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Nitric oxide (NO) expression during annual reproductive activity in buffalo epididymis: A histochemical and immunocytochemical study

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Abstract

The buffalo is one of the few domestic animals that has a seasonal mating cycle, influenced by the photoperiod. It is known that the photoperiod regulates gonadal function probably via the pineal and/or hypothalamus-pituitary axis. Moreover, the hypothalamus (melatonin) and gonads influence the production of the signaling transmitter nitric oxide (NO), suggesting that the NO may have an important role in the regulation of gonadotropin-releasing hormone secretion. This further suggests the hypothesis that NO in the epididymis has an important role in the maturation of spermatozoa and their motility and posterior fertilization capacity. The aim of the present study is to investigate the seasonal variations in the morphology of the epididymis by means histochemical and immunocytochemical techniques. We used the NADPH-d, nitric oxide synthase (NOS) I and NOS III to clarify the relationship between epididymis function and NO signaling activity. The results of this work show that NO is present in the caput of epididymis during short photoperiods, i.e., periods of maximum gonadal activity (winter) and absent during long photoperiods, i.e., periods of gonadal regression according to the previously described role of NO in spermatozoa capacitation and motility in the caput epididymis.

Keywords: Buffalo; Epididymis; Seasonality; NADPH-d; Immunogold labeling

1. Introduction

The buffalo is a domesticated ruminant species of peculiar biological interest because it is one of the few domestic animals that has a seasonal mating cycle. Mating activity only takes place from autumn to winter, and there is a long non-mating season from late spring to the beginning of the autumn. It is well-known that the photoperiod plays an important role in influencing the sexual activity of the adult male buffalo. This period regulates gonadal function, probably via the pineal and/or hypothalamus-pituitary axis by melatonin production. A growing body of recent evidence suggests that the hypothalamus (melatonin) and gonads influence the production of the signaling transmitter nitric oxide (NO) [1,2]. Aydogan, et al. [3] reported that melatonin inhibits the activity of NO synthase in mammals. The anatomical localization of NO neurons is in close proximity to the gonadotropin-releasing hormone neurons in the hypothalamus. This suggests that NO may have an important role in the regulation of gonadotropin-releasing hormone secretion [4]. In fact, NO has been shown to modulate gonadal and adrenal functions in Japanese quail [5]. This further suggests the hypothesis that NO in the epididymis has an important role in the maturation of spermatozoa, including their acquisition of progressive motility and posterior fertil-

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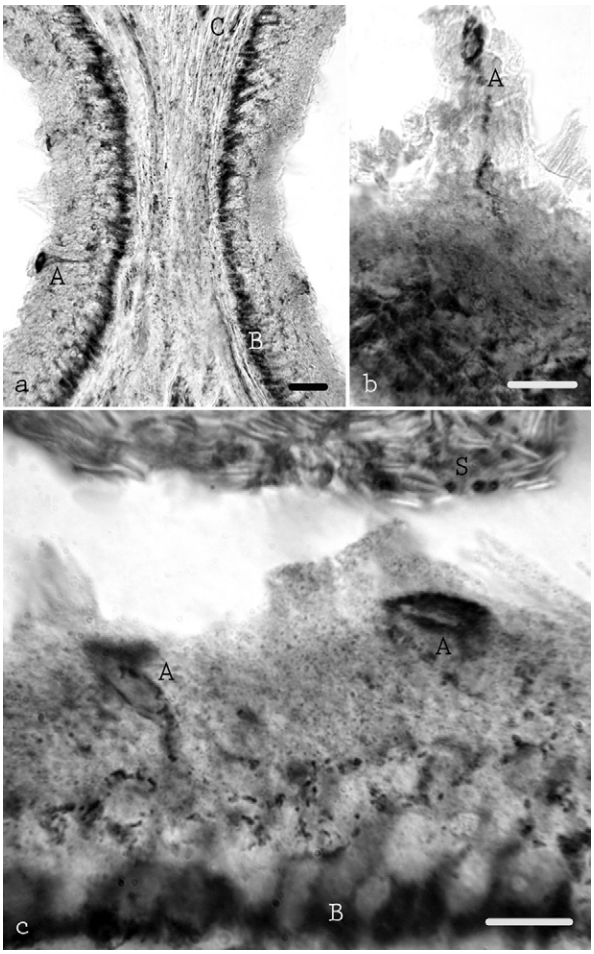

Fig. 1. LM buffalo epididymis in the winter. Intense NADPH-d staining of the caput epididymis. (a) epididymis duct; (b) narrow cell that protrudes in lumen of epididymis duct; (c) nuclei of the narrow cells. A = narrow cell; B = basal portion of the principal cells; C = blood capillary; S = spermatozoa. Scale bars = 10 μm.

ization capacity. In addition, it is also well-known that NO plays a decisive role in regulating the functions of the male reproductive system, where it is produced from the amino acid L-arginine by the enzymatic action of nitric oxide synthase (NOS) and various cofactors, such as oxygen, nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d), flavin mononucleotide, flavin adenine dinucleotide, tetrahydrobiopterina, calmodulin and calcium [6,7]. In mice, NO was observed on the acrosome and tail of non-capacitating spermatozoa suggesting a role for NO in capacitation [8]. Lewis, et al. 1996 and Donnelly, et al. 1997 [9,10] have reported that respectively low concentrations of NO improve and maintain human sperm motility. Moreover, Meiser, et al. 2003 and Leal, et al. 2009 [11,12] have reported that NO is involved in control of sperm motility and spermatozoa capacitation bull. In addition, Herrero, et al. 1999 [13] reported that NO is produced by capacitating human spermatozoa and acting as a cellular messenger by modulating the cAMP pathway and protein tyrosine phosphorylation. Sperm capacitation by protein tyrosine phosphorylation was also observed in buffalo spermatozoa [14,15], although the buffalo epididymis has often been studied to define the morphostructural characteristics that effect reproduction of this species. Scala, et al. 2002 [16] investigated the microvasculature of the buffalo epididymis and found fenestrations, located in the ovoid area of the endothelium post-capillary venules, that appeared to play an important role in the absorption and secretion processes of the epididymal epithelium. There are a few studies on the morphostructural characteristics of mammals epididymis, and buffalo in particular, in relation to seasonal reproduction. Schon and Blottner 2009 [17] reported in *Capreolus capreolus* only seasonal variations of the epididymis. Nitric oxide is currently one of

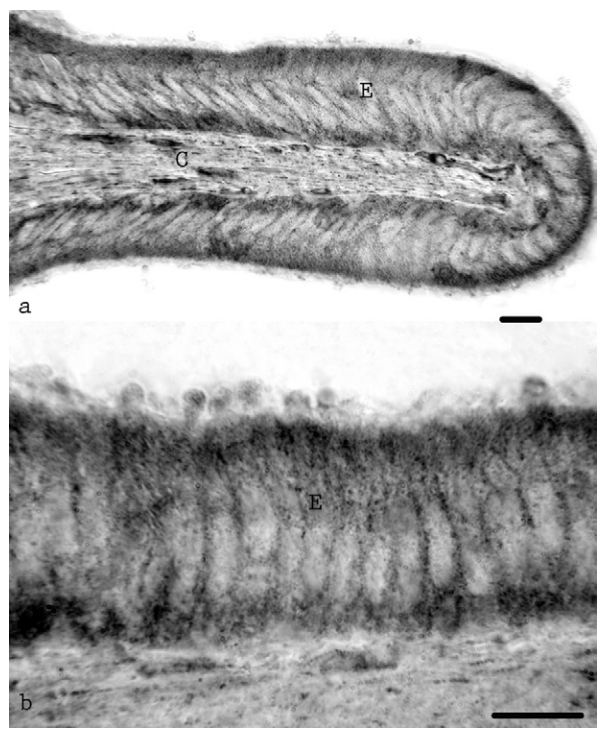

Fig. 2. LM buffalo epididymis in the winter. (a) NADPH-d staining of the corpus epididymis; (b) NADPH-d staining of the cauda epididymis. C = blood capillaries; E = epithelium of duct epididymis. Scale bars = 10 μm.