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Effects of castration on IFN-γ and TNF-α expression in the adrenal gland of Angora goat

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ABSTRACT

In the adrenal gland, there is a mutual interaction between the cells in the cortex and medulla and the cells of the immune system. Sex hormones regulate immune cell functions as well as reproductive and metabolic processes throughout life. Interferon-gamma (IFN-γ) is a cytokine that regulates the maturation and differentiation of many cell types. Tumor necrosis factor-alpha (TNF-α) is a multifunctional cytokine that plays an important role in various physiological and pathophysiological processes and orchestrates cytokines. This study was conducted to investigate the effects of castration on the expression of IFN-γ and TNF-α in the adrenal gland of Angora goat. A total of 16 Ankara goat kids were used as material for the study. Healthy animals were selected for the study by clinical examination. At an average age of 75 days, eight animals were randomly selected from the institute herd and castrated. 85 days after castration (after approximately 160 days old), eight control and eight castrated Angora goats were slaughtered and their adrenal glands were removed. In the analysis of each group, IFN-γ expression was found in the cytoplasm of cells in the adrenal cortex and medulla. The adrenal glands of the castrated group showed a decrease in IFN-γ expression in the zona fasciculata. A distinct brown staining was seen in the cytoplasm of TNF-α-positive responsive secretory cells. TNF-α expression was found to be increased in the zona glomerulosa, zona fasciculata and zona reticularis of the adrenal gland in the castrated group. Accordingly, androgen hormone deficiency was able to increase TNF-α expression, whereas it did not significantly affect IFN-γ expression in the adrenal tissue of the Angora goat.

Keywords: adrenal gland, Angora goat, castration, interferon-gamma, TNF-α

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Introduction

The adrenal glands are embryologically originating in two different regions: the mesodermal cortex and the ectodermal origin medulla (Kerr, 2010). The adrenal cortex consists of three histological zones, which are named according to the arrangement of the secretory cells zona glomerulosa, zona fasciculata, and zona reticularis. These regions secrete hormones such as steroid hormones, glucocorticoids, and mineralocorticoids (Young et al., 2006). The medulla comprises chromaffin cells, which are large epithelioid

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cells, connective tissue, sinusoid blood capillaries, and nerves (Ross and Pawlina, 2016). There is a mutual interaction between the cells in the cortex and medulla and the immune system's cells in the adrenal gland (Kanczkowski et al., 2016).

There is an interaction between the immune system and the endocrine system. This interaction involves both the inhibitory and stimulatory effects of hormones on the immune system (Imura and Fukata, 1994). Cytokines and other mediators play a role in the interaction between these two systems (Blalock, 1994). Tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) are known to induce endocrine and metabolic effects in addition to their effects on the immune system (De Metz et al., 1999).

IFN-γ is a cytokine that controls the maturation and differentiation of various cell types (Schroder et al., 2004). One of the main physiological activities of IFN-γ is its ability to upregulate the expression of MHC class I and II proteins in various cell types during the formation of immune responses (Teixeira et al., 2005). IFN-γ has been shown to increase natural killer cell activity (Mann-Chandler et al., 2005), regulate T cell activation and differentiation and control B cell functions such as immunoglobulin formation (Schroder et al., 2004). In addition to modulating immunological factors, IFN-γ can perform metabolic reprogramming of immune cells (Wang et al., 2018).

TNF-α is a pleiotropic cytokine produced by various cell types (Aggarwal, 2003). It is known to be released as preliminary mediators that activate neutrophils, stimulate other effector cells, and increase chemokine synthesis (Metcalfe et al., 2009). Furthermore, TNF-α is a multifunctional cytokine that plays an important role in various physiological and pathophysiological processes and orchestrating cytokines in a variety of inflammatory conditions (Parameswaran and Patial, 2010).

Throughout life, sex hormones influence immune cell functioning as well as reproductive and metabolic processes (Gilliver, 2010). Steroid hormones like estrogen, progesterone, and glucocorticoids may influence innate and adaptive immunity (Klein and Flanagan, 2016). There is a close relationship between the hormone and cell apoptosis. Androgens are known to stimulate cell proliferation and differentiation (Isaacs, 1984) Furthermore, studies have shown that castration reduces cell proliferation and promotes apoptosis (Hussain et al., 2012).

The aim of the present study was to investigate the effects of castration on the expression of IFN-γ and TNF-α in the adrenal gland of the Angora goat.

Materials and Method

Animal Material: The research was carried out within the ethical committee decision scope taken from the Livestock Central Research Institute (27.06.2013/82). A total of 16 Angora goat kids were used as materials in the study. Healthy animals were selected for the study by clinical examination. At an average age of 75 days, eight heads were randomly selected from the institute herd and castrated with burdizo forceps. Before castration, 0.02mg/ml local anesthesia was applied percutaneously into the funiculus spermaticus. 85 days after castration (after approximately 160 days old), eight control and eight castrated Angora goats were slaughtered and their adrenal glands were removed.

The adrenal glands of the Agrora goat were fixed in 10% formaldehyde solution for histological examination. Following this, they were blocked in paraffin after undergoing routine tissue processing procedures.

Immunohistochemical staining: The presence of IFN-γ and TNF-α was demonstrated in 5 µm thickness adrenal gland sections from paraffin blocks using the streptavidin-biotin complex method (True, 1990). Rabbit monoclonal IFN-γ (1/500 dilution, Shanghai YL Biotech, YID2791) and rabbit polyclonal TNF-α (1/200 dilution, Abcam, AB-9739) primary antibodies were used for immunohistochemical staining. Histostain Plus (Zymed kit: 85-6743) kit was used as a secondary antibody. Serial sections were dewaxed in xylene and hydrated through graded alcohols. Endogenous peroxidase activity was blocked with H2O2 3% in absolute methanol for 15 min. The sections were rinsed with phosphate buffered saline (PBS, pH 7.2) and subsequently heated in citrate buffer (pH 6) in a microwave oven (700 W) for 10 min for antigen retrieval. After washing with phosphate buffer solution (PBS), sections were incubated with primary antibody at +4 ^{0}C for one night. After brief rinsing, the biotinylated secondary antibody was applied to the sections and incubated in the streptavidin-horseradish peroxidase complex. 3,3' diamino-benzidine (DAB) was used as chromogen and sections were counterstained with haematoxylin for 1 min, rinsed with tap water, and mounted with entellan mounting medium. Primary antibodies were omitted from negative control sections, which were incubated with PBS.

Immunoreactivity density:

After immunohistochemical staining, IFN-γ and TNF-α staining intensity in each cell was analyzed as follows: 0, no immunostaining detected; +/-, weak positive staining present; +, moderate positive staining

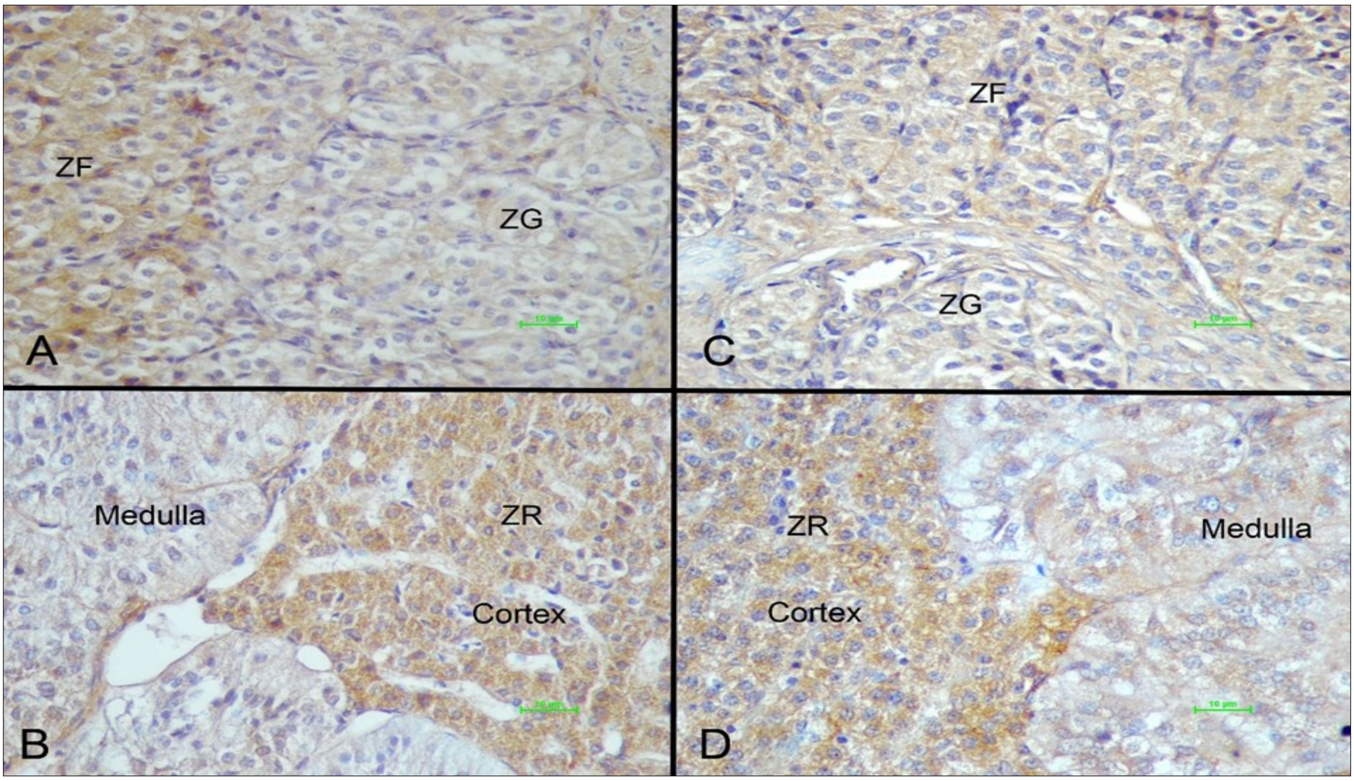

Figure 1. A/B: Control group adrenal gland; C/D: Castration group adrenal gland; Zona Glomerulosa (ZG), Zona Fasciculata (ZF), Zona Reticularis (ZR); expression of IFN-γ, original magnification x40; range bar, 10 µm.

present; ++, strong positive staining present (Platt and Hunt, 1998).

Results

IFN-γ immunohistochemistry: In the analysis of each group, IFN-γ expression was found in the cytoplasm of secretory cells in the adrenal cortex and medulla (Figure 1). There was no significant difference in staining between the two groups in the zona glomerulosa, zona reticularis, and medulla. The zona glomerulosa and medulla were weakly stained, while the zona reticularis was moderately stained. The adrenal glands of the castrated group showed a decrease in IFN-γ expression in the zona fasciculata (Table 1).

Table 1. Immunoreactivity of IFN-γ in histological zones of adrenal glands; +/-, weak positive staining present; +, moderate positive staining present; ++, strong positive staining present.

	Control group	Castration group
Zona Glomerulosa	+/-	+/-
Zona Fasciculata	+	+/-
Zona Reticularis	+	+
Medulla	+/-	+/-

TNF-α immunohistochemistry: A distinct brown staining was seen in the cytoplasm of TNF-α-positive responsive secretory cells (Figure 2). There was a strong expression of TNF-α in the medulla of both groups. TNF-α expression was found to be increased in the zona glomerulosa, zona fasciculata and zona reticularis of the adrenal gland in the castrated group (Table 2).

Discussion

Many pathways connect the immune system with the endocrine and neural systems and integrate the functions of the adrenal glands, gonads, and autonomic nervous systems (González-Díaz et al., 2017). The interaction between the endocrine and immune systems maintains the immune system to function more efficiently (Ortona et al., 2016). TNF-α is known to induce the secretory response of the adrenal cortex by stimulating hypothalamic-pituitary corticotropin-releasing hormone (Judd, 1998). TNF-α contributes to the interaction of the immune system and spermatogenesis by playing a role in communication between Sertoli and germ cells (Miao et al., 2001). IFN-γ is a multifunctional cytokine that acts as an antiviral, anticancer, and immunomodulator. Furthermore, it is critical in the coordination of both innate and adaptive immune responses (Mendoza et al., 2019). IFN-γ activates the immune response and promotes the elimination of pathogens in an inflammatory environment; it also prevents immune system overactivation and tissue damage (Zhang, 2007).

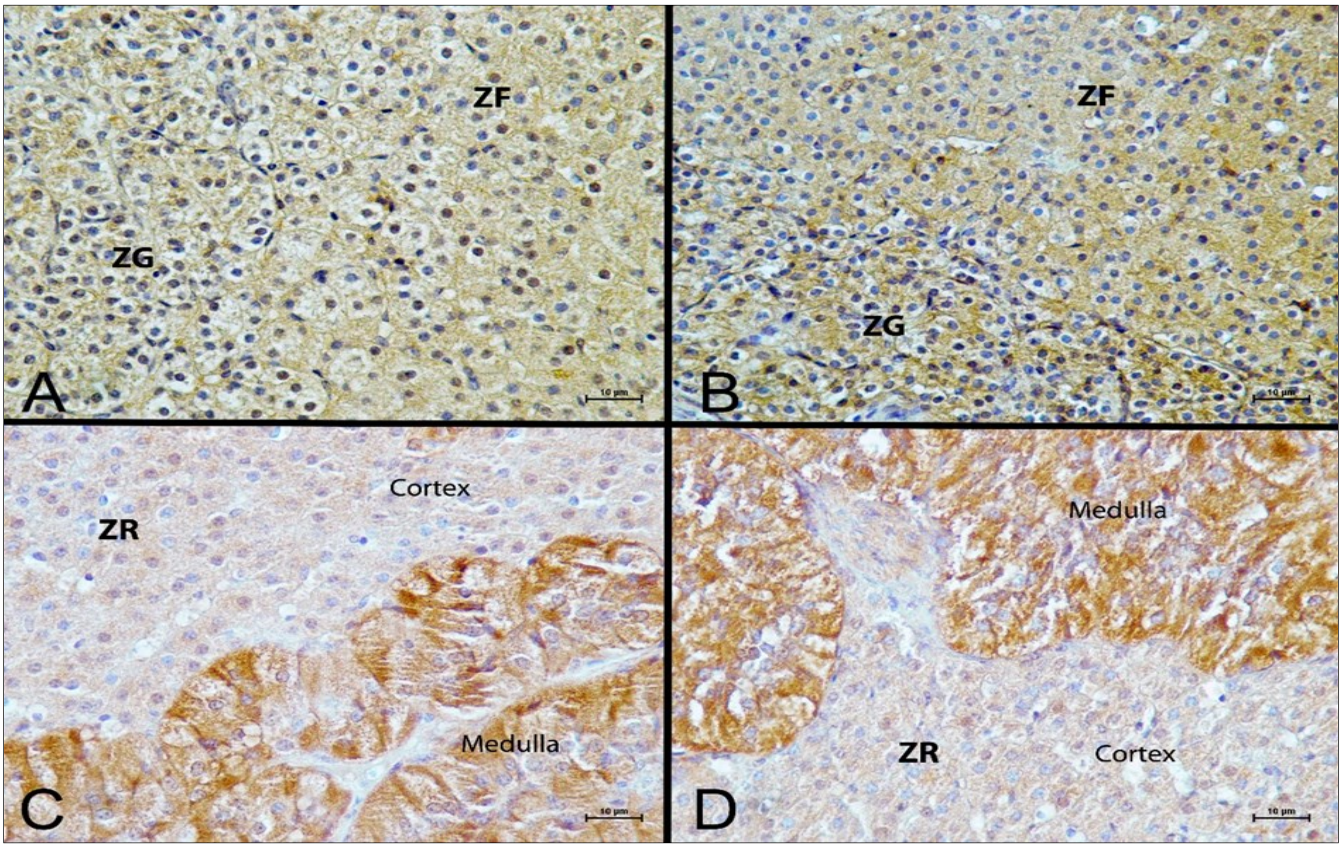

Figure 2. A/C: Control group adrenal gland; B/D: Castration group adrenal gland; Zona Glomerulosa (ZG), Zona Fasciculata (ZF), Zona Reticularis (ZR); expression of TNF-α, original magnification x40; range bar, 10 µm.

Table 2. Immunoreactivity of TNF-α in histological zones of adrenal glands; +/-, weak positive staining present; +, moderate positive staining present; ++, strong positive staining present.

	Control group	Castration group
Zona Glomerulosa	+	++
Zona Fasciculata	+	++
Zona Reticularis	+/-	+
Medulla	++	++

Glucocorticoid hormones are one of the most important anti-inflammatory hormones in the body, and this hormone can also regulate immunity (Bereshchenko et al., 2018). Sex hormones influence both the innate and adaptive immune systems. These hormones act as a transcriptional complex when they bind to their receptors inside cells (Beato and Klug, 2000). Testosterone may modulate the secretion of cytokines from adipose tissue and immune cells to achieve its anti-inflammatory effects (Mohamad et al., 2018). Adrenal cells in the cortex and medulla have been reported to stain intracytoplasmically with IFN-γ (Li et al. 2007). The effects of castration-induced stress on IFN-γ levels have been studied in castrated calves (Fisher et al., 1997) and cattle (Ting et al., 2003). These studies reported no significant difference in IFN-γ levels between castrated animals and the control group. In our study, we observed no significant association between castration and IFN-γ cytokine in the secretory cells of the zona glomerulosa, zona reticularis, or medulla, which is consistent with previous studies. Only the zona fasciculata region of the castrated group showed a decrease in IFN-γ expression. We concluded that the expression of IFN-γ in the adrenal gland of the Ankara goat might be partially affected by castration.

TNF-α has different effects on the adrenal gland depending on the species or developmental stage (Van der Meer et al., 1996). TNF-α is a potent regulator of steroidogenesis and apoptosis in adrenocortical cells (Mikhaylova et al., 2007) and an effective promoter of cell viability (Liu et al., 2004). According to studies, cytokines such as IL-6 and TNF-α influence stress hormone release via direct effects on adrenal cells (Seckl, 2004). TNF-α may act as an important link between the endocrine and immune systems by inhibiting the stimulatory effect on adrenal cells and reducing the synthesis of steroid hormones (Jaattela et al., 1990). Different regulators of adrenal steroids regulate TNF-α expression in different ways. Therefore, it is likely that the cytokine pattern of the adrenal gland changes depending on the physiological situation (Judd et al., 2000). Experimental studies report that TNF-α can inhibit testosterone secretion by modulating the hypothalamic-pituitary-gonadal axis (Norata et al., 2006). TNF-α has been reported to be expressed by the secretory cells of the zona glomerulosa, zona fasciculata, and zona reticularis in the bovine adrenal gland (Call et al., 2000). TNF-α expression in the prostate of castrated rats is increased according to a study in rats (Jia et al., 2015). Moreover, studies indicated that ovariohysterectomy and estrogen deprivation increased TNF-α expression in response to inflammatory signals (Raehtz et al., 2017). In our study, we observed that TNF-α expression increased in the secretory cells of the zona glomerulosa, zona fasciculata, and zona reticularis in the castrated group. The results of our study on TNF-α expression were similar to those of the previous studies. Taken together, based on the results of this study, we can say that castration increases TNF-α expression in a specific region of the adrenal gland of the Angora goat. Furthermore, we can postulate that castration increases TNF-α expression in secretory cells in the cortex of the Angora goat adrenal gland.

Conclusion

As a result, we observed that androgen hormone deficiency increased TNF-α expression in the adrenal tissue of the Angora goat but did not have a major effect on IFN-γ expression. To our knowledge, this is the first study to investigate the association between TNF-α and IFN-γ cytokines and castration in the adrenal gland of the Angora goat. We anticipate that this research will help future cytokine studies in the adrenal gland and contribute to the literature.

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Conflict of Interest

Author has no conflict of interest to declare.

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