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ORIGINAL ARTICLE

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Effects of melatonin and gonadal androgens on cell proliferation in the pituitary of viscachas (*Lagostomus maximus maximus*)

G. J. Rosales[1,2] | F. I. Busolini[1,2] | F. H. Mohamed[1] | V. P. Filippa[1,2]

[1]Laboratorio de Histología, Área Morfología, Departamento de Bioquímica y Ciencias Biológicas, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, 5700 San Luis, Argentina

[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Correspondence
Verónica P. Filippa, Área de Morfología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Av. Ejército de los Andes 950-Bloque I, 1° Piso (5700) San Luis, Argentina.
Email: vpfilipp@unsl.edu.ar

Abstract

Objectives: Melatonin and androgens are involved in the regulation of cell proliferation. However, effects of these hormones on pituitary pars distalis (PD) of male viscachas is not fully understood. In the present study, we analysed melatonin and gonadal androgens' effects on proliferating cell nuclear antigen (PCNA) expression.

Materials and methods: Pituitary glands from foetuses, immature individuals, prepubertal individuals and adult viscachas during their reproductive cycle, after melatonin administration and after castration, were used. PCNA-ir cells were detected by immunocytochemistry and morphometrically quantified using image analysis.

Results: Total percentage of PCNA-ir cells varied seasonally in the adult pituitary, with maximum values during the reproductive period and minima during gonadal regression periods. Percentages of PCNA-ir cells increased after melatonin administration, whereas it decreased after castration. Caudal end and ventral regions were the PD zones which were most affected by seasonal variations and castration. PCNA expression was highest in foetal pituitary from midpregnancy. Numbers of PCNA-ir cells decreased during sexual maturity.

Conclusions: Our results demonstrate the effect of gonadal androgens on cell proliferation during the reproductive period and sexual maturity of these animals. Exogenous melatonin increased PD cell proliferation in adults. Thus, these hormones seem to be involved in different mechanisms that regulate cell renewal of PD in this seasonally breeding rodent.

1 | INTRODUCTION

The proliferating cell nuclear antigen (PCNA) is a 36 kD non-histone nuclear protein. It is highly conserved between species, functions as cofactor of DNA polymerase-delta and plays a fundamental role in the initiation of cell proliferation. Elevated expression of PCNA appears in the nucleus during late G1 phase before the onset of DNA synthesis, becomes maximal during S-phase and declines during the G2 and M phases. The usefulness of PCNA as a marker of proliferating cells has been supported by several studies.[1–3]

In the short term, the pituitary responds to physiological demand by modifying hormone synthetic and secretory activity; and over hours, days and weeks, by altering the relative proportions of the different cellular subtypes that it contains. This modification is achieved by increased or decreased rates of cell division and cell death, by differentiation of cells to specific secretory subpopulations, and by a direct transition from one mature secretory phenotype to another.[4,5]

The identification of the stimuli that seasonally activates cell proliferation in vivo is under study, and melatonin is a leading candidate. In mammals, melatonin is critical for the regulation of major physiological processes including the sleep-wake cycle, pubertal development and seasonal adaptation.[6] In addition, it has been demonstrated that melatonin inhibits cell proliferation, modulates the length of the cell cycle and reduces the metastatic capacity of tumoural cells.[7,8]

Gonadal steroids are involved in the regulation of mitotic and apoptotic events in adenohypophysis. Proliferative activity has been linked to circadian changes, estrous cycle and sex in normal adult rats achieving the cell renewal of the different pituitary cell types.[1] Oestrogen locally generated in the pituitary by aromatization of testosterone stimulates proliferation of some cell types.[9,10] Moreover, differential effects on the mitotic activity in the pituitary have been reported after castration.[3,11,12] Several studies have examined the cell proliferation during the development of the foetal pituitary, resulting in a decrease from the foetal stage to the early stages of post-natal life.[13–15]

Our experimental model, the viscacha (*Lagostomus maximus maximus*), is a hystricomorph rodent with seasonal reproductive patterns. The annual changes in photoperiod length have been shown to constitute the primary signal for synchronizing the annual reproductive cycle of the adult male viscacha,[16–19] which presents three periods: reproductive (summer and early autumn), gonadal regression (winter) and gonadal recovery (spring). Earlier histological studies demonstrated that pituitary cell types changed in relation to gonadal activity and sexual maturity of viscachas.[20–24] Variations in the immunostaining for androgen receptor (AR), luteinizing hormone (LH) and follicle-stimulating hormone cells have been reported in the pituitary of melatonin-administered and castrated adult male viscachas.[25] In addition, androgens and their receptors have been involved in the regulation of the pituitary activity of this rodent.[26]

The hormonal regulation of cell proliferation in the pituitary of foetal, immature and prepubertal viscachas has not been examined, and there is no data on the seasonal cell renewal in the pituitary of this rodent. This study provides insight not only into possible deviations in the physiological response to hormonal stimuli but it might also predict pathophysiological events that contribute to the induction of pituitary adenomas. Based on the above data and on our previous knowledge about the viscacha, it is reasonable to hypothesize that melatonin and gonadal androgens provoke variations of cell proliferation in the pituitary of male viscachas. To test this hypothesis, we performed an immunohistochemical and morphometrical study of PCNA expression in the pituitary PD of male viscachas during the annual reproductive cycle, after melatonin administration and castration, and in animals of different sexual maturity.

2 | MATERIALS AND METHODS

2.1 | Animals and experimental conditions

The viscachas were captured in their habitat near San Luis, Argentina (33°20′ south latitude, 760 m altitude) using traps placed in their burrows. In San Luis, in summer, the light phase is up to 14 hours light daily (14L:10D) with an average temperature of 25°C. In winter, the light phase decreases to 10 hours (10L:14D), and the average temperature is 10°C. In spring, the light phase increases to 12 hours (12L:12D), and the average temperature is 15°C.

All viscachas were intramuscularly anesthetized with a combination of ketamine (Ketamina 50; Holliday-Scott SA®, Beccar, Buenos Aires, Argentina) and xylazine (Vetanarcol®; König SA, Rosario, Santa Fe, Argentina) at a dose of 12 and 0.4 mg/kg, respectively. Then the animals were sacrificed by intracardiac injection of Euthanyle (0.25 mL/kg body weight, sodium pentobarbital, sodium diphenylhydantoin; Brouwer S.A.). The brain was exposed rapidly, and the pituitary processed for light microscopy. The pituitaries were sectioned sagittally and the hemipituitaries fixed in Bouin's fixative, embedded in paraffin and then sectioned sagittally (5 μm), as described in previous studies.[21,22,24]

The experimental designs were approved by the local Ethics Committee of Universidad Nacional de San Luis, and were in agreement with the National Institute of Health (NIH, USA) guidelines for the use of experimental animals. Moreover, the Biodiversity Control Area of the San Luis Ministry of the Environment (Argentina) approved a study protocol for conducting scientific research within the territory of this province (Resolution No. 47.PBD.2015).

2.2 | Annual reproductive cycle

Twelve adult male viscachas weighing 5–7 kg were captured during the most representative months of their reproductive cycle, for a period of over 1 year: four animals during the reproductive period in summer to early autumn (February to April), four animals in the gonadal regression period in winter (July) and four animals in the gonadal recovery period in spring (September). The reproductive condition of viscachas was carefully assessed on the basis of observations by light microscopy of testes.[27]

2.3 | Administration of melatonin

Eight adult male viscachas captured during the month of February (summer) were used. The rodents were kept individually in boxes with free access to food and water, at 20 ± 2°C. They were maintained under a 14L:10D light-dark cycle. The experimental group received two daily subcutaneous injections of melatonin (100 μg/kg body weight in oil solution; Sigma, St. Louis, MO, USA) at 09:00 and 17:00 hours, for 9 weeks. The control group received only the diluent. The experimental design was similar to that reported in previous experiments.[20–22,24,28]

2.4 | Castration

Eight adult male viscachas captured during the reproductive period (summer and early autumn) were used. The animals were divided into two groups: a group of surgically castrated viscachas (n=4) and a control group constituted by intact viscachas (n=4). They were kept individually in boxes for 6 weeks and maintained under a 14L:10D light-dark cycle, with free access to food and water, at 20 ± 2°C, as used in previous studies.[21,25] After 6 weeks, the animals were anesthetized and sacrificed. The reproductive condition of the viscachas of the control group was carefully assessed on the basis of observations by light microscopy of testes. All control male viscachas showed morphological characteristics of gonadal activity according to the data provided by Muñoz et al.[27]

2.5 | Animals of different sexual maturity

A total of eight foetal male pituitary glands were collected. The foetal pituitaries were collected from foetuses of mid (n=4) and late pregnant (n=4) females classified on the basis of foetal weight and crown-heel length.[29–31] The male animals were carefully classified into immature (1–2 kg; n=4) and prepubertal (3–4 kg; n=4) according to body weight and light microscopy observations of testes.[21,32]

2.6 | Immunohistochemistry of PCNA-ir cells in the pituitary

Tissue sections were stained using the streptavidin-biotin peroxidase complex method at 20°C. Sections were first deparaffinized with xylene, hydrated through decreasing concentrations of ethanol, and rinsed with distilled water and phosphate-buffered saline (PBS, 0.01 M, pH 7.4). Antigen retrieval was performed by microwaving the sections for 6 minutes (2 × 3 minutes) at full power in a 900 W microwave oven in sodium citrate buffer (0.01 M, pH 6.0). Endogenous peroxidase activity was inhibited with 3% H_2O_2 in water for 20 minutes. Non-specific binding sites for immunoglobulins were blocked by incubation of sections for 20 minutes with non-immune serum diluted in PBS containing 1% bovine serum albumin, 0.09% sodium azide and 0.1% Tween-20. Sections were incubated with the primary antibody as follows: 12 hours in a humidified chamber at 4°C with mouse monoclonal antibody to PCNA (proliferating cell nuclear antigen, Catalog. No AM 252-5M; BioGenex, San Ramon, CA, USA). After rinsing with PBS for 10 minutes, immunohistochemical visualization was performed using the Super Sensitive Ready-to-Use Immunostaining Kit (BioGenex), as follows: sections were incubated for 30 minutes with diluted biotinylated anti-IgG, and after washing in PBS, were incubated for 30 minutes with horseradish peroxidase-conjugated streptavidin, and finally washed in PBS. The reaction sites were visualized using freshly prepared solution of 100 µL of 3,3′-diaminobenzidine tetrahydrochloride chromogen in 2.5 mL PBS and 50 µL H_2O_2 substrate solution. Sections were counterstained with Harris' haematoxylin for 10 seconds, dehydrated and mounted.

Rat large intestine was used as positive control (Fig. 1a,b). For negative control in the immunohistochemistry procedures performed, 10% non-immune serum and PBS replaced the primary antibodies. No positive structures or cells were found in these sections (Fig. 1c).

2.7 | Morphometric analysis

The distribution of PCNA-ir cells in the PD was quantified in each zone of the pituitary PD parenchyma of foetal, immature, prepubertal and adult male viscachas; and the total percentage of PCNA-ir was calculated for each group. Pituitaries from adult viscachas during their reproductive cycle, after melatonin administration and after castration were studied. The different zones in the pituitary PD were the ventral (anterior), medial and dorsal (posterior, close to Rathke's pouch) regions, and the rostral (or cephalic, superior, connected to the pars tuberalis) and caudal (inferior) ends (Fig. 1d).

A computer-assisted image analysis system was used for the morphometric analysis as reported previously.[22,24] Briefly, the image was displayed on a colour monitor; a standard area of 18 141.82 µm^2 (reference area) was defined on the monitor, and distance calibration was performed using a slide with a micrometric scale for microscopy (Reichert, Austria, Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina). The morphometric study was performed as follows: four regularly spaced serial tissue sections (100 µm each) from a pituitary were used, and microscopic fields were examined under a 40× objective. In each section, 35 microscopic fields were randomly selected throughout the PD (seven from each region or end of the PD: ventral,

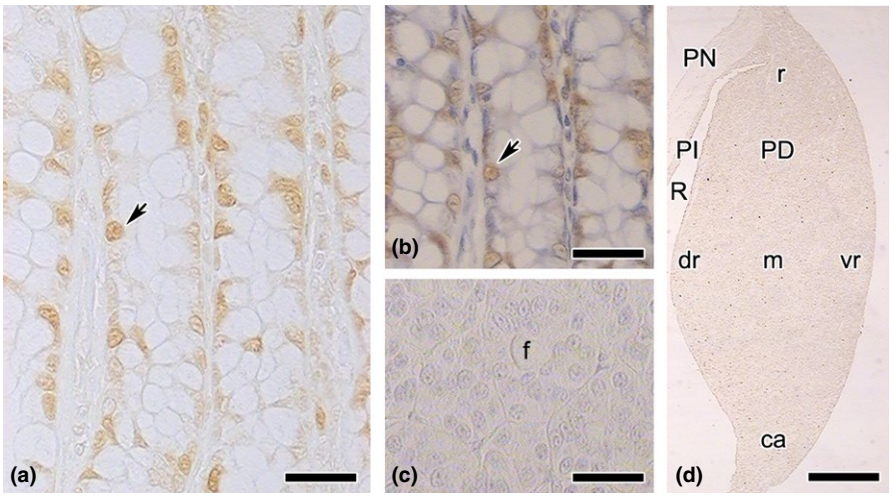

FIGURE 1 Controls of immunohistochemical technique. (a,b) Rat large intestine, a positive control of immunoperoxidase staining with anti-proliferating cell nuclear antigen (PCNA), without haematoxylin staining (a) and counterstaining with haematoxylin (b). (c) Pituitary of viscacha, a negative control of immunohistochemistry counterstained with haematoxylin. Follicular structures, f. (d) Sagittal section of a pituitary from an adult male viscacha showing the different regions. PD, pars distalis; PI, pars intermedia; PN, pars nervosa; R, Rathke's pouch; ca, caudal end; r, rostral end; vr, ventral region; m, medial region; dr, dorsal region. (Immunohistochemical technique with anti-PCNA antibody). (a–c) Scale bar: 25 µm. (d) Scale bar: 500 µm

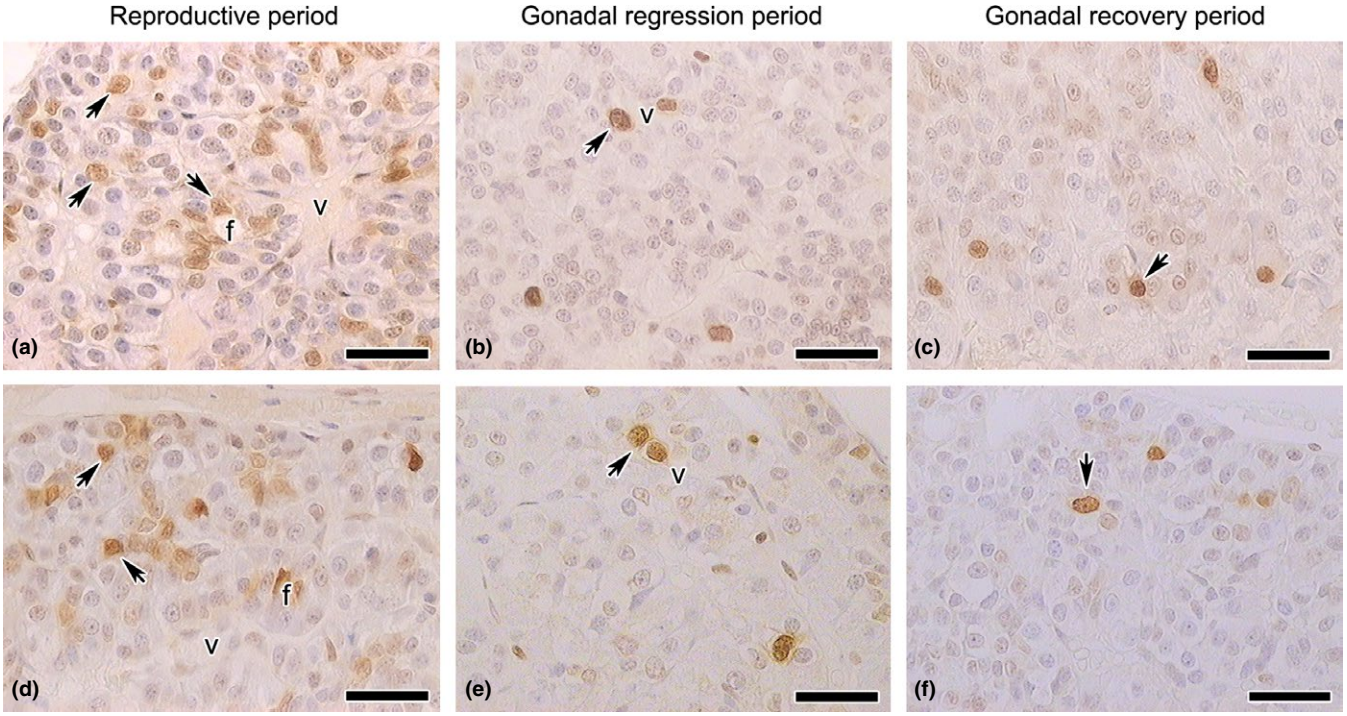

FIGURE 2 Seasonal expression of proliferating cell nuclear antigen (PCNA) in pituitary during the reproductive cycle of adult viscachas. Images of caudal ends (a–c) and ventral regions (d–f). The PCNA-ir cells (arrows) are numerous during the reproductive period, and they are scarce in the gonadal regression and gonadal recovery periods. Follicular structures, f; blood vessels, v. Images counterstained with haematoxylin. Scale bar 25 μm

medial and dorsal regions, rostral and caudal ends). In each image (250–280 cells), the percentage of immunoreactive (-ir) cells in the PD (i.e. percentage of PCNA-ir cell) was determined using the formula $(A/A + B) \times 100$, where A is the number of immunoreactive cells and B is the number of nuclei in immunonegative cells.

2.8 | Statistical analysis

Results are expressed as the mean ± SEM. The significance of differences between groups were evaluated by one-way analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test. Differences between the two groups (control group vs experimental group) were evaluated using Student's *t*-test. A *P* value of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Annual reproductive cycle

In PD of adult viscachas, numerous PCNA-ir cells were mainly located at the caudal end, and in the ventral and dorsal regions during the reproductive period. The nucleus was frequently spherical, and some of them were oval in shape. Positive nuclei near follicular structures, and scarce mitotic figures were observed. In the gonadal regression and gonadal recovery periods, few positive nuclei were found at the caudal end and dorsal region of PD, which were large and oval in shape (Fig. 2).

TABLE 1 Percentage of PCNA-ir cells in pituitary PD of adult viscachas

	Reproductive period	Gonadal regression period	Gonadal recovery period
Total % PCNA-ir cells	1.40 ± 0.16[a]	0.52 ± 0.06[b]	0.36 ± 0.01[b]
Caudal end	1.60 ± 0.29[c,†]	0.51 ± 0.05[d,†]	0.54 ± 0.02[d,†]
Rostral end	0.73 ± 0.18	0.37 ± 0.07	0.46 ± 0.08[†]
Medial region	0.39 ± 0.07	0.34 ± 0.03	0.32 ± 0.01
Dorsal region	1.23 ± 0.25[c]	0.67 ± 0.04[†]	0.37 ± 0.01[d]
Ventral region	1.26 ± 0.25[c]	0.36 ± 0.04[b]	0.26 ± 0.01[d]

Values are the mean ± SEM (n=4). [a] vs [b]: P<.001; [c] vs [d]: P<.05; reproductive period: [†]P<.01 compared with the rostral end and medial region; gonadal regression period: [†]P<.05 compared with rostral end, medial and dorsal regions; gonadal recovery period: [†]P<.05 compared with medial, dorsal and ventral regions (ANOVA followed by the Tukey-Kramer test).

The total percentage of PCNA-ir cells obtained from the whole reproductive cycle presented a significant increase during the reproductive period compared to the other periods (P<.001). Moreover, the percentage of PCNA-ir cells was significantly different among the PD zones during the reproductive cycle. At the caudal end and in the ventral region, the percentages of PCNA-ir cells were higher during the reproductive period compared to the other periods, whereas in the dorsal region the PCNA-ir cells percentage was higher in the reproductive period in relation to the gonadal recovery period (Table 1).

3.2 | Administration of melatonin

PCNA-ir cells were distributed throughout the parenchyma of the pituitary PD in the control group. The nuclei of these cells were oval or spherical in shape, and some of them were irregular and they were found near follicular structures. In melatonin-administered animals, PCNA-ir cells were observed in all PD zones. Numerous PCNA-ir cells were found on the dorsal and ventral edges of PD. Their nuclei were large, intensely immunolabelled and usually found near follicular structures. Besides, mitotic figures were observed (Fig. 3).

The total percentage of PCNA-ir cells increased significantly in melatonin-administered viscachas compared to the control group ($P<.05$). In most PD zones, except at the rostral end, the percentage of PCNA-ir cells was higher in the melatonin-administered group compared to the control group ($P<.05$; Table 2).

3.3 | Castration

In the control group, PCNA-ir cells were observed throughout the PD parenchyma, mainly located at the caudal end. However, these cells were scarce and distributed throughout the parenchyma in the

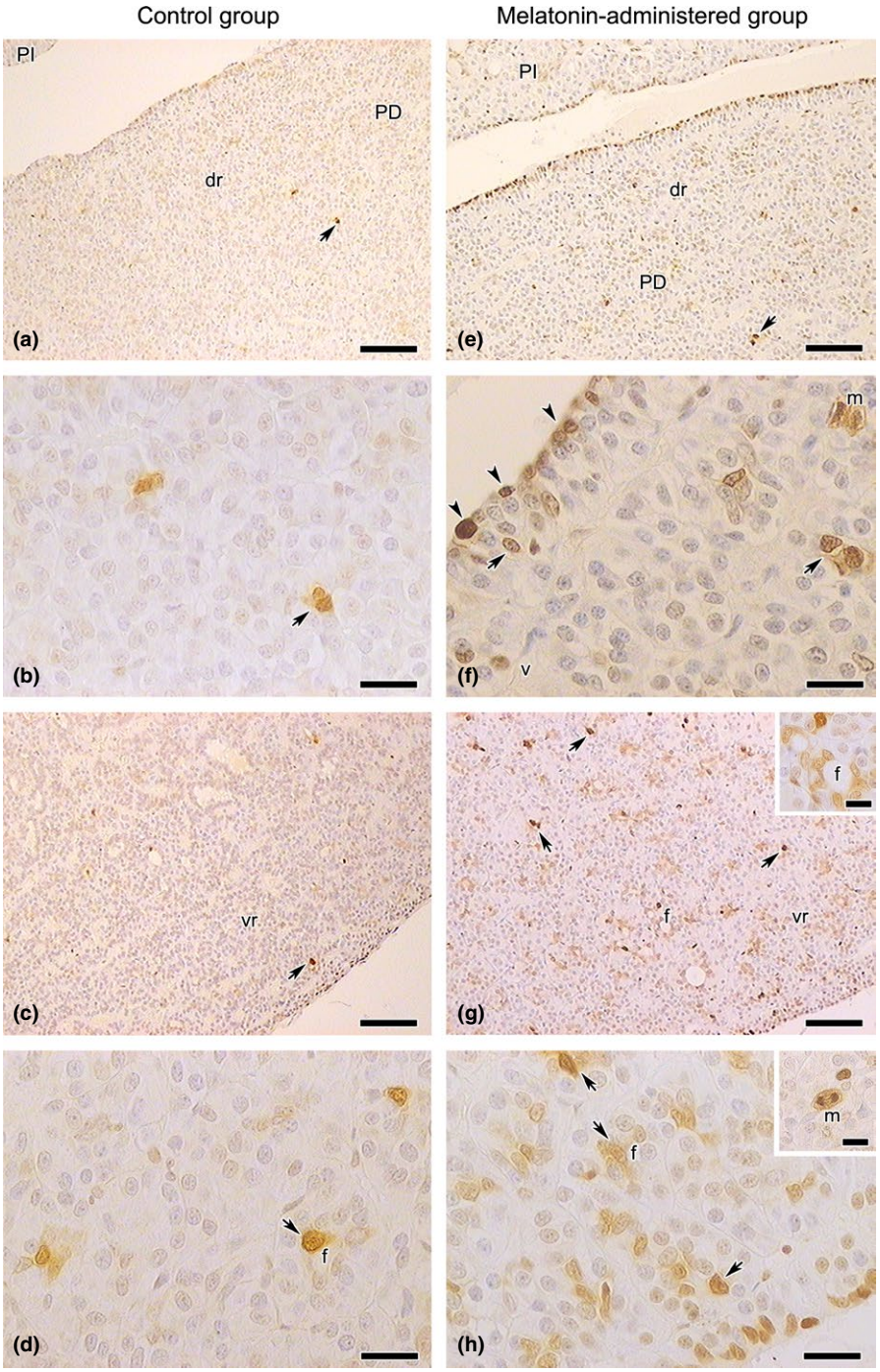

FIGURE 3 (a–d) Pituitaries of adult male viscacha of control group. Few and isolated proliferating cell nuclear antigen (PCNA)-ir cells (arrows) are in the dorsal (a,b) and ventral regions (c,d) of PD. (e–h) Pituitaries of melatonin-administered animals. The number of PCNA-ir cells increased in the dorsal (e, f) and ventral (g,h) regions. There are numerous cells on the dorsal edge (arrowheads). Inset of image g: PCNA-ir cells near follicular structures (f). Inset of image h: a mitotic figure in the ventral region. PD: pars distalis; PI: pars intermedia; dr: dorsal region; vr: ventral region; v: blood vessels; m: mitotic figures. Images counterstaining with haematoxylin. (a, c, e and g) Scale bar 100 μm. (b, d, f and h) Scale bar 25 μm. Insets scale bar 12.5 μm

TABLE 2 Percentage of PCNA-ir cells in PD after melatonin treatment

	Control group	Melatonin-administered group
Total % PCNA-ir cells	1.88 ± 0.36	0.58 ± 0.06*
Caudal end	0.50 ± 0.08	2.30 ± 0.45*
Rostral end	0.64 ± 0.16	1.60 ± 0.44
Medial region	0.55 ± 0.05	1.34 ± 0.28*
Dorsal region	0.66 ± 0.09	2.21 ± 0.52*
Ventral region	0.66 ± 0.02	2.34 ± 0.36**

Values are the mean ± SEM (n=4). *P<.05 and **P<.01 (Student's t-test).

castrated group. The immunolabelled nuclei were spherical or oval in shape, and some nuclei near follicular structures were found (Fig. 4).

The total percentage of PCNA-ir cell decreased significantly in castrated viscachas compared to the control group (P<.05). At the caudal end and the ventral region, the percentage of PCNA-ir cells was lower in the castrated group compared to the control group (P<.05; Table 3).

3.4 | Animals of different sexual maturity

In foetal pituitary PD from midpregnancy, abundant and intensely stained PCNA-ir cells were located in all regions of the PD. The nuclei presented different shapes and sizes. They were frequently oval or spherical, and some of them were irregular. In foetal pituitary PD from late pregnancy, PCNA-ir cells were located at the caudal end and extended towards the rostral end. There were immunoreactive nuclei near follicular structures. Numerous mitotic figures were found in foetal pituitary PD. In pituitary PD of immature animals, the PCNA-ir cells were mainly located at the caudal end. The nuclei were spherical and oval in shape, with intense immunolabelling. The mitotic figures were scarce. In prepubertal viscachas, the PCNA-ir cells were more numerous and intense in comparison to immature animals. The nuclei of these cells were large, oval or spherical in shape, some of them were less intense and localized near follicular structures (Fig. 5).

The total percentage of PCNA-ir cells was higher in the foetal pituitary from midpregnancy. The total percentage of PCNA-ir cells decreased significantly in foetal pituitary from late pregnancy compared to the foetal pituitary from midpregnancy (P<.01); and in immature compared to foetal pituitary from late pregnancy (P<.05). Finally, the percentage of PCNA-ir cells in prepubertal was higher than in adult viscachas during their gonadal regression and recovery periods (P<.01).

TABLE 3 Percentage of PCNA-ir cells in PD of adult after castration

	Control group	Castrated group
Total % PCNA-ir cells	0.50 ± 0.05	0.24 ± 0.03*
Caudal end	0.80 ± 0.10	0.25 ± 0.01*
Rostral end	0.24 ± 0.09	0.27 ± 0.06
Medial region	0.30 ± 0.05	0.20 ± 0.01
Dorsal region	0.47 ± 0.23	0.27 ± 0.05
Ventral region	0.62 ± 0.06	0.26 ± 0.01*

Values are the mean ± SEM (n=4); *P<.05 (Student's t-test).

In all PD zones from late pregnancy, the percentage of PCNA-ir cells decreased significantly compared to percentages of foetal pituitary obtained from midpregnancy (P<.01). At the caudal end, and in the dorsal and medial regions, the percentage of PCNA-ir cells decreased significantly in immature compared to the percentages of foetal pituitary from late pregnancy (P<.05). Besides, at the caudal end and in the dorsal region, the percentage of PCNA-ir cells increased significantly in prepubertal when compared to the immature viscachas (P<.05). In the medial region, the percentage of PCNA-ir cells decreased significantly in adult compared to the prepubertal viscachas (P<.05; Table 4).

4 | DISCUSSION

This study represents the first description of variations in the expression of pituitary PCNA, a nuclear antigen, indicative of cell proliferation, in a seasonal breeding rodent. The effects of melatonin and gonadal androgens on the proliferation of normal pituitary PD were analysed in adult male viscachas throughout their annual reproductive cycle, after the administration of melatonin and castration, and in viscachas during sexual maturity.

Bibliographic reports referring to seasonal variations of PCNA expression are scarce. Migaud et al.[33] have demonstrated seasonal variations of cell proliferation in brain regions and pituitary pars tuberalis of sheep, suggesting that they are involved in the monitoring of seasonal physiological functions.

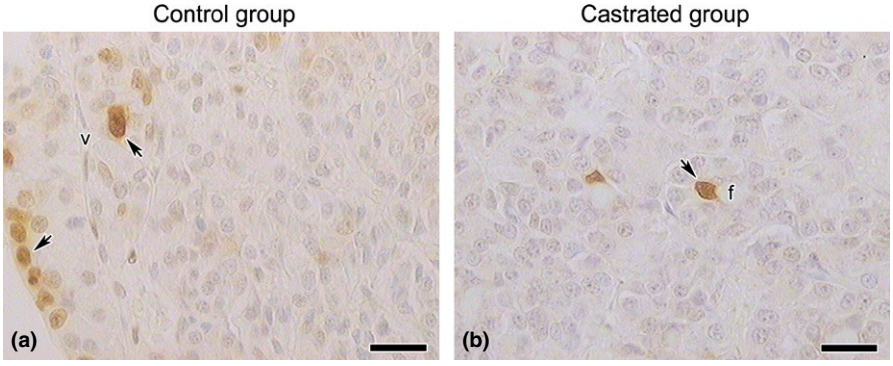

FIGURE 4 PCNA-ir cells in the caudal end of pituitaries from control (a) and castrated (b) viscachas. The number of these cells (arrows) decreases after castration. Follicular structures, f; blood vessels, v. Images counterstained with haematoxylin. Scale bar 25 μm

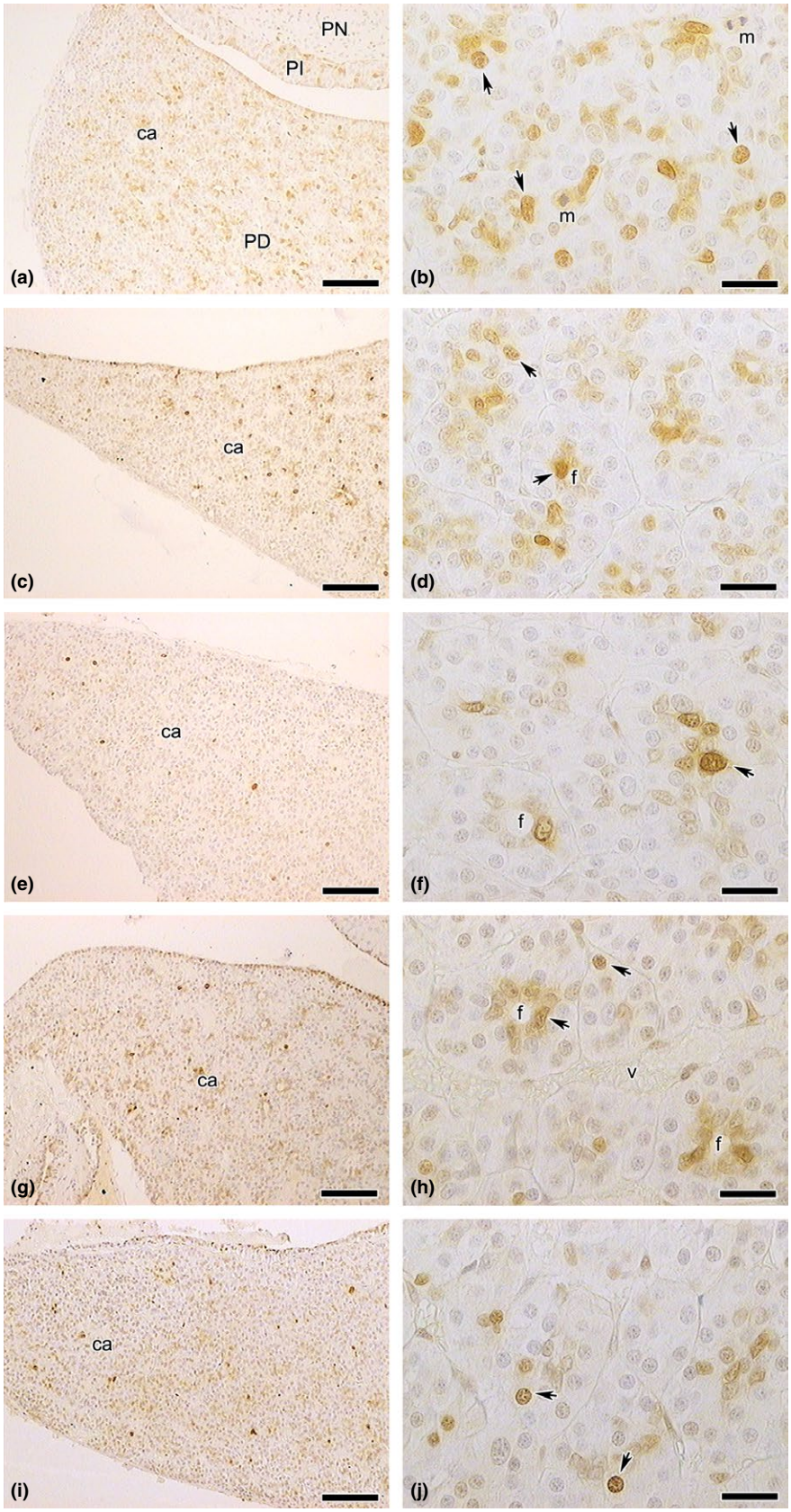

FIGURE 5 Proliferating cell nuclear antigen (PCNA) immunohistochemical staining in the caudal end of viscacha pituitaries during sexual maturity. (a,b) Sections of a foetal pituitary from midpregnancy, where PCNA-ir cells are abundant. There are mitotic figures (m). (c,d) Images of a foetal pituitary from late pregnancy showing a decrease in the quantity of PCNA-ir cell. (e,f) A small number of PCNA-ir cells are present in a pituitary gland of immature viscacha. (g,h) These cells increase in a pituitary gland of prepubertal viscacha. (i,j) Pituitary of an adult viscacha during their reproductive period. Follicular structures, f; blood vessels, v. Images counterstained with haematoxylin. (a, c, e, g and i) Scale bar 100 μm. (b, d, f, h and j) Scale bar 25 μm

More recently, Fredrich et al.[34] have pointed out that melatonin signalling appears to be crucial in both the generation and timing of proliferation and apoptosis regulating the physiological cell turnover in the adult neuroendocrine system. Fornas et al.[35] have demonstrated that melatonin modulates the proliferation mechanisms of an experimental rat pituitary cell line. Some researchers have observed

TABLE 4 Percentage of PCNA-ir cells in PD of male viscacha during sexual maturity

	PM-Foetus	PF-Foetus	Immature	Prepubertal	Adult-Rep. P
Total % PCNA-ir cells	8.17 ± 0.77	1.88 ± 0.29**	0.88 ± 0.16*	1.45 ± 0.19	1.40 ± 0.16
Caudal end	7.65 ± 0.78	2.70 ± 0.04**, †	1.20 ± 0.07*, †	2.12 ± 0.29*, †	1.60 ± 0.29†
Rostral end	7.51 ± 1.20	0.27 ± 0.10**	0.59 ± 0.09	0.45 ± 0.18	0.73 ± 0.18
Medial region	8.52 ± 1.30	1.94 ± 0.36**	0.89 ± 0.18*	1.19 ± 0.07	0.39 ± 0.07*
Dorsal region	7.99 ± 1.23	2.01 ± 0.41**	0.70 ± 0.15*	1.74 ± 0.25*	1.23 ± 0.25
Ventral region	8.34 ± 1.60	1.71 ± 0.28**	0.97 ± 0.09	1.26 ± 0.25	1.26 ± 0.25

Values are the mean ± SEM (n=4); **P<.01 and *P<.05 compared with the value of the previous group (ANOVA followed by the Tukey-Kramer test). PF-Foetus: †P<.01 compared with rostral end and ventral region. Immature: †P<.05 compared with rostral end and dorsal region. Prepubertal: †P<.05 compared with rostral end, ventral and medial regions. Adult-Rep P: †P<0.01 compared with the rostral end and medial regions. Rep P: reproductive period.

an anti-proliferative function of the pineal hormone in pituitary[36] and testes.[37] However, under different conditions, the data indicated that melatonin had proliferative and anti-apoptotic effects.[38]

Different variations on pituitary cell proliferation have been reported in castrated rats. No changes in the mitotic cell number in pituitary from castrated adult rats after 2 weeks were observed by Sakuma et al.[12] Nolan and Levy[5] have reported that gonadectomy resulted in a 3-fold increase in mitotic activity by the fourth post-surgery day, which returned gradually to levels for intact animals over the subsequent 3–4 weeks. In the pituitary, the enzyme aromatase P450 is responsible for the aromatization of androgens into oestrogens, which stimulates proliferation of some cell types. Besides, the expression of this enzyme decreased after castration suggesting that gonadal testosterone could stimulate the pituitary aromatase, thus altering the regulation of adenohypophyseal cytology.[10,39]

Previously, seasonal studies carried out in our laboratory showed a lower melatonin serum level, a higher testosterone serum level and the expression of AR in viscacha pituitary PD during the reproductive period compared to the gonadal regression period.[17,26,40] Moreover, during the gonadal activity, an increase in the percentage of LH-gonadotrophs,[28] corticotrophs,[20] somatotrophs,[21] thyrotrophs,[24] and lactotrophs[22] was observed.

In the present study, seasonal changes of the regionalization of PCNA-ir cells were observed in pituitary PD. The regionalization of PCNA-ir cells is probably related to the maintenance and cellular renewal processes of different cell types depending on the period of their annual reproductive cycle. The total percentage of cell proliferation presented variations during the annual reproductive cycle, being higher in the reproductive period and lower in the gonadal regression (2.7-fold) and gonadal recovery (3.8-fold) periods. The functional relevance of these seasonal changes in cell proliferation rates might contribute to the seasonal variations in hormonal secretion.

The number and size of LH-gonadotrophs increased during the reproductive period,[28] suggesting that the number of these cells might increase. However, it should be considered that other cell populations might also proliferate or be renewed during this period.

Few studies have considered the effects of melatonin on pituitary in vivo. Our data indicated that exogenous melatonin had a proliferative effect on the pituitary PD of viscachas. The significant increase in cell proliferation appears to be primarily on the dorsal and ventral edges, a part from the parenchyma growth from the inner PD. Proliferation from within the parenchyma was observed and follicular structures might be involved in this process because numerous PCNA-ir were found near them. Thus, an increase of PCNA-ir cells was observed in the melatonin-administered animals. These results suggest that melatonin might directly or indirectly affect the PD cells, in order to regulate PD cell proliferation.

The castration was performed during the reproductive period of adult viscachas, allowing for morphological analysis of the effects of the lack of gonadal androgens on pituitary PD cells. Previous studies have demonstrated a reduction in gonadotropic cells and AR expression after castration.[25] In this study, a decrease of the amount of PCNA-ir cells in PD was observed 6 weeks after castration suggesting that gonadal androgen impacts cell proliferation mainly at the caudal end and ventral region of pituitary PD of male viscachas.

In addition to the seasonal study on cell proliferation, we analysed the effect of both melatonin administration and castration. The results obtained under these experimental conditions demonstrated that high levels of exogenous melatonin increase cell proliferation. Instead, castration or lack of gonadal androgens results in a decrease in cell proliferation. Thus, these hormones might have an opposite effect on pituitary PD cells of viscachas.

It has been demonstrated that the proliferative activity of the anterior pituitary of rat decreases with growth.[13,41] In addition, Sakuma et al.[12] have described changes in regionalization of mitotic cells according to age, reaching the highest values in the cells of immature rats due to an increase of somatotroph population.

In the foetal viscacha pituitary from midpregnancy, a large amount of cell proliferation was observed in all areas of PD, indicating that this moment of pregnancy is key to the foetal pituitary development and that it correlates with the previously reported characteristics of maternal pituitary.[30] In addition, higher progesterone and androstenedione levels were observed during pregnancy,[29] which might be related to the increase of PCNA-ir cells in the foetal pituitary. During the late pregnancy, a significant decrease in cell proliferation from the foetal gland was observed, and they were regionalized at the caudal, and in the dorsal and medial region. In the post-natal life, the percentage of PCNA-ir cells in PD exhibited low values, and the regionalization of PCNA-ir cells was maintained at the caudal end. However, many cells appeared at the caudal end and in the dorsal

region of prepubertal pituitary. These variations might be due to the proliferation of different cell types during the sexual maturation resulting from androgen. The testosterone serum level and the nuclear AR expression increased in prepubertal viscachas,[26] which might stimulate some mechanism of cell proliferation in PD. The presence of numerous PCNA-ir cells near follicular structures, in both the prepubertal and melatonin-administered animals, suggests that the cells that are part of the follicles probably have some regulatory action of cell proliferation in PD.

The present morphological study demonstrated that the percentage of PCNA-ir cells varied seasonally, after castration, and during sexual maturity, indicating the effect of gonadal androgens. On the other hand, an increase of PCNA-ir cells was observed after melatonin administration. These results suggest that gonadal androgens and melatonin might act by means of different regulation mechanisms. Future studies are needed to understand the hormonal regulation of cell proliferation in the pituitary PD of viscachas.

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CONFLICT OF INTERESTS

The authors declare that no conflict of interests is present in this paper.

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