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Research paper

Seasonal variation of cell proliferation and apoptosis in the efferent ductules and epididymis of the Neotropical bat *Artibeus lituratus* (Chiroptera, Phyllostomidae)

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ABSTRACT

The balance between cell proliferation and apoptosis is important for maintenance of male fertility, being influenced by a variety of stimuli including androgens and estrogens. However, studies concerning regulation of these processes along the male reproductive tract under physiological conditions are scarce. Therefore, in this study, we investigated the profile of cell proliferation and apoptosis in the efferent ductules and epididymis of the Neotropical bat *Artibeus lituratus*, a seasonal breeder that presents natural variation in components of the androgen and estrogen responsive systems along the circannual cycle. Low rates of cell proliferation and apoptosis were found in the efferent ductules and epididymis of *A. lituratus* during the reproductive period, as few epithelial cells were positive for MCM7 (proliferation marker) and cleaved caspase-3 or TUNEL (apoptosis markers). In contrast, during the regressive period, the rate of both proliferating and apoptotic cells was significantly higher in the epithelium lining the efferent ductules as well as throughout the epididymis. The increased proliferative activity at this phase was positively correlated with the expression of estrogen receptor alpha (ERα), whereas the variation in apoptosis appears to be unrelated to the local expression of androgen and estrogen receptors. Together, these data suggest that cell proliferation and apoptosis are differentially modulated in the efferent ductules and epididymis of *A. lituratus* during the annual reproductive cycle, and support the hypothesis that ERα may be important in preparing the male reproductive tract for sexual recrudescence.

1. Introduction

The equilibrium between cellular proliferation and death is essential for normal development and homeostasis of the male reproductive system. Factors such as photoperiod, temperature, age, and alterations in sex hormone levels may interfere with this equilibrium (Billig et al., 1996; Jara et al., 2002; Carballada et al., 2007; Kim et al., 2015; Gonzaga et al., 2017). Among hormonal factors, it is well established that testosterone plays a central role controlling the reproductive function and homeostasis in males, directly or indirectly via its metabolites dihydrotestosterone (DHT) and estrogen (Hess and França, 2008; Joseph et al., 2011; Cooke et al., 2017). Both testosterone and DHT exert their biological functions through their ability to bind androgen receptor (AR), whereas estrogens act mainly via estrogen receptors alpha (ERα, also known as ESR1) and beta (ERβ, also known as ESR2). AR is widely distributed in the male reproductive system of vertebrates, in contrast to ERα and ERβ expressions that are organ- and species-specific (Hess et al., 2011; Cooke et al., 2017).

The big fruit-eating bat *Artibeus lituratus* (Olfers, 1818) is a widespread Neotropical species of great ecological importance due to its increased role in seed dispersal and forest regeneration (Carvalho et al., 2017). Interestingly, this bat species presents a well-defined annual reproductive and non-reproductive period (also referred as regressive period) (Oliveira et al., 2009). We have demonstrated that, during the non-reproductive phase, *A. lituratus* suffers a significant regression in the testis and epididymis, with decreases in weight, tubular diameter, and epithelial height (Oliveira et al., 2009, 2012). The regressed testis contains a high number of apoptotic figures in both somatic and spermatogenic cells, which occurs in parallel to an increase in ERβ immunoexpression (Oliveira et al., 2009). On the other hand, the expression of ERα, but not ERβ, was significantly higher in the regressed efferent ductules and epididymis, when compared with those of bats in the reproductive period (Oliveira et al., 2012). These data suggest that estrogen receptors may play important and differential roles in the male reproductive system of *A. lituratus* during the annual

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reproductive cycle, since the expressions of ERα and ERβ vary in an organ and reproductive status manner.

Indeed, there is evidence that ERα signaling stimulates cell proliferation in the male reproductive organs, whereas the ERβ functions as a pro-apoptotic and anti-proliferative factor (Cheng et al., 2004; Attia and Ederveen, 2012; Morais-Santos et al., 2015, 2018). Considering the role of estrogens and the natural variation in the expression of their receptors in the male reproductive system of *Artibeus lituratus* during the annual reproductive cycle, it is important to investigate whether there is a relationship between the estrogen signaling pathway and cell proliferation and apoptosis in efferent ductules and the epididymis, as both organs play crucial roles in male fertility and have been shown to be estrogen receptor-dependent (Hess et al., 2011; Cooke et al., 2017). Moreover, studies concerning the regulation of cell proliferation and apoptosis in this region of the male reproductive tract, under natural conditions, are lacking.

Therefore, the present study describes the profile of cell proliferation and apoptosis in efferent ductules and epididymis of the seasonal breeder bat, *Artibeus lituratus,* during the annual reproductive cycle, and correlates the results with previous data showing local variation in estrogen receptors.

2. Material and methods

2.1. Animals and ethical statement

Adult male bats of the *Artibeus lituratus* species were captured along the urban perimeter of Belo Horizonte county (19°55′ S and 43°56′ W), southeastern Brazil, during the reproductive (August to beginning of December) and regressive (middle of December to early April) periods, as previously described (Oliveira et al., 2013). The age of each animal was determined by assessing body weight and the pelage, the degree of ossification in the metacarpal epiphyses and wear of the teeth (Oliveira et al., 2009). The experimental procedures were approved by the Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais (CETEA-UFMG). The captures were authorized by the Brazilian Institute of Natural Environment and Renewable Resources (IBAMA, Brazil).

2.2. Tissue preparation

The investigation was performed on 16 adult bats, which after capture were weighed, anesthetized (i.p. lethal dose of sodium pentobarbital 30 mg/kg and ketamine chloridrate 20 mg/kg), and then perfused intracardially with Ringer's solution and 10% neutral buffered formalin (NBF) for immunohistochemistry and TUNEL assays. For Western blotting analysis, anesthetized animals were perfused only with Ringer's solution, and the fresh efferent ductules and epididymis were immediately frozen in liquid nitrogen and stored at −80 °C until use.

2.3. Immunohistochemistry

Fragments of NBF-fixed efferent ductules and epididymides (caput, corpus and cauda) of bats in the reproductive and regressive periods ($n = 4$ per group) were embedded in paraffin, sectioned at 5.0 μm and stained for MCM7 (also known as CDC47) and cleaved caspase-3, for detection of cell proliferation and apoptosis, respectively. The assays were performed as previously describe (Oliveira et al., 2012). Briefly, after blocking non-specific antibody binding with 10% normal goat serum for 1 h, the sections were incubated overnight at 4 °C with mouse anti-MCM7 (47DC141, Thermo Scientific, Fremont, CA, USA) or rabbit anti-cleaved caspase-3 (Asp 175, Cell Signaling Technology; Beverly, MA, USA) monoclonal antibodies, diluted 1:300 and 1:500 in phosphate buffer saline (PBS, pH 7.4), respectively. For negative controls, the sections received only PBS in place of the primary antibody. After washing in PBS, the slides were incubated for 1 h with the biotinylated goat anti-mouse (for MCM7) or goat anti-rabbit (for caspase-3) polyclonal antibodies (Dako, Carpentaria, CA, USA), diluted at 1:100 in PBS. The slides were exposed for 30 min to avidin-biotin complex conjugated with peroxidase (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA, USA). Finally, the immunoreaction was visualized by immersion in 0.05% 3,3′ diaminobenzidine solution containing 0.01% hydrogen peroxide in 0.05 M Tris-HCl buffer (pH 7.4). The sections were lightly counterstained with hematoxylin. To confirm the results, the staining was performed in triplicate.

2.4. TUNEL (TdT-mediated dUTP nick end Labeling)

In addition to caspase-3, for detection of apoptosis we performed TUNEL assays by utilizing the ApopTag® Plus Peroxidase *In Situ* Apoptosis Detection Kit (Millipore Corporation, Billerica, MA, USA), based on a previous protocol (Gonzaga et al., 2017). For this purpose, NBF-fixed fragments (5.0 μm) of efferent ductules and epididymis of bats in the reproductive and regressive periods ($n = 4$ per group) were processed as described for immunohistochemistry. After antigen retrieval with proteinase K (IHC SELECT® – Millipore Corporation) diluted 1:30 in 0.01 M Tris -HCl (pH 8.0), the sections were immersed in 3% (v/v) H_2O_2 in Tris-HCl buffer saline (TBS) for 30 min. After, the sections were incubated with TdT enzyme and anti-digoxigenin antibody conjugated to peroxidase, following the manufacture's protocol. Negative controls did not receive TdT enzyme. The immunostaining was visualized by immersion in 3,3′ diaminobenzidine solution, and the sections were lightly counterstained with hematoxylin, dehydrated, washed in xylene and mounted. To confirm the results, the TUNEL assay was performed in triplicate.

2.5. Western blotting

Western blot analysis was carried out with tissue samples to confirm specificity of the antibodies used in immunohistochemistry. For this purpose, the proteins were extracted from pooled tissues of animals in the reproductive and regressive periods ($n = 5$ per group) and then quantified by Bradford assay, as previously describe (Oliveira et al., 2012). A total amount of 20 or 25 μg of protein was loaded per lane for separation by continuous electrophoresis using 12 or 15% SDS-PAGE, for MCM7 and caspase-3, respectively. After, the proteins were transferred to nitrocellulose membranes and non-specific binding sites in the membrane were blocked by using 10% normal goat serum for 1 h. The blots were incubated overnight with the primary antibodies diluted 1:500 for mouse anti-MCM7 (Thermo scientific, Fremont, CA, USA) or rabbit anti-cleaved caspase-3 (Asp 175; Cell signaling technology; Beverly, MA, USA). Following this step, the membranes were washed in PBS-Tween 0.05% and incubated with the secondary antibodies (Dako, Carpinteria, CA) goat anti-mouse (for MCM7) or goat anti-rabbit (for caspase-3) diluted 1:1000. After washes in PBS-Tween 0.05%, immunolabeling was visualized with a solution of 0.1% of 3,3′ diaminobenzidine in PBS containing 0.05% (w/v) chloronaphthol, 16.6% (v/v) methanol, and 0.04% (v/v) H_2O_2. The Western blotting assays were performed in triplicate to confirm the results.

2.6. Quantification of cell proliferation and apoptosis

The rates of epithelial cell proliferation and apoptosis in both reproductive and regressive periods were quantified in

immunohistochemical and TUNEL preparations, by using computer-assisted image analysis. For each tissue section, five pictures per region were randomly taken at 400× magnification by using the ZEN 2012 Lite imaging system (Carl Zeiss®, Oberkochen, Germany). Positive and negative epithelial cells of the efferent ductules and epididymis (initial segment, caput, corpus and cauda) were counted, and the results expressed as percentage (Gonzaga et al., 2017).

Our prior study using the same specimens analyzed herein implicated ERα in cell proliferation signaling and the receptor varied significantly during the annual reproductive cycle of *Artibeus lituratus*; therefore, we also compared cell proliferation rates in the efferent ductules and epididymis with the previous ERα data (Oliveira et al., 2012).

2.7. Immunofluorescence

To evaluate the relationship between cell proliferation and ERα expression in the efferent ductules and epididymis epithelium, we also performed immunofluorescence colocalization assays of ERα and a cell proliferation marker, following the protocol described by Morais-Santos et al. (2015). Because both ERα and MCM7 primary antibodies were raised in mouse and thus would hinder colocalization, in this assay we used Ki67 as the marker for cell proliferation. Briefly, after blocking non-specific binding, tissue sections were incubated overnight at 4 °C with the primary antibodies: mouse monoclonal anti-ERα (6F11, Novocastra Laboratories, Newcastle, UK) plus rabbit polyclonal anti-Ki67 (ab15580, Abcam, Cambridge, USA) diluted 1:50 and 1:200 in phosphate buffer saline (PBS, pH 7.4), respectively. After washing, the sections were exposed at room temperature for 2 h to the secondary antibodies: goat anti-mouse Alexa 488 conjugated plus goat anti-rabbit Alexa 546 conjugated (Life Technology, Carlsbad, USA), both diluted 1:100 in PBS. Nuclei were probed with DAPI (Life Technology, Carlsbad, USA). The immunofluorescence signals were examined by using a Zeiss ApoTome microscope (Carl Zeiss, Göttingen, Germany).

2.8. Statistical analysis

Quantitative data were initially submitted to D'Agostino-Pearson normality test, and a QQ plot of the values in each dataset was created. When normality was confirmed, the data were analyzed by Student's *t*-test to compare means between two populations, or ANOVA plus Tukey post hoc test for comparisons between more than two populations. Otherwise, the Mann-Whitney or Kruskal-Wallis plus Dunn's post hoc tests were used to compare non-parametric data from two or more populations, respectively. Moreover, the relationship between proliferating cells stained for MCM7 and ERα-positive epithelial cells was evaluated by Spearman's correlation coefficient. The presence of atypical points (outliers) was verified by Grubbs' test, and the significance level used for all comparisons was $P \leq 0.05$.

3. Results

3.1. Cell proliferation in the male reproductive tract of Artibeus lituratus varied during the reproductive cycle and correlated with ERα distribution

Few MCM7-positive epithelial cells were observed in the efferent ductules and epididymides of animals during the reproductive period (Figs. 1 and 2A). Proliferative activity in the efferent ductule epithelium was mainly observed in the non-ciliated cells, whereas in the epididymis, nuclei of the principal cells were the most immunoreactive for MCM7 (Fig. 1). Other cell types of the bat epididymal epithelium, identified by their nuclear form and position, also presented positive staining for MCM7, albeit fewer in number. Proliferating cells in both efferent ductules and epididymis presented an adjacent pattern of cell division (side-by-side), except for rare epithelial cells that were found dividing parallel to the basement membrane (Fig. 1).

During the regressive period, the percentage of proliferating cells was significantly increased in efferent ductules (4.2-fold) as well as initial segment (6.8-fold), caput (3.8-fold), corpus (4-fold), and cauda epididymis (4.8-fold) (Fig. 2A). At this phase, rows of MCM7-positive cells were commonly found in the epithelium lining all the regressed segments (Fig. 1). Eventually, some cells displaying apoptotic morphology were observed in the vicinity of epithelial cells stained for MCM7 (data not shown).

Considering that (1) ERα has been implicated in cell proliferation and (2) increased expression of this estrogen receptor, but not of ERβ or androgen receptor, has been observed in the efferent ductules and epididymis of *Artibeus lituratus* during regression (Oliveira et al., 2012), we also evaluated the correlation between cell proliferation and ERα expression. The number of proliferating epithelial cells showed a strong positive correlation with the number of ERα-positive cells in the efferent ductules and epididymis (Fig. 3A and B). Moreover, the co-localization of ERα and Ki67 revealed epithelial cells expressing ERα, which were actively proliferating in both efferent ductules and epididymis (Fig. 3C and D). Positivity for ERα was much more frequent in the efferent ductules than the epididymis epithelium.

3.2. Apoptosis in the efferent ductules and epididymis of Artibeus lituratus varied during the reproductive cycle

The efferent ductules and epididymis presented low rates of apoptosis during the reproductive period of *Artibeus lituratus* (Figs. 2 and 4). At the regressive phase, there was a significant increase in the apoptosis throughout the efferent ductules and epididymis compared to the reproductive period. The increase in apoptosis was greater in efferent ductules (8-fold) and initial segment (10-fold), compared to the caput (2-fold), corpus (2.4-fold), and cauda epididymides (3.2-fold) (Fig. 2B and 4A). Cells with morphological features of apoptosis, such as cell shrinkage and fragmentation into apoptotic bodies, which were unreactive for cleaved caspase-3, were also observed (Fig. 4A). Therefore, besides the well-defined apoptotic marker cleaved caspase-3, we also used TUNEL assays to complement the apoptosis assessment.

TUNEL assays confirmed the low apoptosis rates in efferent ductules and the epididymis (Fig. 2C and Fig. 4B). Some segments of the bat male tract, such as the efferent ductules, initial segment and cauda epididymis, presented rare or undetectable epithelial cells that were TUNEL-positive, especially during the reproductive period. However, similar to caspase-3, an increased number of TUNEL-positive cells was observed during the regressive period, compared to the reproductive period (Fig. 2C and Fig. 4B). We also found some unreactive epithelial cells displaying morphology of apoptosis (Fig. 4B).

3.3. Western blotting

The immunohistochemical results and the specificity of the antibodies used for detection of MCM7 and cleaved caspase-3 were confirmed by Western blotting. The assays detected specific bands of 80 kDa and 17 kDa, respectively (Fig. 2D), which are consistent with the molecular weights for MCM7 and activated caspase-3 (Kwong et al., 1999; Ren et al., 2006). MCM7 immunoreaction was highest in the efferent ductules and epididymis during the

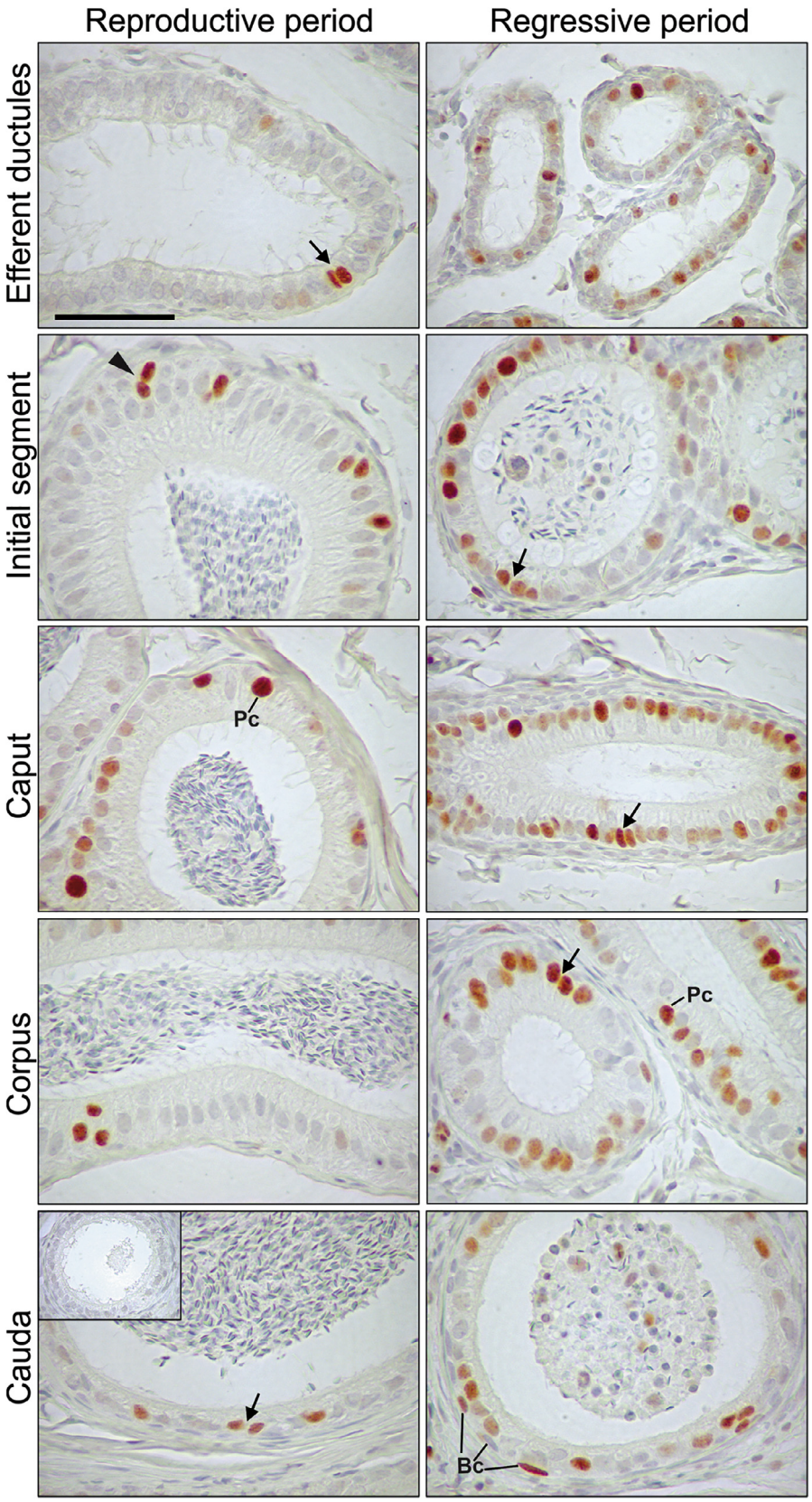

Fig. 1. Proliferating cells in efferent ductules and the epididymis of the bat *Artibeus lituratus* during the annual reproductive cycle. A higher number of epithelial cells with reactive nuclei for the proliferating marker MCM7 was found in all segments of bats in the regressive period, compared to those during the reproductive period. Arrows = MCM7-positive cells showing an adjacent pattern of cell division; Arrowheads = cell division parallel to the basement membrane (however, this was rare in the epithelium of all segments analyzed). Pc = principal cells. Bc = basal cells. Upper insert = negative control. Scale bar = 50 μm.

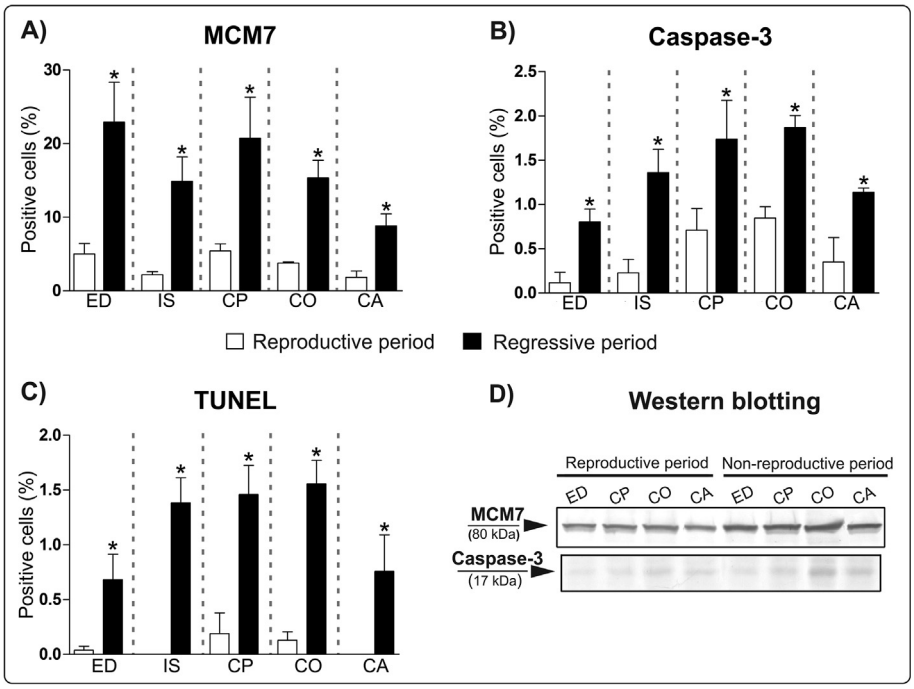

Fig. 2. Quantification of cell proliferation and apoptosis in efferent ductules and the epididymis of the bat *Artibeus lituratus* during the annual reproductive cycle. (A) Rate of epithelial cells positive for the proliferating cell marker MCM7. (B) Rate of epithelial cells positive for cleaved caspase-3. (C) Rate of epithelial cells TUNEL-positive. (D) Representative Western blotting showing reactive bands consistent with the molecular weights for MCM7 (80 kDa) and cleaved caspase-3 (17 kDa). ED = efferent ductules; CP = caput; CO = corpus; and CA = cauda. * = P < 0.05 when reproductive and regressive periods were compared.

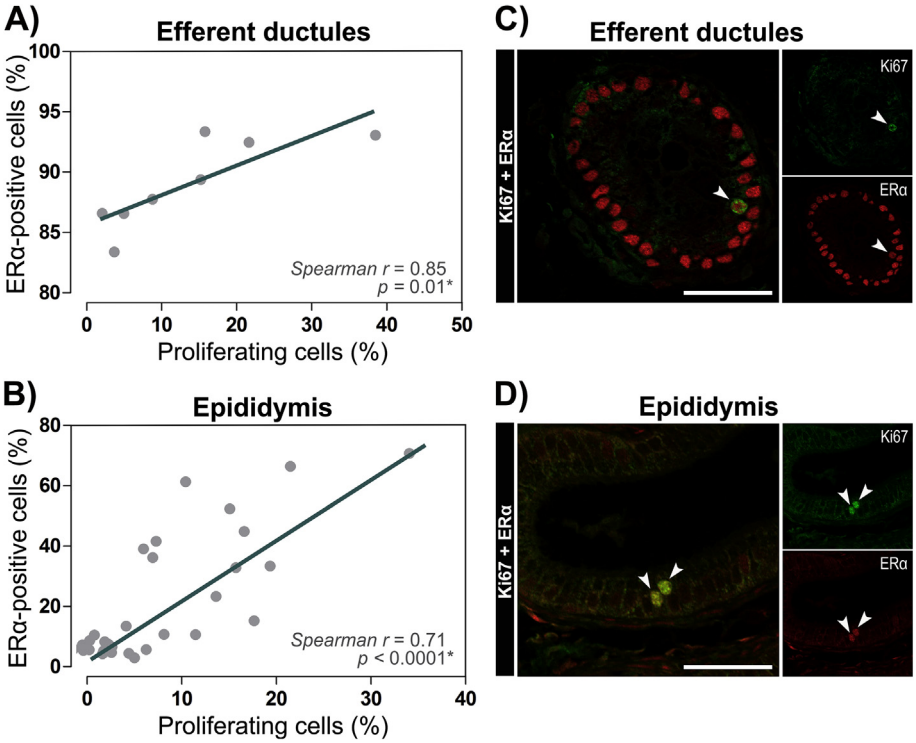

Fig. 3. Relationship between cell proliferation and ERα expression in efferent ductules and the epididymis of the bat *Artibeus lituratus*. (A and B) Significant positive correlation between the percentages of proliferating cells and ERα-positive cells in the efferent ductules and epididymis epithelium. (C and D) The colocalization of ERα and Ki67 (proliferation marker) revealed the presence of proliferating cells also expressing ERα (arrowheads) in both efferent ductules and epididymis epithelium. Scale bars = 50 μm.

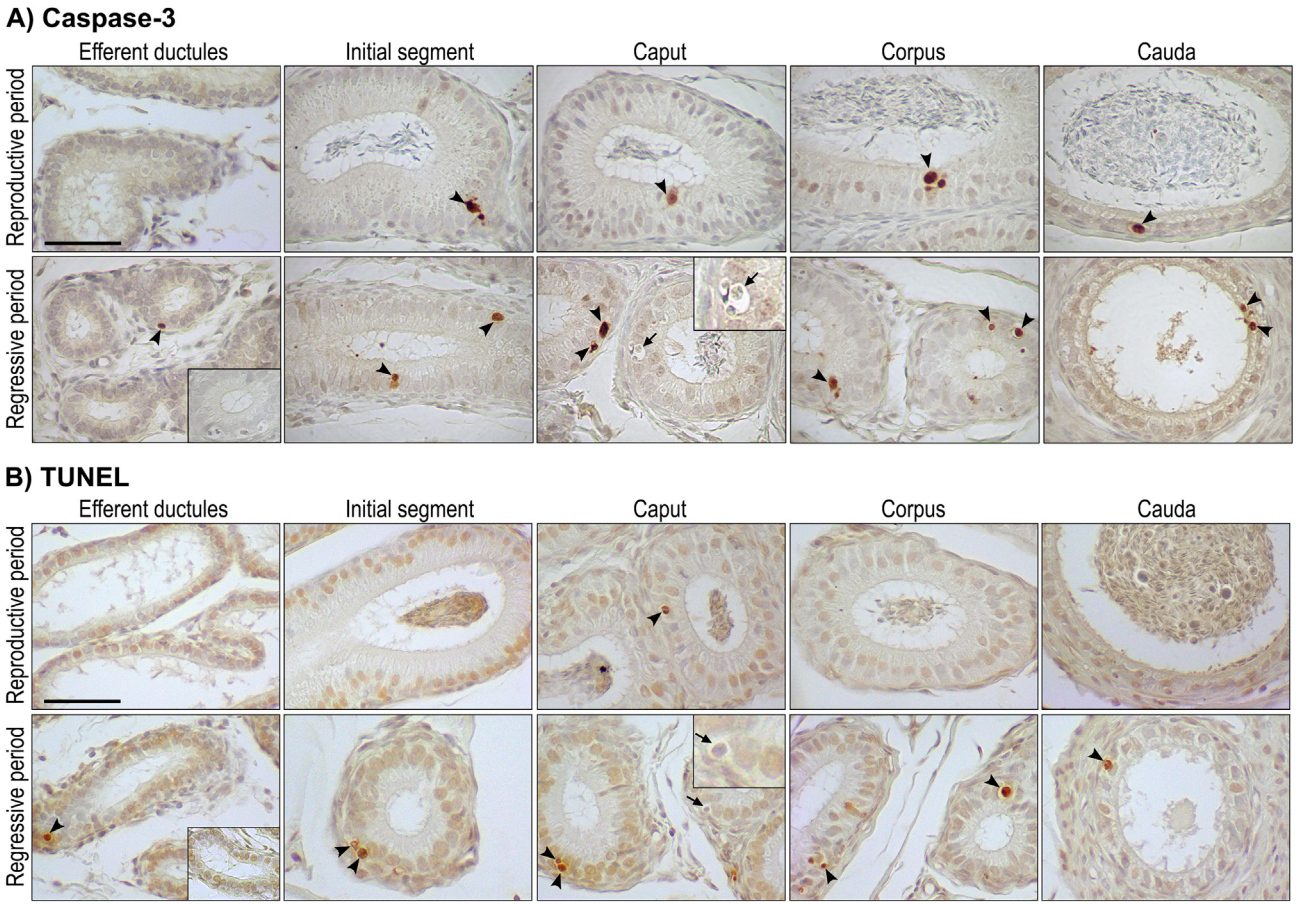

Fig. 4. Apoptotic cells in efferent ductules and the epididymis of the bat *Artibeus lituratus* during the annual reproductive cycle. (A) Immunodetection of cells stained for cleaved caspase-3. (B) Immunodetection of TUNEL-positive cells. Few epithelial cells were reactive (arrowheads) for apoptosis markers, but when found they were present mainly during the regressive period. Upper inserts highlight the presence of some unreactive epithelial cells that display apoptotic morphology (arrows). Lower inserts show the negative controls. Scale bars = 50 μm.

regressive period, compared to the reproductive period (Fig. 2D). Cleaved caspase-3 was barely detectable in the efferent ductules and epididymis during both periods, except in the regressed corpus and cauda (Fig. 2D).

4. Discussion

This is a pioneer study concerning cell proliferation and apoptosis along the male reproductive tract in a seasonal breeding bat species. Herein, we found a remarkable increase in the rates of cell proliferation and apoptosis in the efferent ductules and epididymis, which was detected under natural conditions, without surgical, chemical, or genetic interference.

During the reproductive period, few proliferating cells (MCM7-positive) were detected in the epithelium of all epididymal segments, corroborating previous findings in other adult mammals, such as mouse (Holschbach and Cooper, 2002; Kim et al., 2015), rat (Clermont and Flannery, 1970; Sun and Flickinger, 1982; Hamzeh and Robaire, 2009), hamster (Nagy and Edmonds, 1975), pig (Bernal-Manas et al., 2014), and the flat-faced fruit-eating bat *Artibeus planirostris* (Beguelini et al., 2013). It is commonly accepted that proliferative activity occurs mainly in the principal cells, being low or absent in other epithelial cell types of the epididymis (Holschbach and Cooper, 2002; Hamzeh and Robaire, 2009; Kim et al., 2015). A similar pattern was presently found for *Artibeus lituratus* during both periods analyzed.

Information regarding cell proliferation in the efferent ductules are so far scarce. As observed for the epididymis during the reproductive period, few proliferating cells were also detected in the efferent ductules epithelium of *Artibeus lituratus*. This finding is consistent with a recent study in the adult Wistar rat showing a very low proliferation rate in efferent ducts under normal conditions (Martins-Santos et al., 2018).

The efferent ductules are well-recognized for their dependence on ERα and expression of this receptor at levels that exceed even female tissues, across all species, including *Artibeus lituratus* (Hess et al., 1997; Oliveira et al., 2012; Cooke et al., 2017). Therefore, we hypothesized that estrogen could be involved in the regulation of epithelial proliferation in these ductules. Others have shown that ERα is a potent proliferation factor in several cellular systems, including organs of the male reproductive system (Zhou et al., 2011; Attia and Ederveen, 2012; Gong et al., 2014). Therefore, it was interesting to find that the proliferative activity in the bat efferent ductules epithelium was mainly observed in non-ciliated cells, which were also ERα positive and previously shown to be the primary cell type to express ERα in *Artibeus lituratus* (Oliveira et al., 2012).

We have shown that ERα expression, but not that of ERβ or AR, exhibited marked seasonal variation and was increased in the efferent ductules and epididymis epithelium of *Artibeus lituratus* during the regressive period (Oliveira et al., 2012). Data presented herein are consistent with those previously observed for ERα levels, and supports the hypothesis that modulation of epithelial

proliferation may be another local function of estrogens acting through ERα, as there was a strong positive correlation between the percentage of proliferating cells and ERα-positive cells. Furthermore, ERα co-localized with the cell proliferation marker in epithelial cells of both efferent ductules and the epididymis.

It has been known since 1991 that during development, efferent ductules are the first region of the male reproductive tract to show estrogen receptor binding, but also to show the most intense binding activity (Cooke et al., 1991). Thus, it is reasonable to suggest that ERα may be important for the maintenance of cell proliferation in the efferent ductules, as *Artibeus lituratus* prepares for the next reproductive period, following regression (Oliveira et al., 2012). Its role in the epididymis, however, remains controversial (Hess et al., 2011), as ERα has been detected in the developing epididymis of several species (Jefferson et al., 2000; Nielsen et al., 2000 – mouse; Atanassova et al., 2001 – rat; Albrecht et al., 2004 – baboon; Shapiro et al., 2005 – human; Parlevliet et al., 2006 – stallion), but in most adult epididymides, ERα is present at lower levels and in select cell types (Atanassova et al., 2001; Saunders et al., 2001; Nie et al., 2002; Joseph et al., 2011; Cooke et al., 2017).

It was interesting that cell proliferation occurred primarily side-by-side or in small groups within the epithelium and in rare proliferating single cells lying parallel to the basement membrane. This pattern of cell division was also found in the efferent ductules and epididymis of rodents (Holschbach and Cooper, 2002; Martins-Santos et al., 2018). Although this observation may seem trivial, it is reported that spatially restricted or adjacent dividing cells lead to an increase in tubular diameter, whereas those dividing parallel to the basement membrane or to the ductal axis, will increase its length (Hinton et al., 2011). Moreover, it has been suggested that cells dividing in small groups ("hotspots") might contribute to coiling of the epididymis (Joseph et al., 2009). Previous data have shown a significant reduction in tubular diameter of 30–50% in the *Artibeus lituratus* epididymis during regression (Oliveira et al., 2012). Therefore, the increased proliferative activity during this period and the unique pattern of cell division in hotspots would be consistent with the reestablishment of tubular coiling that would be needed in the next reproductive phase.

Few epithelial cells undergoing apoptosis were detected along the male reproductive tract of *Artibeus lituratus* during the reproductive period. Similarly, apoptosis is also low in the efferent ductules (Martins-Santos et al., 2018) and epididymis of other mammals under normal conditions (Fan and Robaire, 1998; Carballada et al., 2007; Kim et al., 2015).

The initial segment was the epididymal region that showed the highest proportion of apoptotic cells during the regressive period (increased 10-fold, compared to 2-fold for caput, 2.4-fold for corpus, and 3.2-fold for cauda). This was not surprising, as a high apoptotic index has been consistently detected in the initial segment of mouse and rat after experimental deprivation of luminal factors of testicular origin, due to castration or efferent ductules ligation (Fan and Robaire, 1998; Turner and Riley, 1999; Smith et al., 2014). Of these experimental models, androgen withdrawal appears to be the major factor that induces apoptosis along the epididymis, except in the initial segment, where other luminal factors such as growth factors and components of key signaling pathways (ERK, AKT, STAT, and NFκB) are required for modulating cell survival (Xu et al., 2011, 2013, 2014). In contrast to that seen in rodents, it is not clear whether apoptosis in the epididymis of bat species is regulated by androgens. Indeed, in the *Artibeus lituratus*, high plasma and tissue levels of testosterone and DHT, concomitantly with the expression of androgen receptor in the testis, efferent ductules and epididymis were found during both the reproductive and regressive period (Oliveira et al., 2012, 2013). Therefore, androgens appear to be unrelated to the seasonal changes in the apoptosis rate observed in the male reproductive tract of this bat species along the annual reproductive cycle. Moreover, the expression of ERβ, an estrogen receptor that has been related to a pro-apoptotic stimulus (Helguero et al., 2005; McPherson et al., 2010; Jia et al., 2015), including in the testis of *Artibeus lituratus* (Oliveira et al., 2009), did not vary in the efferent ductules and epididymis of this bat species. Due to the paucity of information about the reproductive physiology of bats, the stimulus that triggers apoptosis in the male reproductive tract of *A. lituratus*, remains unknown.

Interestingly, the percentage of apoptotic cells significantly increased in the efferent ductules and all epididymal segments of bats in the regressive period, compared to sexually active animals. The increase in apoptotic activity at this period occurs simultaneously with the increase in cell proliferation, a fact that may reflect a strategy for epithelial cell renewal in the efferent ductules and epididymis. Corroborating this point of view, it was not uncommon to find apoptotic cells in the vicinity of proliferating cells along the male reproductive tract of *Artibeus lituratus*. Therefore, crosstalk between these opposing cellular processes may be crucial for maintaining epithelial homeostasis in efferent ductules and the epididymis, thus assuring the eminent recrudescence.

5. Conclusion

Rates of cellular proliferation and apoptosis were higher in efferent ductules and the epididymis of *Artibeus lituratus* during the regressive period compared to the reproductive period. This would indicate that a parallel increase in these opposing processes in the regressed organs may function as a strategy to maintain tissue homeostasis. Although the stimulus triggering apoptosis in efferent ductules and epididymis of the bats remains unknown, seasonal variation in cell proliferation was strongly correlated with the expression of ERα, thus supporting the hypothesis that this estrogen receptor may be important in the preparation of the male reproductive tract of *A. lituratus* for sexual recrudescence.

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Conflicts of interest

None.

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