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Short- and Long-term Effects of Weaning on Adrenocortical and Functional Response of Lambs

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ABSTRACT

***Background*:** Weaning period is considered a crucial event in the management of ewes and lambs, resulting in a number of significant challenges. Although multiple stresses significantly affected adaptative capability of ewes in terms of changes in physiological mechanisms insufficient knowledges are available to understand the adrenocortical and functional responses of lambs to adapt to weaning stress. The main objective of the present study was to evaluate the effect of weaning associated with maternal separation on circulating cortisol concentrations, respiratory rate and rectal temperature changes after the first 24 h weaning period and 2 weeks later in cross-bred lambs.

***Materials, Methods & Results*:** Thirty-four cross-bred lambs were assigned randomly to two treatments: 17 (unstressed control group) were housed in standard farming conditions with their mothers and 17 lambs (treated group) were submitted to weaning and separation from maternal care. Both groups were studied and sampled 2 weeks before (weaning) in baseline conditions (T0), 24 h (T1) and 2 weeks (T2) after (weaning). In treated group lambs showed decreases of cortisol concentrations 24 h after ($P < 0.001$) and 2 weeks after ($P < 0.05$) weaning, compared to baseline values. Weaning effects were shown for cortisol changes ($P < 0.0001$) in treated lambs. The comparison between control and treated lambs showed lower cortisol concentration both 24 h after weaning ($P < 0.001$) and 2 weeks later ($P < 0.05$) in treated than control subjects. Treated lambs showed decreases of respiratory rate 24 h after ($P < 0.01$) and 2 weeks after ($P < 0.05$), compared to baseline values, with a significant weaning effects for RR changes ($P < 0.001$). No significant changes were observed for RT at different times in treated lambs. Negative and significant correlations were observed between RR and RT (r= -0.674; $P < 0.05$) for all measurement periods in control group.

***Discussion*:** Results obtained showed that the ability of the adrenocortical gland to modulate cortisol concentrations was different in control and treated groups. In fact, this ability remained unmodified in unstressed animals, but significantly decreased in weaned lambs. It was concluded that the presence of maternal care did not appear to influence cortisol concentrations, as confirmed by no significant cortisol changes observed at T0, T1 and T2 in unstressed control lambs. The general higher no significant RT values of control group than treated group would be in agreement with results observed in suckling lambs, with increased heat production, and could explain the existence of negative correlation between RR and RT only in control group. The decreases of cortisol concentrations after weaning were the evident effect of persistent stress suffered by lambs both early and late phases of weaning. These results showed that short- and long-term perturbations of the post-weaning has measurable effects on adrenocortical and functional response of weaned lambs. These findings suggest that circulating cortisol concentrations and RR values of lambs play an important role in providing additional information for comparative evaluation of weaning effects and related coping responses in small ruminants. Data obtained indicate that both the short- and long-term effects of weaning and maternal separation may be perceived as stressful and aversive stimuli.

Keywords: lamb, total cortisol, respiratory rate, rectal temperature, weaning.

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INTRODUCTION

Weaning period is a crucial event in the management of ewes and lambs, resulting in a number of significant challenges, including deprivation of maternal care, confinement, discontinuation of mother-young contact and the reluctance to eat new foods.

Numerous studies have investigated the physiological, emotional and behavioural effects of various acute or chronic, potentially alarming events, related to social context, shepherd knowledge, negative or gentling handling that can occur under different farming conditions in lambs [3-5,23]. The activity of HPA axis during isolation and restraint is reduced in lactating ewes, and the presence of lambs increases this level of attenuation [21]. Behaviour, cortisol and cardiac activity of lambs to controllable versus uncontrollable aversive events showed that an aversive situation is perceived as less stressful by sheep when they can exert control over it and this effect depends on the degree of control [10,11]. Scottish Blackface lambs showed elevated cortisol concentrations at the 1st day after weaning compared to those of the day before, at 60 min and at 24 h after weaning, but no at 48 and 72 h after weaning, compared to basal level at 0 min, with a return to pre-weaning level at 2 days after weaning in Kivircik lambs [9].

Aim of the present study was to evaluate the effect of weaning on circulating cortisol concentrations, respiratory rate and rectal temperature changes after the first 24 h weaning period and 2 weeks later in Comisana cross-bred lambs.

MATERIALS AND METHODS

Animals

Thirty-four Comisana cross-bred lambs (14 males and 20 females), 8 weeks old were studied. Lambs were assigned randomly to two treatments over a period of 24 days: 17 (unstressed control group) were housed in standard farming conditions with their mothers, 17 lambs (treated group) were submitted to weaning and separation from maternal care. Both groups were studied 2 weeks before (weaning) in baseline conditions (T0), and 24 h (T1) and 2 weeks (T2) after (weaning). From delivery to weaning treated lambs were maintained with their mothers and were fed on their ewe's milk. All treated lambs were weaned at the same time of 8 weeks, during late autumn. On the weaning day, occurred in October, the lambs were weaned at approximately 08.00 a.m.; they were separated from their ewes all at once and were housed in paddocks (30 m x 60 m) in presence of con-specifics. The ewes were drastically removed out of sight and hearing range of the lambs. At weaning the lambs received the same *ad libitum* pelleted alfalfa ration. They also had free access to native grass pasture or grass hay and water when outside. The environmental temperature and humidity were recorded with a Hygrothermograph ST-50[1]. The weather was mild but wet (temperatures and relative humidity fluctuating between 18.6-19.8°C, and between 33.5-34.5%, respectively. Sicilian farm was allocated at 37°33'56"N, and 14°16'30" E, respectively (Italy). The rectal temperature was measured with a clinical thermometer for 1 min. Respiration rates were obtained prior to the measurement of the rectal temperatures; this was accomplished by counting the flank movements for a 30 s. interval while the lambs were in a resting state. During the experimental period, individual live body weights (BW) were recorded using large animal scales, at the same times but after the blood samplings.

Hormone analysis

Samples of jugular blood were taken between 08:00 and 09:00 a.m. to minimize the effect of circadian rhythm on hormone measurements. In addition, blood samples were taken in baseline conditions 2 weeks before weaning (T0), and 24 h (T1) and 2 weeks (T2) after weaning, respectively. All samples were taken in quiet conditions by the same operator. Immediately after withdrawal, blood samples were refrigerated at 4°C and were subsequently (within 1 h) centrifuged for 15 min at 1,500 x *g*. Plasma was harvested and stored in polystyrene tubes at -20°C and assayed for cortisol analyses. Serum total cortisol concentrations were determined in duplicate with EIA Kits supplied by SEAC[2]. Intra- and inter-assay coefficients of variation were 4.6% and 6.9% respectively.

Statistical analysis

Data are presented as mean values ± sd of duplicate measurements. Statistical analysis was done by one way repeated measures analysis of variance (ANOVA). Significant differences between baseline (T0) and post-weaning values (T1-T2) were established using Bonferrroni's multiple comparison test. Further changes due to the gender of the lambs were assessed by Student's unpaired t-test. The level of significance was set at $P < 0.05$. All calculations were performed using the PRISM package (GraphPad Software Inc., San Diego, CA). The relation between different parameters was evaluated by correlation and linear regression.

RESULTS

Circulating total cortisol concentrations in lambs before and after weaning period were presented in Figure 1. Treated Group, before and after weaning treatment, showed decreases of cortisol concentrations 24 h after (62.38 ± 15.15; $P < 0.001$) and 2 weeks after (74.18 ± 23.67; $P < 0.05$) weaning, compared to baseline values (95.38 ± 20.83). Weaning effects were shown for cortisol changes ($P < 0.0001$) in treated lambs.

Unstressed control group (Figure 1) showed weak increases of cortisol concentrations at T1 (100.90 ± 16.10 nmol/L) and at T2 (90.90 ± 20.17 nmol/L), compared to baseline values at T0 (87.33 ± 20.00 nmol/L). No significant effects were shown for cortisol changes in control lambs.

The comparison between control and treated lambs showed lower cortisol concentration both 24 h after weaning ($P < 0.001$) and 2 weeks later ($P < 0.05$) in treated than control subjects.

Males and females of treated group (Figure 2) showed the typical trend of total cortisol concentrations observed in total subjects. Both males and females of treated group showed lower serum cortisol concentrations respectively 24 h ($P < 0.001$) and 2 weeks ($P < 0.05$) after weaning, as compared to baseline values. Compared to control male and female lambs, weaning males and females showed lower cortisol concentrations both 24 h ($P < 0.001$) and 2 weeks ($P < 0.05$) after weaning. Gender effects were shown for cortisol changes only for treated lambs ($P < 0.0001$). There were no cortisol differences between males and females with-in the single group ($P > 0.05$).

RR values in lambs before and after weaning period were presented in Figure 3. Treated group, before and after weaning treatment, showed decreases of RR values 24 h after (54 ± 13 btp/min; $P < 0.05$) and 2 weeks after (42 ± 12 btp/min; $P < 0.001$) weaning, compared to baseline values (58 ± 12 btp/min). Weaning effects were shown for RR changes ($P < 0.001$) in treated lambs.

Unstressed control group (Figure 3) showed RR values equal to 55 ± 15 btp/min at T0, to 54 ± 13 btp/min at T1 and 54 ± 12 btp/min at T2. No significant effects were shown for RR changes in control lambs. No significant differences were obtained between RR values of control and weaning lambs.

Males and females of treated group (Figure 4) showed the typical trend of total RR values observed in total subjects. Both males and females of treated group showed lower RR values 24 h (P 0.05 and $P < 0.05$), respectively, and 2 weeks ($P < 0.001$) after weaning, as compared to baseline values. Compared to control male and female lambs, weaning males and females showed higher RR values both 24 h ($P < 0.05$) and 2 weeks ($P < 0.001$) after weaning. Female treated lambs showed higher RR values at T0 in baseline conditions ($P < 0.05$) than males. Female control lambs showed higher RR values at T0, T1 and T2 ($P < 0.05$) than males. No gender effects were shown for RR changes of treated and control lambs at different experimental times ($P > 0.05$).

The average rectal temperatures was respectively 38.7°C (T0) and 39.1°C (T2) for control lambs, and 38.6°C (T1) and 38.9°C (T2) for treated lambs. The comparison between control and treated lambs did not shown significant differences between treated and control groups.

No significant differences were observed for RT between males and females of control and treated groups. No gender effects were shown for RR and RT changes of treated and control lambs at different experimental times ($P > 0.05$).

Respiration rate was negatively correlated with rectal temperature (r= -0.674; $P < 0.05$) for all measurement periods in control group.

Body weight was equal to 15.42 ± 0.24 in males and 14.91 ± 0.11 in females in baseline conditions; equal to 15.28 ± 0.11 in males and 13.94 ± 0.07 in females 24 h after weaning; equal to 16.04 ± 0.18 in males and 15.50 ± 0.11 in females 2 weeks after weaning.

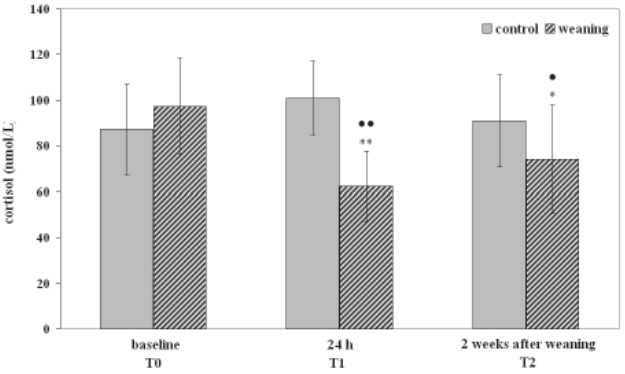

Figure 1. Mean (sd) circulating concentrations of total cortisol in control and weaning lambs. Asterisks indicate significant (*$P < 0.05$; **$P < 0.001$) differences in average hormone concentrations versus baseline values. Symbols indicate significant (•$P < 0.05$; ••$P < 0.001$) differences in average hormone concentrations versus control group.

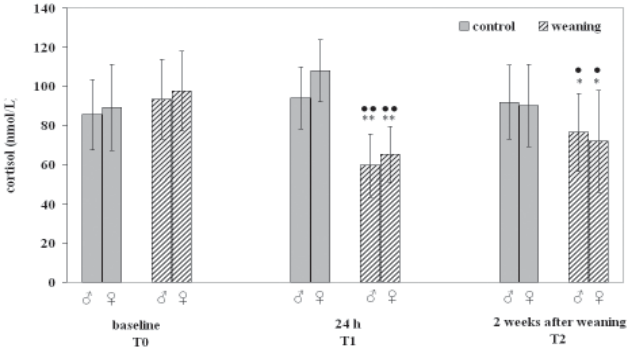

Figure 2. Mean (sd) circulating concentrations of total cortisol in control and weaning male and female lambs. Asterisks indicate significant (*$P <$ 0.05; **$P < 0.001$) differences in average hormone concentrations versus baseline values. Symbols indicate significant (•$P < 0.05$; ••$P < 0.001$) differences in average hormone concentrations versus control group.

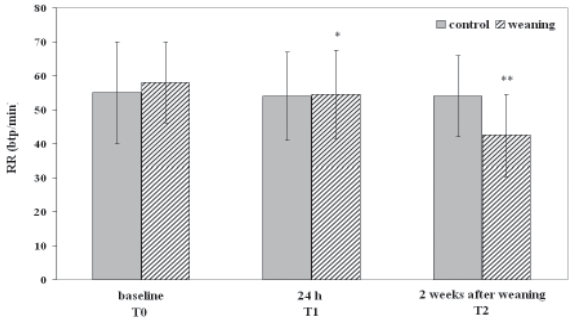

Figure 3. Mean (sd) respiratory rate (RR) values (btp/min) in control and weaning lambs. Asterisks indicate significant (*$P < 0.05$; **$P < 0.001$) differences in average hormone concentrations versus baseline values.

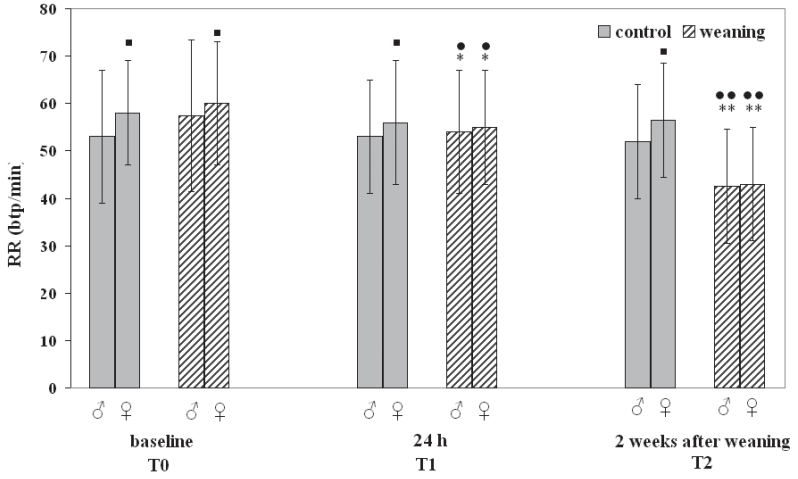

Figure 4. Mean (sd) respiratory rate (RR) values (btp/min) in control and weaning male and female lambs. Asterisks indicate significant (*$P < 0.05$; **$P < 0.001$) differences in average hormone concentrations versus baseline values. Symbols indicate significant (•$P < 0.05$; ••$P <$ 0.001) differences in average hormone concentrations versus control group. Symbol indicates significant (•$P < 0.05$) differences in average hormone concentrations versus males.

DISCUSSION

Sheep have been used as experimental subjects in such different fields of study as endocrinology, physiological function, thermoregulation, nutrition and animal production [15,17,18,20]. The study of the sheep fetus has also been extensive, and much of knowledge of fetal physiology derives from these researches. Although multiple stresses significantly affected coping capability of ewes and lambs in terms of changes in physiological mechanisms, many laboratories have established reliable reference values for cortisol concentrations in blood of healthy animals. In fact, many endogenous and exogenous factors that affect adrenocortical function may lead to misinterpretation of test results when values for individual subjects are compared with physiological ranges. Comparisons of our data with published references for growing lambs were in agreement with data previously reported in Kivircik lambs 8-week-old, stabled in paddock and in stalls [9].

Results obtained showed that the ability of the adrenocortical gland to modulate cortisol concentrations was different in control and treated groups. In fact, this ability remained unmodified in unstressed animals, but significantly decreased in weaned lambs. Thus, it suppose that the presence of maternal care did not appear to influence cortisol concentrations, as confirmed by no significant cortisol changes observed at T0, T1 and T2 in unstressed control lambs. In addition, lower cortisol concentration observed in treated than unstressed lambs confirmed previous data observed in Comisana lambs reared with their mothers but suckling denied by covering udders, compared to

lambs separated from their mothers [14], and in lambs housed in standard farming conditions, compared to treated group exposed daily to various aversive events [8]. Nevertheless, cortisol decreases of weaned lambs did no confirm previous data [16], which showed that plasmatic cortisol levels in lambs were not modified by the two different weaning methods (total and partial separation). In addition, neither higher plasma concentration at the 1st day after weaning compared to day before weaning in lambs nor the increase in plasma cortisol concentration at 60 min and at 24 h after weaning compared to basal values were in agreement with our results.

However, some discrepancies might be ascribed to differences in methods and are the subject of speculation; in addition, some differences may also possibly be explained by physiologic (lambs from different genetic groups and phenotypic traits, domesticated and wild phenotype) and/or environmental modifications (different climatic conditions), related to different experimental conditions (suckling length, rearing single or twin lambs, etc.).

One hypothesis is that the lowest cortisol concentrations found at 24 h and 2 weeks after weaning could suggest adrenocortical depletion arising from short- and long-term weaning stress, respectively. These findings could probably assume that a daily exposure for 2 weeks to various unpredictable and uncontrollable aversive events related to weaning and separation from maternal care induced a concomitant decrease of cortisol concentrations, pointing to a chronic stress state.

Though, it is well known that psychological and physiological stressors can alter the acute pain response to castration and tail docking in lambs [6], with sex differences in the cortisol responses, suggesting that the mechanisms for the sex difference in response to tail docking may involve the adrenal glands [22], in our experimental design no sex differences were observed in cortisol response to weaning stress.

The animals used in this study had generally a placid nature and relate positively to handlers, possibly a result of their adaptation to domestication and regular daily management which occurred over many generations. On the basis of well known glucocorticoid's metabolic effects, the lowest cortisol concentration after weaning may be beneficial to exposed lambs to aversive stimuli, thereby reducing the detrimental impact of weaning stress on hearth, welfare and immune function of lambs. Thus, it conclude that decreased cortisol concentrations may be used as a sign of chronic stress in farm animals, with their involvement in early and late weaning periods.

The higher RR values observed in females than males of treated and control groups were in agreement with data obtained in male and female lambs the fourth post-natal week [19].

The absence of significant RT differences between control and treated lambs, and between males and females, probably represented the time at which the thermal regulatory mechanism of healthy young lambs was effectively capable of maintaining a body temperature within homeothermic physiological limits. The general higher no significant RT values of control group than treated group would be in agreement with results observed in suckling lambs, with increased heat production, and could explain the existence of negative correlation between RR and RT only in control group; in addition, the highest RT observed in males confirmed previous data observed in lambs 4 weeks old [19].

The influences of circadian rhythms on cortisol concentrations and functional variables were not investigated because single measurements of these parameters performed between 08.00 and 09.00 a.m. are insufficient and inadequate to evaluate them, even if many endogenous and exogenous factors, environmental conditions and physiological variables can affect or modify the course and amplitude of rhythms.

These findings suggest that circulating cortisol concentrations and RR values of lambs play an important role in providing additional information for comparative evaluation of weaning effects and related coping responses in small ruminants.

Data obtained indicate that both the short- and long-term effects of weaning and maternal separation may be perceived as stressful and aversive stimuli by lambs.

CONCLUSIONS

In conclusion, data obtained confirm that animals vary in their sensitivity and mode of response to external stimuli and show that differences of adrenal function exist between treated and control groups, as reported in previous studies related to reference values for physiological responses to psychological [1,2,7] and physical [6,12,13] stressors.

Hence, the decreases of cortisol concentrations after weaning were the evident effect of persistent stress suffered by lambs both early and late phases of weaning. These results showed that short- and long-term perturbations of the post-weaning has measurable effects on adrenocortical and functional response of weaned lambs.

Further research is needed to elucidate the interaction of endocrine and functional changes after weaning and maternal separation and their relationship to overall animal welfare and future productive performance.

These findings indicate that weaning represents a severe stress in lambs, and the presence of conspecifics probably does not enough reduce psychological stress within 2 weeks. Hence, the adrenocortical responses may, therefore, be either the physiological consequence of active resistance to post-weaning stress.

SOURCES AND MANUFACTURERS

[1]Sekonic Corporation, Tokyo, Japan.

[2]SEAC, Florence, Italy.

Ethical approval. All procedures, treatments and animal care were in compliance with the guidelines of the Italian minister of health for the care and use of animals (D.L.27/171992 n.116) and UE (Directive 86/609/CEE).

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

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