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Biagi, G.; Bartalena, L.; Valentini, A.; Bagliacca, M.;  
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SERUM CORTISOL LEVELS IN ITALIAN FRIESIAN CALVES  
DURING THE FIRST 6 MONTHS OF LIFE.

TOMO II

SERUM CORTISOL LEVELS IN ITALIAN FRIESIAN CALVES  
DURING THE FIRST 6 MONTHS OF LIFE<sup>(\*)</sup>.

G. Biagi<sup>1</sup>, L. Bartalena<sup>2</sup>, A. Valentini<sup>2</sup>, M. Bagliacca<sup>4</sup>,  
G. Della Croce<sup>1</sup>, S. Baccarini<sup>2</sup>, V. Bassi<sup>2</sup> and A. Romagnoli<sup>1</sup>

- (<sup>1</sup>) Istituto Clinica Medica Veterinaria, Università di Pisa, Viale delle Piagge, 2 - 56100 PISA Italy.  
 (<sup>2</sup>) Istituto Endocrinologia, Università di Pisa, Viale del Tirreno, 64 - 56018 TIRRENIA (PI) Italy.  
 (<sup>3</sup>) Istituto Zootecnia, Università di Viterbo, Via de Lellis - 01100 VITERBO Italy.  
 (<sup>4</sup>) Dipartimento Scienza delle Produzioni Animali, Università di Pisa, Viale delle Piagge, 2 - 56100 PISA Italy.  
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INTRODUCTION

Cortisol, with corticosterone, is the main corticosteroid formed in the adrenal (3, 5) and found in jugular (12, 20, 23) venous plasma of dairy cattle. Furthermore, Balfour (3) reported that only cortisol can be detected in calves at birth.

The changes in the plasma concentration of cortisol have been studied in neonate animals of different species. In calf the serum cortisol levels increase during the last days of pregnancy (9, 13), probably in relation to an increased glucocorticoid secretion by the fetal adrenal cortex. The cortisol concentrations progressively decline from birth to 11, 12 days or 20 days of postnatal life (11, 15, 19).

Our study was carried out to investigate the changes in serum cortisol concentrations in Italian Friesian calves from birth to 6 months of life to ascertain when cortisol secretion becomes relatively constant and comparable to that of adult cattle.

MATERIALS AND METHODS

Animals. Forty five Italian Friesian calves (19 males and 26 females) were included in this study. The animals were born at the end of normal pregnancies (272±3 days). The animals had no clinical problems, were housed in individual boxes until 30<sup>th</sup>-40<sup>th</sup> day of life and then reared in single stalls, yoke tying. The calves were fed maternal colostrum twice a day for the first 4 days; on the 5<sup>th</sup> day, when

the average animal weight was about 36 Kg, they received 8l of a commercial milk replacer (4 l in the morning and 4 l in the afternoon). At the 30<sup>th</sup> day (average live weight 70 Kg) the milk replacer was substituted by 400-500 g of a weaning

Table I - Chemical composition of the feed used during the trial (a.f.b.)

	commercial milk replacer	weaning mixture	concentrated food
Moisture.....%	4.5	12.0	13.0
Crude protein...."	23.5	16.3	15.7
Ether extract...."	13.0	3.0	5.7
Crude fiber....."	0.5	14.5	9.4
Ash....."	7.0	7.5	9.6

mixture (the animals could eat the weaning mixture till from the second week). After 2-3 months the weaning mixture was gradually substituted by 1-1.5 Kg of concentrated food and 1-2 Kg of lucerne hay (alfalfa). These quantities were gradually increased up to the 6<sup>th</sup> months (average live weight 170 Kg). (See table 1 for the chemical composition of the employed feed).

Blood samples were collected from all calves by puncture of the right jugular vein in the following way: the first and the second sample were collected at 24 and 48 h after the birth, respectively; the following 10 samples at weekly intervals and the last 4 samples every month up to 6th month. Blood was drawn in the morning (from 8:00 to 9:00 h) with the exception of the first 2 samples which were obviously related to the delivery time. In fact, if the calf birth took place during the day, the blood sample was collected 2-3 h after delivery and before sucking the maternal colostrum; if the calf birth took place at night, the blood sample was collected 12-14 h after delivery.

The births were distributed over a period of 6 months (January - June 1985) as follows: January (3 males and 4 females); February (3 males and 5 females); March (5 males and 3 females); April (2 males and 7 females); May (3 males and 3 females); June (3 males and 4 females).

Assays. Sera were frozen until analyzed. The assay was carried out in duplicate. All samples belonging to the same animal were run in the same assay to avoid interassay variations. The interassay coefficient of variations was less than 5%. Serum cortisol concentrations were measured by specific RIA (Cortisol Test IV807, Cambridge Medical Technology, InnoVet Division, MA - USA).

The data were analyzed by the following statistical model:

$$CBL_{ijk} = \mu + \text{sex}_i + \text{month}_j + b_1 * (1/\text{day})_k + (\text{subj}_{ij} * \text{day}_k)_{ijk}$$

where: CBL = cortisol blood level;  
sex = sex of calf;  
month = month of birth;  
day = age of the calf in days;  
subj = subject I.D.

The interaction between subject and day was considered as the error term.

## RESULTS AND DISCUSSION

This study represents the first report on the variations of serum cortisol concentrations in Italian Friesian calves in the postnatal life. Serum cortisol values were high on the first day ( $5.5 \pm 1.6 \mu\text{g/dl}$ , mean  $\pm$  SD) and sharply declined thereafter (mean value at 48 h:  $2.6 \pm 0.9 \mu\text{g/dl}$ ,  $p < 0.01$ ). A further decrease occurred at the end of the fourth week ( $0.8 \pm 0.03 \mu\text{g/dl}$ ,  $p < 0.01$  vs. the 2<sup>nd</sup> day value) while the mean serum cortisol levels remained fairly stable thereafter (Figure 1).

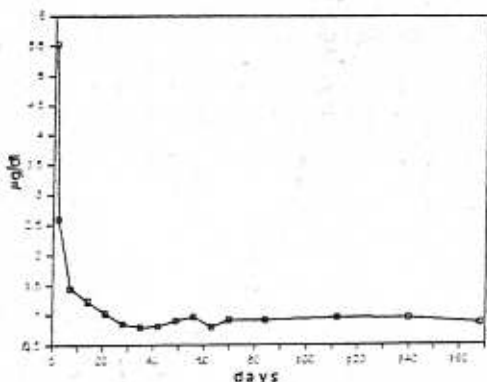


Figure 1 - Serum cortisol levels.

These results are in good agreement with those reported by other authors, who in other bovine breeds also found the highest serum cortisol concentrations shortly after birth (2, 4, 6, 7, 10, 11, 14, 15, 16, 18). This hypercortisolemia can be explained by cortisol hypersecretion which occurring in the fetus, probably to induce parturition (9) and to favour the adaptation to the extrauterine life (17). Alternatively, an accelerated catabolism, rather than a diminished synthesis, may intervene (11). Finally, it cannot be excluded that other factors, such as the hemodilution occurring in the neonatal period, may contribute to these changes (16). The permanence of high serum hormone levels during the first week of life might be explained by the stress due to the absence of the mother and by the replacement of colostrum with a commercial milk replacer; in addition the differences between intrauterine and extrauterine environment should not be forgotten.

It is interesting to note that the mean cortisol values at birth reported in different series were quite scattered, ranging from 5 to 12  $\mu\text{g/dl}$  (2, 4, 6, 7, 10, 11, 14, 15). These differences may be related, on one hand, to the different animal species evaluated, and on the other hand, to differences in the timing of blood sampling. For example, our mean values were similar than those reported by Cabello (6, 7), Gonchorova (14) and slightly lower than those reported by Agarwal et al. (2), Bosc & Fèvre (4), Dvorak (10), Eberhart et al. (11) and Hudson et al. (15). The reason of these differences may be attributed, as suggest by Cabello (6) and confirmed by the trend of our estimated means (Table 2), to the fact that serum cortisol concentration quite substantially decreases within the first 24 h after birth.

Table 2 - Regression of cortisol blood level by 1/age. The coefficients  $b_a$  and  $b_r$  are different ( $p < .05$ ).

	parameter	est.mean	std.err.
all animals	constant	0.798	0.028
	$b$	4.506	0.099
males	constant	0.672	0.036
	$b_a$	4.800	0.123
females	constant	0.887	0.040
	$b_r$	4.279	0.145

Our cortisol data on first day (5.5  $\mu\text{g/dl}$ ) are much lower than those reported in male calves by Lopez & Phillips (18) (33.16  $\mu\text{g/dl}$ ); this difference persists at 7-8 days (1.4  $\mu\text{g/dl}$  compared to 10.75  $\mu\text{g/dl}$ ). In this case, we believe that the explanation is in the different method of analysis used. In fact, for the measurement of serum cortisol concentrations we used a specific radioimmunoassay while Lopez & Phillips (18) employed a competitive protein-binding technique assayed after initial purification by descending paper chromatography.

Estimated cortisol values in the animals born from January to March were significantly higher than those in the calves born from April to June (5.33  $\mu\text{g/dl}$  vs. 5.26  $\mu\text{g/dl}$ ,  $p < 0.05$ ) (see table 3 for the estimated values

Table 3 - LS-means estimated at 1 day old

Factor	LS-mean	Std.Err.	
Month	January	5.367	0.062
	February	5.294	0.059
	March	5.313	0.059
	April	5.208	0.057
	May	5.247	0.066
	June	5.317	0.064
Males	5.208	0.039	
Females	5.374	0.033	

of each month). It is conceivable that the different temperature be responsible for these differences, since heat is a factor known to depress cortisol secretion (1, 7, 22).

Finally, the estimated values were slightly lower in male than in female calves (5.21  $\mu\text{g/dl}$  vs. 5.37  $\mu\text{g/dl}$  in the first day after birth). This fact may be due to the effect of gonadal hormones (21).

In conclusion, these results demonstrate that also in Italian Friesian calves the highest serum cortisol levels are found immediately after birth and sharply decline thereafter. The period of birth (winter vs. spring) and the sex of calves in the first day may affect the mean serum cortisol concentrations and must be taken into account when establishing the "normal" values.

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## SUMMARY

The aim of the present study was to evaluate the variations of serum cortisol concentrations during the first 6 months of life in Italian Friesian calves (IFC). For this purpose serum cortisol levels were measured by specific RIA in 45 IFC (19 males and 26 females) born from January to June. Blood was drawn, once a day, for the first 2 days, then at weekly intervals for 10 weeks, and then at

monthly intervals up to 6 months. The data were analyzed by a split-plot least square method for repeated hormonal measures over time. Serum cortisol concentration (mean  $\pm$  standard dev.) 24 h after birth was  $5.5 \pm 1.6$   $\mu\text{g/dl}$ ; a significant reduction of cortisol level was observed just after 48 h ( $2.6 \pm 0.9$   $\mu\text{g/dl}$ ), and continued up to the 4<sup>th</sup> week of life ( $0.8 \pm 0.03$   $\mu\text{g/dl}$ ). After this age the cortisol concentration remained unchanged. These data confirmed in IFC a progressively decrease of serum cortisol concentration from the highest values in the perinatal period to the normal adult values after the first month of life. The neonatal hypercortisolemia may be due to the hypersecretion of cortisol by fetal adrenals which precedes and probably induces parturition. The high serum hormone levels in the first week of life may be related to the stress due to the absence of the mother and to the replacement of colostrum with a commercial milk replacer; in addition the differences between intrauterine and extrauterine environment should not be forgotten.

## SUMÁRIO

O objectivo do presente estudo foi avaliar as variações das concentrações de hidrocortisona no soro durante os primeiros 6 meses de vida nos Vitelos Italianos da Frísia (VIF). Para este fim, os níveis de hidrocortisona no soro foram medidos por Radioimuneanálise específicos em 45 VIF (19 machos e 26 fêmeas) nascidos desde janeiro até junho. O sangue foi obtido, uma vez por dia, durante os 2 primeiros dias, depois com intervalos de uma semana durante 10 semanas e depois com intervalos de um mês até aos 6 meses. Os dados foram analisados por um diagrama duplo com o método dos mínimos quadrantes por medidas hormonais naquele período. A concentração de hidrocortisona no soro (valor médio  $\pm$  variante do padrão) 24 h depois do nascimento era  $5.5 \pm 1.6$   $\mu\text{g/dl}$ ; uma redução significativa do nível de hidrocortisona foi observada logo a seguir às 48 h ( $2.6 \pm 0.9$   $\mu\text{g/dl}$ ) e continuou até 4<sup>a</sup> semana de vida ( $0.8 \pm 0.03$   $\mu\text{g/dl}$ ). Depois desta idade a concentração de hidrocortisona ficou inalterável. Estes dados confirmaram nos VIF uma diminuição progressiva da concentração de hidrocortisona no soro desde os valores mais altos no período pós-natal até aos valores adultos normais depois do primeiro mês de vida. O hiper valor de hidrocortisona neo-natal foi interpretado com a hipersecreção de hidrocortisona pelas glândulas suprarenais fetais que precede e provavelmente motiva o parto. Os altos níveis de hormona no soro na primeira semana de vida foram relacionados com o stress devido à ausência da mãe e com a substituição de colostro por leite comercial substituinte, além disto as diferenças entre ambiente intrauterino e extrauterino não devem ser esquecidas.

## ZUSAMMENFASSUNG

Diese Untersuchung wurde durchgeführt, um die Schwankungen der Serumkonzentrationen von Hydrocortison bei schwarz gefleckten Kälbern (SGK) während der ersten 6 Lebensmonate auszuwerten. Zu diesem Zweck wurden mit einem speziellen Radioimmuntest die Serumkonzentrationen von Hydrocortison bei 45 SGK (19 männliche und 26 weibliche Tiere) untersucht, die in der Zeit von Januar bis Juni

geboren waren. In den ersten beiden Tagen wurden täglich eine Blutprobe entnommen, danach 10 Wochen lang einmal wöchentlich und im folgenden bis zum 6. Monat einmal pro Monat. Die Daten wurden mit der Doppelkurvenmethode der kleinsten Quadrate für wiederholte Messung des Hormonspiegels über die gesamte Zeitspanne hin analysiert. Vierundzwanzig Stunden nach der Geburt waren die Serumkonzentrationen von Hydrocortison  $5.5 \pm 1.6 \mu\text{g/dl}$  (Mittelwert  $\pm$  Standardabweichung); nach 48 Stunden konnte ein starkes Absinken der Hydrocortisonkonzentration beobachtet werden ( $2.6 \pm 0.9 \mu\text{g/dl}$ ), das bis zur 4. Lebenswoche anhielt ( $0.8 \pm 0.03 \mu\text{g/dl}$ ). Danach blieben die Hydrocortisonkonzentrationen unverändert. Diese Ergebnisse bestätigen, daß die Serumkonzentrationen von Hydrocortison bei SGK schrittweise absinken, von den Höchstwerten sofort nach der Geburt bis zu Normalwerten für ausgewachsene Tiere, die nach dem ersten Lebensmonat erreicht werden. Der übermäßige Hydrocortisongehalt bei den Neugeborenen wurde als Hydrocortisonhypersekretion der Nebennieren des Fötus interpretiert, die der Geburt vorausgeht und sie wahrscheinlich einleitet. Der hohe Hormonspiegel im Serum während der ersten Lebenswochen ist auf den Streß zurückzuführen, der durch die Abwesenheit der Mutter und durch die Substitution des Kolostrums mit handelsüblicher Ersatzmilch hervorgerufen wird; außerdem sollte nicht der Unterschied zwischen intrauterinem und extrauterinem Milieu vergessen werden.

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