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LONG-TERM ADMINISTRATION OF PROLACTIN OR TESTOSTERONE INDUCED SIMILAR PRECANCEROUS PROSTATE LESIONS IN RATS

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Evidence indicates that prolactin plays a crucial role in the normal function and development of the prostate, but abnormal high levels of the hormone are associated with hyperplasia and cancer of the gland. *Aims:* The present study was designed to describe the progressive specific histological abnormalities in the prostate of rats with chronic hyperprolactinemia. *Material and Methods:* Prolactin was administered during 4; 12 or 24 weeks, and the resulting prostatic alterations were compared with control rats, and also with those treated with testosterone, or the combination of prolactin + testosterone. *Results*: Rats treated with prolactin, testosterone or prolactin + testosterone expressed precancerous histological abnormalities in the dorsolateral and ventral portions of the prostate as early as in 4 weeks of treatment, but in all cases the malignancy increased after 12 or 24 weeks of treatment. *Conclusion*: Our study confirms that chronic hyperprolactinemia is a cause of prostate precancerous pathologies.

***Key Words*: prolactin, prostate, cancer, dysplasia, testosterone.**

Among all types of tumors in men, prostate cancer (CaP) is the second most common worldwide (≈ 15%) [1]. Every year, hundreds of thousands of new cases of CaP are detected and up to 6.6% of them result in death [1]. The etiology of the disease is diverse, and may include family history, age (> 65) [2], race (> african-ancestry) [3], diet (> high-fat products) [4], obesity (body mass index (BMI) > 25) [5], and bad habits, such as smoking [6]. For that reason, treatments are also diverse and may include surgery, radiation, chemotherapy, vaccines, or hormone treatments. The latter is mainly focused on the androgen deprivation therapy (ADT), and it is perhaps, the first choice therapy [7].

The goal of ADT is to reduce the levels of androgens that reach the prostate. ADT can slow down the growth of the gland and may decrease its size. CaP has been classically associated with androgens not only because they induce cell division, but also because they are associated with spontaneous mutations that result in more and abnormal cells within the gland [8, 9]. Paradoxically, by the age of 65 the blood levels of androgens, i.e. testosterone (T) decrease by more than 50% as compared to the levels observed at age 20 [10–13], and other hormones such as prolactin (PRL) increase by at least 30% above normal levels in elderly [14]. A similar shift in the concentrations of those hormones is observed in studies of the prostate of laboratory rats. For instance, treatment with dopamine blockers results in a twofold increase of serum PRL in males, but also results in a twofold decrease in T [15]. PRL has both direct and indirect effects on the gland, and interacts with androgens in normal and abnormal processes [16, 17]. In fact, CaP patients express higher blood levels of PRL and less T [18], as compared to healthy people of the same age [19–21].

Animal models and cell lines support the idea that PRL, via its receptor (PRLR), activates the Jak2-Stat5a/b pathway that may result in tumorigenesis [22]. So far, studies on laboratory rats have shown that treatment with PRL results in enlargement of the prostate [17, 23], suggesting that high levels participate in the histological alterations in the gland. However, the timed progression and the specific histological alterations caused by high levels of PRL during long periods have not been described yet. Thus, in the present study we assessed the effects of treatment with PRL during 4; 12 or 24 weeks on laboratory rats in comparison with treatment with T alone, or PRL + T combination. We hypothesized that PRL would induce prostatic precancerous lesions as severe as those induced by T. In addition, we hypothesized that PRL + T combination would induce more severe alterations than either of the two hormones given alone.

MATERIAL AND METHODS

Animals. Fifty-four Wistar male rats were used (*Rattus norvegicus albinus)*. They were purchased from a certified animal supplier in Mexico (Rismart®) and were 12 weeks old at the start of the study. The rats were housed in large plexiglas cages (50 × 30 × 20 cm), and kept in a colony room at the Center for Brain Research, University of Veracruz in a 12–12 h reverse Light-Dark cycle (lights off at 8:00 h). Water and commercial feed (Rismart® rat chow) were provided *ad libitum*. All the experimental procedures were approved by an admission committee of the graduate program in neuroethology, following the Official

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Abbreviations used: ADT – androgen deprivation therapy; AR – androgen receptor; BPH – benign prostatic hyperplasia; BMI – body mass index; CaP – prostate cancer; DLP – dorsolateral prostate; DNA – desoxyribonucleic acid; i.p. – intraperitoneal; N:C – nucleus:cytoplasm ratio; PRL – prolactin; PRLR – prolactin receptor; s.c. – subcutaneous; T – testosterone; VP – ventral prostate.

Mexican Norm NOM-062-ZOO-1999 for use and care of laboratory animals.

Hormone treatments. Rats were randomly organized in 5 groups, depending on the hormone treatment they received. 1) The PRL group received intraperitoneal (i.p.) injections of ovine PRL (SIGMA®), of a dose of 1.7 IU diluted in 100 ml of injectable grade water, equivalent to 50 µg/100 ml of PRL every 12 h. 2) The T group received a subcutaneous (s.c.) silastic implant in the back (Dow Corning Corp®. 062X0125 ID); filled with T propionate (100 mg/kg of body weight). The implant allowed the T propionate (T) to be constantly released, which maintained high plasma levels of the hormone as previously shown [24]. 3) The PRL + T group received both treatments. 4). The control group was injected with saline solution (0.9%) 100 µl, i.p. every 12 h, and received an empty s.c. implant in the back. 5) The intact group comprised the animals that received no treatment, but were handled during the same time as experimental animals. Each group was further divided in 3 subgroups, depending on the number of weeks they received treatment (4; 12, or 24).

Prostate samples and histology. One day after the end of hormone treatment of each group, the rats were deeply anesthetized with sodium pentobarbital (35 mg/kg i.p.). Then, the accessory sexual organs were carefully removed and placed into a container with isotonic saline solution. Anesthetized rats were then sacrificed with a second overdose of pentobarbital. The prostate was identified under a dissecting microscope (MEJI, EMZ-TR®), and was further divided into ventral (VP) and dorsolateral (DLP) portions. The VP and DLP were immersed in formol 10% during 24 h, then dehydrated in alcohol 70% and 80% (1 h each), 95% (3×2 h each), and ethanol 100% overnight, and two more changes (1 h each) the following day, and finally in xylene (3×1 h each). The tissue was then embedded in paraffin wax 2×2 h each), sliced (5 µm thick) with a microtome (RM 2125RT Leica®), mounted on slides in a bath at 52 °C (containing pork skin-based gelatin 2.5 mg/100 ml) and then processed for Hematoxylin-Eosin dye technique as follows: 1 h at 57 °C, deparaffinization in xylene (3×5 min each), rehydrated in alcohol/xylene (1:1) 5 min, ethanol 96% 3 min, hematoxylin (10 min), water (30 s), acid alcohol (quick immersion), water (10 s), lithium carbonate (30 s), water (10 s), and eosin (4 quick immersions). Dehydration occurred in ethanol 96% (3 min), ethanol 100% (2 min), ethanol/xylene 1:1 (2 min), and xylene (5 min). Then, permount was added and slides were coverslipped, air dried, and observed under a light microscope (Olympus Ax70). Photomicrographs were taken at × 40 magnification and analyzed by the same experimenter (DH).

RESULTS

A thorough analysis did not demonstrate any histological abnormalities in rats from groups 4 and 5 (control and intact, respectively) after 4; 12 or 24 weeks. In the DLP of intact rats the epithelium was cubic, homogeneous in size, with scarce papillae. The interstitial space was homogeneous and contained collagen-like eosinophilic reticular areas. The cell nuclei were homogeneous in size, had a polar position towards the base with a nucleus-cytoplasm ratio (N:C) of 1:4, and chromatins were homogeneous and dispersed. The myoepithelium was euplasic and the pattern observed at low magnification was tubular (Fig. 1, *a*, *e*, *i* and Table 1). The VP of intact rats was also normal at 4; 12 or 24 weeks of observation. The epithelium was columnar, homogeneous in size, and formed scarce papillae. The interstitial space was homogeneous and contained collagen-like eosinophilic reticular areas. Nuclei were homogeneous in size, with a polar position location towards the base, with a N:C ratio of 1:4, and the chromatins were homogeneous. The myoepithelium was euplasic and the pattern observed at low magnification was tubular (Fig. 2, *a*, *e*, *i* and Table 2).

Unlike the rats from the intact or control groups, those treated with PRL, T or PRL + T displayed histological abnormalities in the DLP and VP as early as in 4 weeks of treatment, but the degree of malignancy in the lesions increased after 12 or 24 weeks. For instance, after 4 weeks of treatment with PRL alone, the epithelium of the DLP was not longer cubic, but columnar (metaplasic). There was hyperthrophy and hyperplasia, the interstitial space was not longer observable, but was rather compressed, and the cell nuclei were heterogeneous in size (anisokaryiosis) [25, 26]. Other features such as myoepithelium, pattern, lumen content and chromatin were apparently normal at 4 weeks of treatment, but were abnormal after 12 or 24 weeks. Specifically, after 12 or 24 weeks of treatment with PRL alone the epithelium showed dysplasia, there were many papillae, the interstice contained many mononuclear cells, there was anisokaryosis, the nuclei were not longer polar, the N:C ratio was abnormal 1:1, the chromatin was compact, and the myoepithelim was proplastic (Fig. 1, *b, f, j* and see Table 1). In the VP similar abnormalities were observed after 12 weeks of PRL (Fig. 2, *b, f, j* and see Table 2). The effects of treatment with T were identified ahead of those induced by PRL treatment. For example, with only four weeks of treatment rats that received T or PRL + T expressed more severe histological alterations than both the DLP and VP rats treated with PRL alone. The epithelium showed dysplasia (with T) or severe dysplasia (with PRL + T) within the first month. In addition, the N:C ratio was 1:1 in T or PRL + T groups, and the polarity of the nucleus was completely lost. Altogether, these data indicate that PRL alone was sufficient to induce precancerous alterations in the prostate of rats. The lesions were similar to those caused by T alone at 12 or 24 weeks, but the latter induced more alterations during the first 4 weeks. In addition, the combination of PRL + T increased the degree of malignancy, even after 4 weeks of treatment (Fig. 1, *c, d, g, h, k, l* and 2, *c, d, g, h, k, l*).

DISCUSSION

Normal levels of PRL contribute to the growth and functioning of the prostate [27]. PRL and its receptor (PRLR) are present in the gland during both fetal

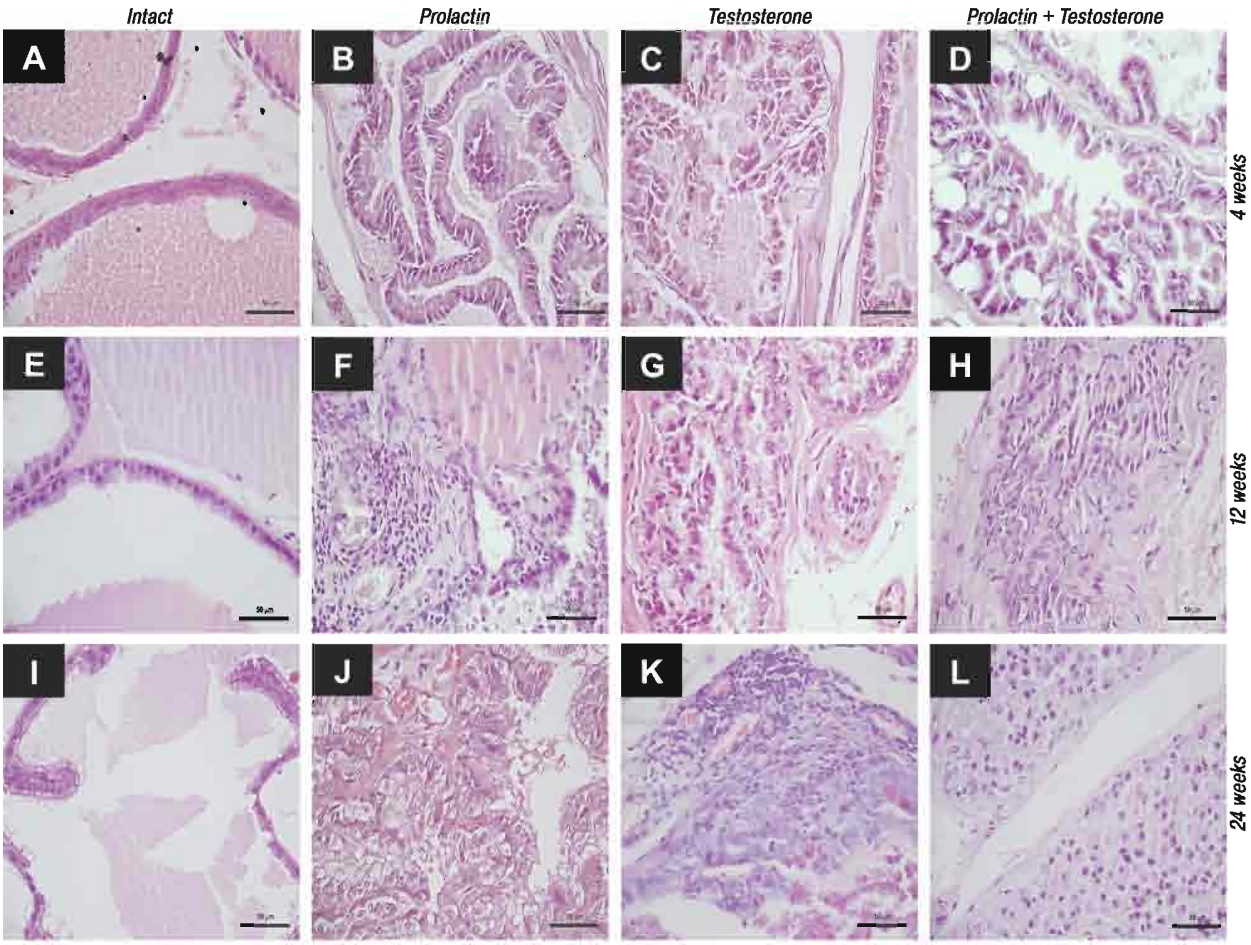

Fig. 1. Dorsolateral prostate (× 40). Rats were treated during 4; 12 or 24 weeks with prolactin (PRL), testosterone (T), both (PRL + T), or served as controls. Histological abnormalities were observed as early as 4 weeks of treatment (see Table 1 for details). Bar indicates 50 μm

development and adulthood [22, 28–30], and they are needed to maintain a normal weight and functions of the prostate. In one study with pup rats the low levels of PRL (reduced with injections of a rabbit anti-rat PRL serum) resulted in significant reduction of the weights of the prostate and seminal vesicles [31]. By contrast, abnormal high PRL levels result in an enlargement of the prostate [17, 23, 24, 32]. Earlier we have reported that prostate enlarges in rats when PRL in blood during two continuous weeks

Table 1. Dorsolateral prostate

Dorsal-lateral prostate	4 weeks				12 weeks				24 weeks			
	Intact	PRL	T	PRL + T	Intact	PRL	T	PRL + T	Intact	PRL	T	PRL + T
Epithelium												
Form	cubic	columnar	dysplasia	Severe dysplasia	cubic	dysplasia	dysplasia	severe dysplasia	cubic	dysplasia	dysplasia	severe dysplasia
Size	even	HT, HP	anisocytosis	anisocytosis	even	HT, HP	anisocytosis	anisocytosis	even	HT, HP	anisocytosis	anisocytosis
Papillae	scarce	few	few	few	few	few	few	many	few	many	many	many
Interstice												
Space	even	compressed	compressed	compressed	even	compressed	compressed	compressed	even	compressed	compressed	compressed
Content	collagen-like	collagen-like	collagen-like	collagen-like	collagen-like	mononuclear cells	mononuclear cells	mononuclear cells	collagen-like	mononuclear cells	mononuclear cells	mononuclear cells
Nucleus												
Size	even	anisokaryosis	anisokaryosis	anisokaryosis	even	anisokaryosis	anisokaryosis	anisokaryosis	even	anisokaryosis	anisokaryosis	anisokaryosis
Location	polar	polar	no polar	no polar	polar	no polar	no polar	no polar	polar	no polar	no polar	no polar
N:C ratio	1:4	1:4	1:1	1:1	1:4	1:1	1:1	1:1	1:1	1:1	1:1	1:1
Myoepithelim	euplasia	euplasia	proplasia	proplasia	euplasia	proplasia	proplasia	proplasia	euplasia	proplasia	proplasia	proplasia
Pattern (4X)	tubular	tubular	tubular	cribriform	tubular	tubular/ cribriform	tubular	amorphous	tubular	tubular/ cribriform	amorphous	amorphous
Lumen												
Content	amorphous	amorphous	amorphous	amorphous	amorphous	amorphous	nothing	nothing	amorphous	nothing	nothing	nothing
Chromatin	dispersed	dispersed	compact	compact	dispersed	compact	compact	compact	dispersed	compact	compact	compact

Rats were treated during 4; 12 or 24 weeks with prolactin (PRL), testosterone (T), both (PRL + T), or served as controls. Histological abnormalities were observed as early as to 4 weeks of treatment. HT = hypertrophy, HP = hyperplasia. Nucleus-Cytoplasm ratio (N:C).

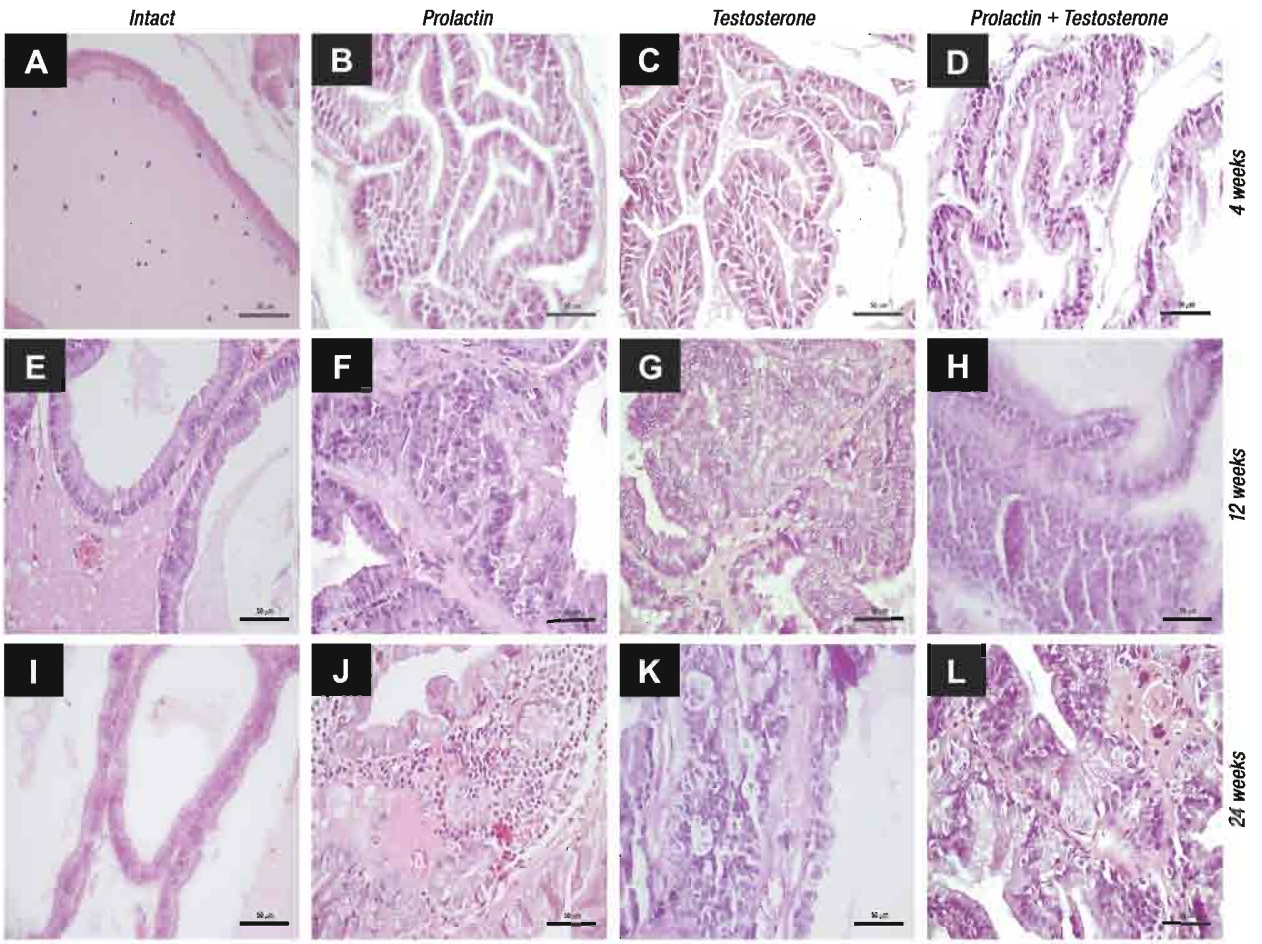

Fig. 2. Ventral prostate (×40). Rats were treated during 4; 12 or 24 weeks with prolactin (PRL), testosterone (T), both (PRL+T), or served as controls. Histological abnormalities were observed as early as 4 weeks of treatment (see Table 2 for details). Bar indicates 50 μm

reaches chronic steady levels of about 40 ng/ml [33] exceeding only by 5% the normal highest PRL levels expressed in every circadian cycle (12–38 ng/ml) [24]. Human patients with benign prostatic hyperplasia (BPH) have higher blood levels of PRL, and the same occurs in patients with CaP [21]. Prostate may not depend on pituitary PRL for its growth since the gland itself can produce PRL by its secretory epithelium [29]. Furthermore, high levels of PRL are also found in obese individuals, suggesting that the adipose tissue itself may function as an endocrine organ [34–36]. Interestingly, the expression of PRLR is reduced

Table 2. Ventral prostate

Ventral prostate	4 weeks				12 weeks				24 weeks			
	Intact	PRL	T	PRL + T	Intact	PRL	T	PRL + T	Intact	PRL	T	PRL + T
Epithelium												
Form	columnar	columnar	dysplasia	dysplasia	columnar	dysplasia	dysplasia	dysplasia	columnar	dysplasia	dysplasia	severe dysplasia
Size	even	HT, HP	anisocytosis	anisocytosis	even	HT, HP	anisocytosis	anisocytosis	even	HT, HP	anisocytosis	anisocytosis
Papillae	scarce	few	few	few	few	few	few	many	few	many	many	many
Interstice												
Space	even	compressed	compressed	compressed	even	compressed	compressed	compressed	even	compressed	compressed	compressed
Content	collagen-like	collagen-like	collagen-like	collagen-like	collagen-like	mononuclear cells	mononuclear cells	mononuclear cells	collagen-like	mononuclear cells	mononuclear cells	mononuclear cells
Nucleus												
Size	even	anisokaryosis	anisokaryosis	anisokaryosis	even	anisokaryosis	anisokaryosis	anisokaryosis	even	anisokaryosis	anisokaryosis	anisokaryosis
Location	polar	polar	no polar	no polar	polar	polar	no polar	no polar	polar	no polar	no polar	no polar
N:C ratio	1:4	1:4	1:1	1:1	1:4	1:1	1:1	1:1	1:1	1:1	1:1	1:1
Myoepithelim	euplasia	euplasia	proplasia	proplasia	euplasia	proplasia	proplasia	proplasia	euplasia	proplasia	proplasia	euplasia
Pattern (4X)	tubular	tubular	tubular	cribriform	tubular	tubular/ cribriform	tubular	amorphous	tubular	tubular/ cribriform	amorphous	amorphous
Lumen												
Content	amorphous	amorphous	amorphous	amorphous	amorphous	amorphous	nothing	nothing	amorphous	nothing	nothing	nothing
Chromatin	dispersed	dispersed	compact	compact	dispersed	compact	compact	compact	dispersed	compact	compact	compact

Rats were treated during 4; 12 or 24 weeks with prolactin (PRL), testosterone (T), both (PRL+T), or served as controls. Histological abnormalities were observed as early as to 4 weeks of treatment. HT = hypertrophy, HP = hyperplasia. Nucleus-Cytoplasm ratio (N:C).

in prostate of the patients with the high grade cancer (>4 in Gleason scale) [28]. However, it has been argued that such reduction of PRLR may be associated with the presence of poorly differentiated prostatic cells, which express less PRLR [28].

Our results indicate that in rats, high levels of PRL during periods of 4 weeks or more result in histological alterations of the prostate that may be considered as precancerous. The PRL-induced lesions are very similar to those induced by T alone, but the combination of both hormones results in more severe histological alterations after 4 weeks. Anysokaryosis, polarity and alterations in N:C ratio indicate the changes in chromosome organization, which in turn can affect gene expression [37] resulting in dysplasia. In addition, the myoepithelium was proplastic, and the chromatin was compact, which indicate that both hormone treatments did activate cell division. In addition, the presence of mononuclear cells in the interstice and the absence of the luminal content (see Tables 1, 2), suggest the activation of inflammation mechanisms as well. The combination of PRL + T resulted in similar, but more severe alterations. These results also indicate a synergistic effect of two hormones in the development of prostate pathology.

Therefore, according to the present study and others [22, 38–40], individuals with chronic high levels of PRL for 4 weeks or more may express abnormal precancerous histological features in the prostate. Altogether, the data support the idea that regardless of the source of PRL (e.g. systemic, pituitary, prostatic, or adipose) it may be responsible of histological alterations of the prostate that may become precancerous, even in individuals with low levels of androgens. In the last decade, some preclinical trials have also contributed to understanding the positive effect of anti-PRL treatment on CaP [41–44]. However, better outcomes have been found with the combined suppression of androgens and PRL together [45]. Further research is needed to understand the specific role of these hormones in the development and maintenance of prostate pathologies including cancer [46, 47].

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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