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Short Communication: Study of Immune Parameters in Three Greek Dairy Sheep Breeds During the Periparturient Period

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ABSTRACT

The objective of the present study was to evaluate whether immunosuppression occurs in 3 different Greek dairy sheep breeds during the periparturient period. A total of 33 ewes from 3 breeds [i.e., the low-producing Boutsiko breed (n = 11), which is highly adaptable to harsh environments; the high-producing but environmentally fragile Chios breed (n = 11); and an intermediate synthetic breed (50% Boutsiko, 25% Arta, and 25% Chios, n = 11)] were used. Blood samples were collected at 18 and 2 d before parturition and at 15 d after parturition. Total cell-associated and membrane-bound urokinase plasminogen activator (U-PA) activity, free U-PA binding sites on cellular membranes, and superoxide anion (SA) production by activated phagocytes were determined. Results indicated that all immune parameters measured remained constant during the periparturient period for the Boutsiko breed. In contrast, there were reductions in total cell-associated and membrane-bound U-PA activity by both monocytes-macrophages and neutrophils and in SA production by monocytes-macrophages at d 2 before parturition for the Chios breed. In the synthetic breed, there were reductions in total cell-associated and membrane-bound U-PA activity by monocytes-macrophages and in SA production by both monocytes-macrophages and neutrophils at d 15 after parturition. Thus, mild immunosuppression during the periparturient period was observed in the 2 breeds with the highest milk production.
Key words: periparturient period, urokinase plasminogen activator, superoxide anion, ovine

Sheep farming is a very important animal production activity in Greece, contributing to approximately 40% of the sector's gross income (Hadjigeorgiou et al., 2002). Two typical indigenous breeds and a synthetic breed were used in this study. The Chios breed is characterized by high milk yield and litter size. On the other hand, the Boutsiko breed is characterized by low milk yield, but it is known for its adaptability under harsh environmental conditions. Furthermore, the Boutsiko breed is less susceptible to mastitis (Hatziminaoglou et al., 1990; Simos et al., 1996; Kominakis et al., 1998; Ploumi et al., 1998). The synthetic breed (50% Boutsiko, 25% Arta, and 25% Chios) has been developed recently in an attempt to upgrade the Boutsiko breed by combining the high productivity of the Chios and Arta breeds with the robustness of the Boutsiko breed.

Several studies have demonstrated a decline in immune function during the periparturient period in dairy cows, but the causes of immunosuppression are not completely understood. It is certain, however, that immunosuppression has been linked to endocrine changes associated with parturition, metabolic stresses associated with lactogenesis, and availability of critical nutrients, including vitamin E and calcium (Kehrli and Goff, 1989; Goff and Horst, 1997; Smith et al., 1997; Kimura et al., 1999). To our knowledge, only one study has dealt partly with immunosuppression during the periparturient period in dairy sheep. Caroprese et al. (2006) reported that cell-mediated and humoral immune responses and plasma IL-6 concentrations underwent marked fluctuations in periparturient ewes. There is a major unfulfilled need for more studies on the suppression of the immune system in periparturient dairy sheep.

Phagocytes (monocytes-macrophages and neutrophils) are critical for a proper immune response, because these cells are capable of migrating from blood circulation to any infected or injured tissue to confront pathogens (Politis et al., 2002). The rapidity of the migratory response depends on the ability of phagocytic cells to overcome the mechanical barriers imposed by the basement membranes. To achieve this objective, monocytes-macrophages and neutrophils use limited proteolytic activity at the regions of contact with the endothelial cells. Ovine monocytes-macrophages and neutrophils express a specific urokinase-plasminogen activator (**U-PA**) receptor (CD 87; Politis et al., 2002). The U-PA can bind to this receptor and convert the abundant proteolytically inactive proenzyme plasmino-

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Table 1. Least squares means (±SEM) of total cell-associated urokinase plasminogen activator (U-PA) activity of macrophages and neutrophils for the Boutsiko breed (B), the synthetic breed (S; 50% Boutsiko, 25% Arta, and 25% Chios), and the Chios breed (C)

Item	Days from parturition	U-PA, ΔA/h per 10^6 cells			Probability within rows		
		B	S	C	B-S	B-C	S-C
Monocytes-macrophages	−18	$0.32^{a} \pm 0.01$	$0.32^{a} \pm 0.01$	$0.27^{a} \pm 0.01$	NS[1]	NS	NS
	−2	$0.31^{a} \pm 0.01$	$0.30^{ab} \pm 0.01$	$0.22^{b} \pm 0.01$	NS	<0.01	<0.01
	+15	$0.32^{a} \pm 0.01$	$0.27^{b} \pm 0.01$	$0.28^{a} \pm 0.01$	NS	NS	NS
Neutrophils	−18	$0.64^{a} \pm 0.03$	$0.64^{a} \pm 0.03$	$0.51^{a} \pm 0.03$	NS	NS	NS
	−2	$0.62^{a} \pm 0.03$	$0.62^{a} \pm 0.03$	$0.39^{b} \pm 0.03$	NS	<0.001	<0.001
	+15	$0.61^{a} \pm 0.03$	$0.62^{a} \pm 0.03$	$0.52^{a} \pm 0.03$	NS	NS	NS

[a,b]Means within the same column and item followed by different letters differ at $P < 0.01$.

[1]NS = nonsignificant ($P > 0.05$).

gen to active plasmin. Plasmin is capable of degrading certain matrix components, in addition to activating other matrix-degrading enzymes such as metalloproteinases.

The objective of the present study was to determine changes in the dynamics of the U-PA system as well as in the production of superoxide anion (**SA**) by monocytes-macrophages and neutrophils in 3 Greek dairy sheep breeds during the periparturient period.

A total of 33 ewes were used in this study; 11 from the Boutsiko breed, 11 from the synthetic breed (50% Boutsiko, 25% Arta, and 25% Chios), and 11 from the Chios breed. Animals were housed within the premises of the experimental farm of the Agricultural University of Athens. The ewes lambed between November 10, 2005, and January 10, 2006. Furthermore, all ewes lambed within ±1 d of anticipated lambing. All ewes that lambed outside this period were excluded from the experiment.

Blood samples were collected from all ewes on 18 and 2 d before parturition and on d 15 after parturition. Monocytes-macrophages and neutrophils were isolated as described by Politis et al. (2002). Total cell-associated, membrane-bound U-PA activity and U-PA binding sites in activated monocytes-macrophages and neutrophils were determined by using the methods described by Politis et al. (2002). Activation of phagocytes is achieved following incubation with 81 μM phorbol myristate acetate for 30 min at 37°C. Superoxide anion production by phagocytes, as a direct indicator of respiratory burst activation, was measured by using the superoxide dismutase-inhibitable reduction of ferricytochrome C. All details are as described by Politis et al. (1995).

Significant departures from normal distribution for all variables and possible outliers were initially assessed by using quantile-quartile plots. Calculation of the extreme Studentized deviates for all variables was performed according to Grubbs (1969), and outliers were excluded at a 2-tailed probability of 0.05. Logarithmic transformation for SA production and for free U-PA binding sites (by adding the value of 1 because of negative values) was then used in an attempt to meet ANOVA assumptions (i.e., normal distribution and homogeneity of variance within class effects). Transformed values of variables were then analyzed by using a linear mixed model appropriate for repeated measurements per subject (animal) with the compound symmetry for modeling the error covariance structure. The fixed effects part of the model included the effects of breed (Chios, synthetic, and Boutsiko), parturition stage (3 stages: 18 d before, 2 d before, and 15 d after parturition), and the breed by parturition stage interaction, whereas the effect of animal nested within breed was fitted as a random effect. The Satterthwaite method (Steel and Torrie, 1980) was used for computing the denominator degrees of freedom for the tests of fixed effects. The Tukey-Kramer adjustment was used for the *P*-values when performing multiple comparisons. Results of mixed ANOVA are presented as least squares means with standard errors. All mixed ANOVA analyses were carried out by PROC MIXED in SAS, version 9.0 (SAS Institute, 2004).

Total and membrane bound U-PA activities in monocytes-macrophages and neutrophils during the periparturient period for the 3 Greek dairy sheep breeds are presented in Tables 1 and 2, respectively. Total and membrane-bound U-PA activities remained constant during the periparturient period for the Boutsiko breed for both monocytes-macrophages and neutrophils. In contrast, there was a decline in total and membrane-bound U-PA activities of both monocytes-macrophages and neutrophils at d 2 prior to parturition in the Chios breed. In the synthetic breed, the lowest values for total and membrane-bound U-PA activities of monocytes-macrophages were observed at d 15 after parturition, and the highest were observed at d 18 prior to parturition. There were no significant differences in total and