffusion from blood capillaries present on the basement membrane during its active growth phase (Pragst & Balíková 2006), and these hormones may remain detectable for a long time (Webb et al. 2010).

The interest for the hair is focussed mainly on the fact that unlike other biological matrices, the hair provides long-term information that allows us to reconstruct what the history of the individual has been a few months prior to sampling. Shavings and re-samplings of hair in the same area allow monitoring of the hormonal concentrations for weeks or months by providing a 'window into the past.' This phenomenon allows us to retrospectively examine hormonal production without needing to take a sample right at that time (Kirschbaum et al. 2009).

Hair has long been used in toxicology, forensic science, doping control and other fields as a biological matrix for the detection of environmental agents, drugs or toxins (Kirschbaum et al. 2009).

To date, cortisol measurement in hair samples has been reported as validated methods to evaluate the

HPA axis activity in humans (Raul et al. 2004; Gow et al. 2010; Thomson et al. 2010). The evaluation of the allostatic load through HCC has been reported in ruminants as goats (Battini et al. 2015) and wild deers (Caslini et al. 2016). Cortisol measurement in hair samples has been already reported as a validated method in cows (Comin et al. 2011; Del Rosario González-de-la-Vara et al. 2011; Comin et al. 2012a; Peric et al. 2013; Biancucci et al. 2016). As concern hair testosterone concentrations (HTC) they have been evaluated both in humans (Kintz et al. 1999; Pozo et al. 2009) and bovine (Gleixner & Meyer 1997; Stolker et al. 2009).

The main objectives of this study were: (1) to evaluate the usefulness of HCC in revealing the activation of HPA axis due to the passage of young bulls from genetic rearing stations to artificial insemination (AI) centre and the influence of this passage on HTC, (2) to evaluate the effects of changes in HCC on semen quantitative and qualitative characteristics.

Materials and methods

Semen was collected twice a week during routine production activities. Although hair sampling is a neither invasive and nor troublesome procedure, all experimental procedures and the care of the animals complied with the Italian legislation on animal care (Legislative Decree n. 116, 27/1/1992) and adhered to the internal rules of University of Udine. The approval for conducting this study was also granted by the veterinarian responsible of animal welfare of the Department of Agricultural and Environmental Science of the University of Udine.

Bulls

The study was carried out over a twelve-month period on a total of 33 yearling bulls. Data were collected from 23 Italian Simmental bulls (age = 17.5 ± 1.3 months) and 10 Brown Swiss bulls (age = 15.6 ± 3.5 months). Before the experimental period, all animals were maintained in free stall boxes at a rearing performances test station, were animals were maintained and feed uniformly. In accordance to the regulations of the national veterinary health surveillance service for AI centre, the animals, before the start of semen production, were quarantined in a tight stall for 30 days. After quarantine, they were moved to production stall where they were kept in individual boxes (2.5 m \times 5 m with straw bedded floor), provided with water and ration of maintenance with an adequate intake of vitamins and minerals. The bulls were all

in good health and certified free from all the diseases listed in the WHO Terrestrial Animal Code (Article 4.6.2.).

Hair and semen collection

Hair samples were carefully obtained from the scapular region of the bulls with a razor (Lister Shearing Equipment Ltd Units, Gloucestershire, UK). Hair samples were collected at the time of arrival (ST1) at the quarantine station inside the AI centre. At this time, the single hairs collected were at a different physiological phases (anagen, catagen and telogen). Because of this, these samples reflected the hormone concentrations of at least one month prior to the start of the experiment. The following three hair samples (ST2, ST3, ST4) were collected at monthly interval from the same skin area in order to ensure that an adequate amount of hair was present for hormonal analysis. During the experimental period, after the end of quarantine, each animal was submitted to the standard procedures for collection and production of semen. In particular, semen from sires was collected biweekly, by artificial vagina, and each collection consists of a double ejaculate sampled 30 min apart in order to maximise semen production.

Hair samples were stored in dry tubes at room temperature until analysis.

Hair hormonal assay

The hair strands were washed in 5 mL isopropanol (Sigma -Aldrich, St. Louis, MO) and approximately 60 mg of trimmed hair was extracted in a glass vial with 3 mL of methanol (Sigma-Aldrich, St. Louis, MO). The vials were incubated at 37 °C for 18 h. Next, the liquid in the vial was evaporated to dryness at 37 °C under an airstream suction hood. The remaining residue was dissolved in 0.6 mL of phosphate-buffered saline (PBS), 0.05 M, pH 7.5. The HCC (Peric et al. 2013) and HTC were measured using a solid-phase microtiter RIA assay. In brief, a 96-well microtiter plate (Optiplat, Perkin-Elmer Life Science, Boston, MA) was coated with goat anti-rabbit γ -globulin serum (Analytical Antibodies - Bologna, Italy) diluted 1:1000 in 0.15 mM sodium acetate buffer, pH 9, and the plate was incubated overnight at 4 °C. The plate was then washed twice with RIA buffer, pH 7.5, and incubated overnight at 4 °C with 200 μ L of the anti-hormone serum diluted 1:12,000 for cortisol and 1:160,000 for testosterone. Cortisol was analysed using a commercial rabbit anti-cortisol antibody (Biogenesis (Poole, UK) that showed the following cross-reactions: cortisol, 100%;

corticosterone, 1.8%; and aldosterone, <0.02%. Testosterone was analysed using a commercial anti-testosterone-3-carboxymethyloxime-BSA (Analytical Antibodies - Bologna, Italy) that showed the following cross-reactions: testosterone 100%, 5 α -dihydrotestosterone 43.2%, 5 α -androstenedione 33.1%, 5 β -androstenedione 11.4%, 5 α -androstan-3 α ,17 β -diol 9.4%, androstenedione 0.4%, progesterone, DHEA, oestradiol 0.01%, cortisol <0.001%.

After washing the plate with RIA buffer, standards (Sigma-Aldrich, St. Louis, MO) (5–200 pg/well), a quality control extract, the test extracts, and tracer (cortisol, Perkin-Elmer Life Sciences (Boston, MA), specific activity: 72.4 Ci/mmol, 23 pg/well; testosterone, Perkin-Elmer Life Sciences (Boston, MA), specific activity: 83.4 Ci/mmol, 30 pg/well) were added, and the plate was incubated overnight at 4 °C. Bound hormone was separated from free hormone by decanting and washing the wells in RIA buffer. After addition of 200 μ L of scintillation cocktail, the plate was counted on a β -counter (Top-Count, Perkin-Elmer Life Sciences), Boston, MA.

For cortisol, intra- and inter-assay coefficients of variation (CV) were 3.6 and 9.8%, respectively. The detection limit of the assay, as calculated by the software Riasmart (Perkin-Elmer Life Sciences, Boston, MA), was 1.23 pg/well. The relationship between hair cortisol concentrations and the standard cortisol curve determined through linear regression was linear: the correlation coefficient (r) was 0.99 and the model was given by the equation $y = 0.96x + 5.572$; the slope of the line was 0.96.

For testosterone, intra- and interassay CV were 3.5 and 12.8%, respectively. The detection limit of the assay, as calculated by the software Riasmart (Perkin-Elmer Life Sciences, Boston, MA), was 0.88 pg/well.

To evaluate assay accuracy, possible interference of components within the extract with antibody binding was analysed through recovery of exogenous testosterone added to pooled bull hair extracts. The percentage of recovery was determined as follows: amount observed/amount expected \times 100, where the amount observed is the value obtained from the spiked sample and the amount expected is the calculated amount of standard hormone added plus the amount of endogenous hormone in the unspiked sample. Recovery rate was $103.6 \pm 3.3\%$ (mean \pm SD). The measured hormone concentrations in the spiked samples correlated with the expected concentrations: the correlation coefficient (r) was 0.99 and the model was given by the equation $y = 1.041x - 0.37$. To determine the parallelism between testosterone standards and endogenous testosterone in bulls, hair samples containing high concentrations of endogenous

testosterone were serially diluted in 0.05 M PBS, pH 7.5. The relationship between HTC and the standard testosterone curve determined through linear regression was linear: the correlation coefficient (r) was 0.99 and the model was given by the equation $y = 0.966x - 2.80$.

Semen evaluation and freezing

Immediately after collection all the semen samples were submitted to standard evaluation procedures. In brief, ejaculate volume was estimated by weighing the ejaculates and within 5 min upon collection and incubation at 35 °C semen concentration and motion characteristic were determined by means of computer assisted semen analysis system (CASA, Sperm Vision, Minitübe GmbH, Germany). The technical settings used in the present study are provided in Table 1 (Schäfer-Somi & Aurich 2007).

Immediately after collection semen samples were diluted to a final concentration of 40 to 80 × 10⁶ spermatozoa/mL with a commercial freezing extender (Tryladil, Minitübe GmbH, Germany), before packaging in 0.5 ml or 0.25 ml straws, respectively.

Motion parameters determined by SpermVision were: percentage of motile (Mtot), percentage of progressively motile (Mpro), curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN = VSL/VCL), straightness (STR = VSL/VAP), wobble (WOB = VAP/VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), curvilinear distance (DCL), straight line distance (DSL) and distance of average path (DAP).

Straws containing extended semen were placed on freezing trays and incubated at 5 °C overnight. Freezing was started by transferring the straws into a fixed temperature freezing chamber (Minitübe GmbH, Germany) at -140 °C for 15 min and subsequently plunged into liquid nitrogen.

Table 1. Technical settings for CASA SpermVision (Minitübe GmbH, Germany), as used in the present study.

Parameter	
Depth of sample chamber	20 µm
Light adjustment	100–155
Temperature of analysis	37 °C
Dimension of sperm heads	22–99 µm ²
Frame rate	60 s ⁻¹
Immotile	AOC < 3.5 µm s ⁻¹
Lateral motile	DSL < 15 µm s ⁻¹ , VSL < 20 µm s ⁻¹ and VAP < 24.9 µm s ⁻¹
Progressively motile	STR > 0.5 and LIN > 0.35
Non linear	STR < 0.5 and LIN < 0.35
Curvilinear	DAP/Radius = 3 and LIN < 0.5
Round pat area	average < 40 µm ² , BCF = 0 and AOC > 8

Statistical analysis

The statistical analysis was performed using SPSS for Windows (version 7.5.21, Inc 1989–1997). Normality of data distribution was tested using Shapiro-Wilk test, and when appropriate, non-parametrically distributed data were log-transformed for parametric testing. The effect of sampling period (ST1, ST2, ST3 and ST4) and breed (PRI, BS) on hair hormones concentration were analysed using linear mixed models for repeated measures considering breed as fixed effect and sampling period as repeated measurements factor. For pairwise comparisons, the p -values were adjusted using the Ryan-Holm-Bonferroni procedure. Hair hormones concentrations reflected the hormone concentrations from at least 40 to 21 days before the sample collection, thus the hormone concentration was related with the average of the characteristics of semen collected between two sampling times, considering a lag time of 21 days. To assess the relevance of HCC on semen characteristics, 2 groups of bulls were created based on the concentration of cortisol in the hair: Low < 4.17 pg/mg and High ≥ 4.17 pg/mg. The ranges were created considering that the cut-off value of 4.17 pg/mg was found to discriminate between clinical normal and clinical compromised dairy cows (Comin et al. 2013). In our knowledge, the only hair cortisol data available on bulls were reported by Moya et al. (2013) that showed as HCC ranges between 0.30 and 5.31 pg/mg. The effect of breed and HCC group (Low, High), treated as fixed factors, on semen characteristics was studied using a two way ANOVA design. The associations between variables were determined using partial correlation in order to control the breed effect. Moreover, in order to assess if hair hormones concentration was related to semen quality, the semen characteristics were transformed in dichotomous variables, according to the thresholds highlighted by Brogliatti et al. (2005) and by Kathiravan et al. (2011), and correlated with hair hormone concentrations by using point biserial correlations (De Ayala 2009).

Results

The effect of sampling time and breed on hair hormones concentrations are showed in Table 2. Overall HCC were significantly affected by period of collection ($p < .01$). In particular, cortisol was higher in samples of hair obtained after finishing the quarantine period (ST2) compared with those obtained on ST1. After ST2, the HCC decreased reaching at ST4 a value similar to that recorded at ST1. An effect of sampling time on HTC was not found ($p > .05$). The effect of breed was

observed only for HTC that was higher in PRI than BS ($p < .01$). Interactions between sampling time and breed were not found ($p > .05$), it means that the trends of the concentrations of the hormones considered were similar between PRI and BS.

The effect of breed and HCC on semen characteristics is shown in Table 3. The HCC group have not influenced the semen variables ($p > .05$). On the contrary, the effect of breed was evident. In particular, PRI showed higher Mtot ($p < .01$), Mpro ($p < 0.01$), LIN ($p < .01$), and ALH ($p < .05$), but lower WOB than BS ($p < .01$). Moreover, PRI tended to have a higher TNS ($p = .06$), DCL ($p = .08$), VCL ($p = .07$), and lower STR ($p = .08$) than BS. Interaction between factors was not found ($p > .05$).

Significant correlations between hair hormones concentrations and semen characteristics were not found. Conversely, point biserial correlations between hormones concentrations and semen quality as proposed by Kathiravan et al. (2011) were highlighted between HCC and VSL ($r = -.272$; $p = .04$; data not reported in Tables), and HCC and ALH ($r = -.393$; $p < .01$; data not reported in Tables).

Discussion

Effect of sampling period

The HPA axis is a neuroendocrine system primarily involved in metabolic homeostasis (Mormède et al. 2007). Different authors claim that this axis is strictly connected also to the functionality of the HPG axis (Brkovich & Fisher 1998; Hardy et al. 2005). In fact, the cost of the allostasis of an organism being forced to adapt to adverse situations and reaching thus homeostasis it is reflected on energy that is spendable in biological processes related to the reproductive sphere. Thus, an organism not adapted to the environment in which it may suspend/modify the reproductive activity in order to use the energy available to allow the body to react to the unfavourable situation would represent a genotype 'unsuitable' to survive under certain conditions and would not be maintained because not advantageous.

We investigated changes on HCC as an indicator of the HPA axis activity relative to environmental variations connected to the transport and different housing condition of the yearling bull. The animals passed

Table 2. Estimated marginal means of hair cortisol and testosterone concentrations recorded monthly in Italian Simmental (PRI) and Brown Swiss (BS) bulls breed.

	Breed		Sampling time				CV	p-Value		
	PRI	BS	ST1	ST2	ST3	ST4		B	ST	B × ST
Cortisol, pg/mg	4.51	4.84	3.68 ^a	6.79 ^b	4.85 ^{ab}	3.93 ^a	0.687	ns	**	ns
Testosterone, pg/mg	10.94 ^a	6.95 ^b	11.32	8.15	7.53	8.36	1.017	**	ns	ns

CV: coefficient of variation; B: breed; ST: sampling time; B × ST: breed × sampling time; ns: not significant.
^{a,b}, ** $p < .01$.

Table 3. Estimated marginal means of semen variables recorded in Italian Simmental (PRI) and Brown Swiss (BS) bulls as related to Low (<4.17 pg/mg) and High (≥4.17 pg/mg) hair cortisol concentrations.

	Breed		Hair cortisol group		CV	p-Value		
	PRI	BS	Low	High		B	CG	B × CG
Concentration ($\times 10^9$)	1386	1186	1238	1314	0.418	ns	ns	ns
TNS ($\times 10^9$)	5.61	4.02	4.72	4.91	0.645	.06	ns	ns
Fresh								
Mtot, %	78.77	68.31	71.52	75.55	0.190	**	ns	ns
Mpro, %	67.42	56.58	59.91	64.09	0.242	**	ns	ns
DAP	47.66	46.77	47.70	46.73	0.114	ns	ns	ns
DCL	81.15	76.63	79.15	78.64	0.123	.08	ns	ns
DSL	43.42	42.95	43.71	42.67	0.120	ns	ns	ns
VAP	106.91	104.41	106.68	104.64	0.119	ns	ns	ns
VCL	181.84	170.93	176.87	175.91	0.128	.07	ns	ns
VSL	97.38	95.91	97.75	95.55	0.125	ns	ns	ns
STR	0.905	0.912	0.910	0.907	0.017	.08	ns	ns
LIN	0.584	0.557	0.549	0.539	0.056	**	ns	ns
WOB	0.590	0.606	0.599	0.591	0.051	**	ns	ns
ALH	4.49	4.07	4.25	4.30	0.159	*	ns	ns
BCF	35.42	36.43	36.05	35.79	0.082	ns	ns	ns

CV: coefficient of variation; B: breed; CG: cortisol group; B × CG: breed × cortisol group; TNS: total number of spermatozoa in the ejaculate; Mtot: total motility; Mpro: progressive motility; DAP: distance of average path; DCL: curvilinear distance; DSL: straight line distance; VAP: average path velocity; VCL: curvilinear velocity; VSL: straight line velocity; STR: straightness (STR = VSL/VAP); LIN: linearity (LIN = VSL/VCL); WOB: wobble (WOB = VAP/VCL); ALH: amplitude of lateral head displacement (ALH); BCF: beat cross frequency (BCF); ns: not significant.

* $p < .05$.
 ** $p < .01$.

from the genetic rearing station where they were kept in pen with outdoor to the tie stall condition in the quarantine barn inside the centre for semen production. Successively, animal were moved within the centre from the quarantine to the semen production area in single pens with free stall condition.

Hair samples collected at ST2 reflected the hormone concentrations of one month before and precisely between the time of arrival (ST1) at quarantine station inside the AI centre to the time of beginning of semen collection. Thus, after the ST1 sampling there was only hair in anagen phase.

The significantly higher HCC in samples obtained on ST2 compared with those obtained at ST1 could be related to the change in environment and management (including transport and different housing condition) to which the animals were subjected arriving at the AI centre. In fact, all the animals included in this study were yearling bulls transferred from the genetic rearing station, where they were kept on freestall, into the AI centres' quarantine barn where they were kept in tie stall condition, before being moved in the production area of the centre.

The results of this study replicated the effects of a major environmental stressor on mean cortisol concentrations assessed in hair, and add to the growing body of evidence that HCC is an effective and simply collected marker for long-term activity of the HPA system in response to persistent environmental challenge. A similar increase in HCC was found following relocation to a new environment in rhesus monkeys. Different hair samples were obtained to represent a period before the relocation, the period immediately after relocation, the period of one year after the relocation. A significant increase in HCC was observed in the post-relocation samples compared to baseline concentrations and one year following the relocation the HCC approximated the pre-relocation concentrations again. Those results documented that not only stress is associated with increased HCC, but also that hair cortisol content responds dynamically to changes in cortisol over time (Davenport et al. 2006, 2008). A similar increase in HCC was found following relocation to a new environment in vervet monkeys (Fairbanks et al. 2011; Dettmer et al. 2012) and in rabbits has been investigated how long relocation modified hair cortisol concentrations (Peric et al. 2017). In human medicine, an assessment of cortisol in hair revealed that elevated HCC were found in participants who reported serious life events (e.g. death of a close relative, serious illness, etc.) during the months that were represented in the hair samples compared to participants without such an event. Major life events elicited a distinct stress

response, which influenced HCC so heavily that the average cortisol concentration of three months distinguished the affected participants from the unaffected participants (Karlén et al. 2011).

HCC after two months (ST3) has declined to values similar to those observed before the movement of animals (ST1). Similarly, in a study on bear's HCC showed a decline after arrival (≥ 163 days) and acclimation at the rehabilitation facility from a bile farm. Declined HCC, if compared with the initial HCC, in relocated bears might result from their ability to adapt to new surrounding at the rehabilitation facility resulting in a declined activity of the HPA axis following the relocation (Malcolm et al. 2013). In our study, a new hair collection was done after a period of 30 days and cortisol concentrations at that time (ST4) were still close to those recorded at ST3. As recorded, the environment conditions that characterised the bull staying at the AI centre were stable throughout the study period. Consequently, the absence of stressful stimuli that could activated the HPA axis were recorded by HCC still showing decreased concentrations if compared with those recorded at ST2.

The evaluation of HTC showed only a numerically and not significant reduction after the first sampling, remaining then constant. Since 1998 (Wheeler et al. 1998) different studies reported testosterone (Dettenborn et al. 2013, 2016; Kapoor et al. 2016) and testosterone esters (Kintz et al. 1999; Pozo et al. 2009) measurement in human hair. On the other hand, only few studies regarding the cattle species can be recorded. A study has been carried out about the incorporation of intact steroid esters in hair following pour-on treatment with a hormone cocktail containing testosterone esters (Stolker et al. 2009). More interesting for our study could be the determination of natural testosterone that has been addressed by Gleixner and Meyer (1997) in which were included bulls older than two years, an animal age averagely close to that described for our bulls. The HTC reported by Gleixner and Meyer (1997) (in average 15 ng/g) are close to those evaluated in our study even if no relocation studies could be detected in the literature in which HTC were evaluated.

Effect of cortisol concentrations in the hair on semen characteristics

The cortisol concentrations in the hair of bulls did not have any influence on characteristics of semen and spermatozoa.

The semen traits evidenced in this research are similar to those reported in the literature both for

Simmental and Brown Swiss bulls (Fuerst-Waltl et al. 1996; Stolla & Trombach 1999). In particular, the differences in quantitative characteristics between the two breeds, although not significant, were previously observed (Stolla & Trombach 1999) and our results confirmed the prevalence of Simmental bulls in term of concentration and total number of collected spermatozoa compared to the Brown Swiss.

Conclusions


The measure of the activation of HPA activity could be important for evaluating animal welfare, but the moderate activation evidenced in our condition did not influence the HPG axis and semen production traits.


Disclosure statement


The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.


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