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Effect of acute stressors, adrenocorticotropic hormone administration, and cortisol release on milk yield, the expression of key genes, proliferation, and apoptosis in goat mammary epithelial cells

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ABSTRACT

Cortisol is essential to milk synthesis; however, different acute stressors and the exogenous administration of adrenocorticotropic hormone (ACTH) decrease milk yield. Therefore, the effect of cortisol on milk yield and its influence on the survival of mammary epithelial cells have not been fully elucidated. In this context, the objective of this study was to evaluate the effect of cortisol on the expression of growth hormone receptor (*GHR*), insulin-like growth factor type 1 (*IGF1*), insulin-like growth factor type 1 receptor (*IGF1R*), insulin-like growth factor-binding protein 3 and 5 (*IGFBP3* and *IGFBP5*), *BAX*, and *BCL2* genes on the proliferation and apoptotic rates of mammary epithelial cells, and on milk yield in Saanen goats. In the present study, 3 experiments were conducted: (1) comparing the in vivo effects of first milking, vaccination, vermifugation, preventive hoof trimming, and the administration of ACTH or a placebo on cortisol release in dairy goats; (2) studying the in vivo effects of immediate increases in cortisol on the mammary gland of lactating goats; and (3) studying the in vitro effects of a prolonged increase in cortisol on mammary epithelial cells obtained from lactating goats. Cortisol release by goats increased significantly after ACTH administration compared with that observed after a placebo, and the cortisol profiles after first milking, vaccination, vermifugation, hoof trimming, and ACTH administration were similar. However, there was no effect of the immediate increase in cortisol in vivo on IGF-1 release, milk yield, milk quality, or the apoptosis and proliferation rates, nor was there any effect on the expression of the target genes. Furthermore, no interaction was observed between IGF-1 and cortisol in either the in vivo or in vitro experiments. However, the addition of cortisol in vitro significantly increased the expression of the *GHR* and *IGF1R* genes, which stimulate cell proliferation, and the *BAX* gene, which causes apoptosis. These contrasting results can explain why cortisol did not change the rates of proliferation or apoptosis in epithelial cells. Indeed, cortisol supplementation in vitro did not change the number or apoptotic rate of epithelial cells over the course of 5 d. Finally, further studies must be performed to understand the effect of cortisol on the expression of the *GHR*, *IGF1R*, and *BAX* genes by epithelial cells and the roles of these genes in milk synthesis during early lactation.

Key words: milk yield, mammary gland, cortisol, cell apoptosis

INTRODUCTION

Cortisol is essential for copious milk synthesis (Neville et al., 2002; Casey and Plaut, 2007). However, an increase in hypothalamic-pituitary-adrenocortical activity is a characteristic of the stress response (Mormede et al., 2007; Negrao, 2008; Brown and Vosloo, 2017). Furthermore, exogenous glucocorticoids and ACTH administration decrease the plasma concentration of IGF-1 and milk yield in cows (Maciel et al., 2001; Ollier et al., 2016; Ponchon et al., 2017). In fact, administration of a large amount of glucocorticoid over a long period disrupts the growth hormone (**GH**)/IGF-1 axis, resulting in inhibition of the action of GH and IGF-I (Lembessis et al., 2004; David et al., 2011; Feng et al., 2013). Indeed, GH and IGF-1, IGFBP-3, and IGFBP-5 also influence the expression of *BCL2* (anti-apoptotic) and *BAX* (pro-apoptotic) genes and have important roles in the regulation of epithelial cell survival (Plath-Gabler et al., 2001; Sakamoto et al., 2007; Flint et al., 2008). Therefore, the effect of cortisol on milk yield and its influence on the survival of mammary epithelial cells have not been fully elucidated.

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At the same time, farm management practices such as vaccination, vermifugation, first milking, weaning, hoof trimming, and transport may cause acute stress, cortisol release, and decreased milk yield (Sevi et al., 2001a,b; Canaes et al., 2009; Caroprese et al., 2010). Usually, stress is negatively associated with milk yield, but it is important to understand the relationship between cortisol release and milk yield, because cortisol is necessary to maintain homeostasis and promote animal adaptation (Negrao and Marnet, 2003; Trevisi and Bertoni, 2009; Brown and Vosloo, 2017). In this context, stress and cortisol release are associated with the expression of proteins that regulate cell survival, including BCL2, which prevents apoptosis, and BAX, which initiates the cascade that promotes apoptosis in mammary epithelial cells (Green and Streuli, 2004; Portt et al., 2011; Tao et al., 2015). These contrasting findings concerning the physiological responses related to lactation and stress show that the effects of cortisol on milk synthesis and the survival of epithelial cells remain controversial.

Although exogenous ACTH and glucocorticoid administration has been shown to decrease milk yield in cows (van der Kolk, 1990; Ollier et al., 2016; Ponchon et al., 2017) and ewes (Caroprese et al., 2010), and increase the SCC in milk in dairy ewes (Sevi et al., 2001a,b; Caroprese et al., 2010), the effects of ACTH and glucocorticoid administration on milk yield in goats remains ambiguous. Several authors have reported the absence of a negative effect of administering ACTH and glucocorticoid on the milk yield of dairy goats (Stewart and Thompson, 1984; Anderson et al., 1991; Shamay et al., 2000). In this context, the objective of this study was to evaluate the effect of cortisol on the expression of the *IGF1*, *IGF1R*, *GHR*, *IGFBP3*, *IGFBP5*, *BAX*, and *BCL2* genes, the rates of proliferation and apoptosis of mammary epithelial cells, milk quality, and milk yield in Saanen goats.

MATERIALS AND METHODS

All the animal procedures complied with the ethical code of the Faculty of Animal Science and Food Engineering of the University of São Paulo, Faculty of Animal Science and Food Engineering (**FZEA**) of the University of São Paulo (**USP**). The experiment was carried out at the Laboratory of Animal Physiology of the Basic Sciences Department in the Faculty of Animal Science and Food Engineering (FZEA/USP) in Pirassununga, Brazil (at latitude 21° 57′ 02″ S, longitude 47° 27′ 50″ W). The climate in this area is subtropical, with an average annual temperature of 23°C and humidity of 73%, and a rainy season from November to March (with annual rainfall varying from 1,300 to 2,000 mm), and the experimental collections were carried out between June and September.

Housing, Diets, and Milking Routine

All experimental goats were distributed into covered, collective pens with feeding troughs, mineral salt, and water. The feed contained 60% concentrate and 40% roughage, providing 100% of the animals' requirements (NRC, 2007). Milking was conducted twice daily by the same team throughout the experimental period. The milking machine was regulated to maintain a vacuum level of 48 kPa and a pulse rate of 120 cycles/min. The milking routine was performed in accordance with the following protocol: before milking, the animals were attached to a milking machine, and their teats were predipped and dried. The teat cups were attached at time 0 and detached at the end of milking. Afterward, their teats were postdipped, the individual milk yield was recorded, and the animal exited the milking parlor.

Organization of Experiments

In the present study, 3 experiments were conducted: (1) comparing in vivo the effects of milking, vaccination, vermifugation, hoof trimming, and the administration of ACTH or a placebo on cortisol release in dairy goats; (2) studying in vivo the effects of immediate increases in cortisol on the mammary gland of dairy goats; and (3) studying in vitro the effects of a prolonged increase in cortisol levels on the epithelial cells of dairy goats.

Experiment 1 (Acute Stress and ACTH Administration)

Twenty-four healthy, homogeneous Saanen goats (mean ± SD; BW of 55.6 ± 1.3 kg, BCS of 3.5 ± 1.0, lactating and not pregnant) were used to conduct this experiment. Experimental goats were randomly distributed according to the previous number of parturitions, milk yield, BW, and BCS. These 24 goats were subjected to 6 treatments: first milking, vaccination, vermifugation, preventive hoof trimming, and placebo (saline solution) or ACTH administration. The ACTH dose tested (0.6 UI of ACTH/kg of BW per animal) was based on previous studies that demonstrated that cortisol returned to basal levels just 6 h after ACTH administration (Fulkerson and Jamieson, 1982; Alam et al., 1986; Schwinn et al., 2016). This cortisol profile was considered similar to other acute stressors related to farm management (Fulkerson and Jamieson, 1982; Negrao et al., 2004; Negrao, 2008).