

# Morphological and immunohistochemical compare of three rat prostate lobes (lateral, dorsal and ventral) in experimental hyperprolactinemia.

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**Abstract:** The prolactin plays an important role in the regulation of growth and differentiation of prostate gland besides androgens. The goal of this study was to reveal the influence of elevated prolactin concentration on epithelial cells of prostate. We compared the morphology of epithelial cells of prostate dorsal, lateral and ventral lobes and expression of androgen receptors in these cells in rats with hyperprolactinemia and in control rats. We used sexually mature male Wistar rats. The experimental rats received metoclopramide; the control group received saline in the same way. The prostate dorsal, lateral and ventral lobes were collected routinely for light and electron microscopy. The intensity of immunohistochemical reaction of androgen receptor in epithelial cells of dorsal, lateral and ventral lobes was evaluated by measure of optical density with computer image analysis. The light and electron (transmission and scanning) microscopes were used for morphological observations. Results: In experimental rats twofold increase in prolactin and twofold decrease in testosterone found. In experimental group the expression of androgen receptor was lower in columnar epithelial cells of dorsal and ventral lobes but higher in lateral one. We observed morphological abnormalities in columnar epithelial cells of lateral and dorsal lobes. The columnar epithelial cells of ventral lobes didn't show any morphological changes in hyperprolactinemia.

**Key words:** hyperprolactinemia, androgen receptor, prostate lobes, testosterone, prolactin

## Introduction

General pattern of prostate gland is common to all the rodents and human, however there are important details differing species. Rat prostate reveals evident lobular structure, whereas adult human one is encapsulated with connective tissue, which enables recognition of distinct lobes. The rat prostate is divided into distinct ventral, dorsal and lateral lobe pairs (VP, DP, LP) according to relative position to urine bladder. [1]. Human prostate reveals lobes only in early development. Afterward connective tissue joins lobes in one solid gland. In the 1980-ies McNeal introduced clinical

division of prostate into four zones: the anterior, central, peripheral and transitional according to urethra position [2,3]. Each lobe of the rat prostate is formed with secretory portions and branching excretory ducts system, embedded in delicate stroma. In ventral and lateral lobes there are a few main ducts with innumerable but wide branches similar to oak tree branching, whereas in dorsal lobe there are numerous main duct with narrow, short but numerous branches similar to palm tree branching [4]. There also is visible unequal proportion epithelium/connective tissue between rodents 5/1 and human 1/1. [1]

The function of prostate gland is mainly regulated by androgens. These steroid hormones are responsible for growth, proliferation and inhibit death (survival) of epithelial cells [5]. Their biological effects are mediated by androgen receptors (AR). This receptor is located both in man and woman in different age and also in

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different organs, but not only in male and female reproductive system [5,8]. There are two forms of these receptors: nuclear and cytosolic [9]. In rats AR were detected in all three lobes of prostate, mainly in epithelial but also in stromal cells. The androgen receptors were not detected in basal cells of rats, but in human – these receptors were detected in basal and columnar cells [5,10,11]. In rats the regulation of AR expression is different in three separate lobes. Nuclear AR expression, in ventral and dorsal lobes, is primary regulated by androgens, but in the lateral lobe it is androgen independent. Cytosolic AR has not been detected in lateral lobe. In dorsal and ventral lobes cytosolic AR expression is regulated by androgen [9]. The other hormone, which regulates the expression of AR is prolactin (PRL), but this only a case of lateral lobe epithelial cells [12].

Prolactin affects the morphology and function of prostate [13-19]. The hormone is derived not only from pituitary gland but also is synthesized locally in prostate [20,22]. Nevalainen *et al.* [20] observed locally synthesis of PRL in lateral and dorsal lobe of rat prostate. The PRL is produced in epithelial cells [21] and in smooth muscles of human prostate too [22].

Prolactin is a physiological regulator of prostate activities and plays an important role in prostate growth, development, proliferation and function. The lateral lobe of rat prostate is an essential target for PRL. The lateral, dorsal and coagulating lobes are homologous with the human prostate [13,15]. The biological effect of PRL is mediated by membrane bound receptors [23]. In rats the prolactin receptors are located in epithelial cells of all three lobes, in basal, lateral and apical parts of the cells [10,24,26]. The major function of the prostate is the production and secretion of high levels of citrate. This unique function in lateral lobe is dependent of PRL, but in ventral lobe is dependent of testosterone [15]. The PRL and T are response of prostate uptake of zinc. The lateral lobe accumulates the highest amount of zinc as compared to other tissues of the organism [15,17]. The PRL stimulates secretion of prostate proteins and conversion T to DHT [14,15,27].

The hyperprolactinemia influences morphology and function of prostate [28,30]. Prins [10,12] showed that hyperprolactinemia affects the morphology and the function of rat prostate lateral lobe only. Van Coppenolle *et al.* [25] observed that chronic hyperprolactinemia induced enlargement and inflammation of the lateral lobe without any histological changes on ventral and dorsal lobes. Ahonen and *et al.* [19] showed that PRL acts as a survival factor for epithelial cells of dorsal and lateral lobe, but not for ventral prostate. The prolactin does not influence on the ventral lobe [15,25,26,29]. However, the ventral lobe is more sensitive for androgens and estrogens [31,32].

**Table 1.** Prolactin (PRL) and testosterone (T) serum concentrations in control rats and in rats receiving MCP (mean  $\pm$ SD).

Group	PRL (ng/mL) M $\pm$ SD	T (ng/mL) M $\pm$ SD
Control	13,7 $\pm$ 3,4	3,42 $\pm$ 1,9
Experimental	28,6 $\pm$ 5,2 ***	1,35 $\pm$ 0,8 *

\*\*\* –  $p < 0,001$  ; \* –  $p < 0,05$

We have reported previously that hyperprolactinemia following administration of different drug in male rats influenced the expression of AR and caused morphological changes in the epithelial cells of lateral prostate and dorsal lobes independently from decrease of testosterone serum level [28-30,33]. The goal of this study was to compare the expression of AR in epithelial cells of lateral, dorsal and ventral lobes and morphology of these cells in experimental hyperprolactinemia.

## Materials and methods

**Animals.** The experiments were performed on 20 sexually mature male rats of Wistar strain. The animals were randomly divided into control and experimental group. To induce hyperprolactinemia the experimental rats were given metoclopramide (MCP, Polfa Starogard Poland) intraperitoneally in a dose of 2.2 mg/kg b.w. for 14 days (duration of rat seminiferous epithelium cycle). The rats of control group were given saline in the same way. The three lobes of rat prostate (lateral, dorsal and ventral) were routinely collected for light and electron microscopy. The blood was collected from heart for evaluation of serum hormones concentrations.

**Hormone analysis.** Prolactin (PRL) serum levels were measured with rat prolactin ELISA enzyme immunoassay kit (Spi-Bio, France). Testosterone (T) serum levels were measured with radioimmunoassay kit (Farnos Diagnostika, Finland).

**Morphology.** Prostate lobes were obtained during section. For morphological studies prostate lobes were fixed in Bouin's solution and embedded in paraffin. Morphological analysis was carried out after hematoxylin-eosin (H+E) staining.

**Transmission electron microscopy (TEM).** Prostate dorsal lobe was cut into 1 mm<sup>3</sup> pieces, fixed in 0.25 mol/l glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4) for 2 h at 4°C, postfixed in 1% OsO<sub>4</sub>, dehydrated in ethyl alcohol (30-96%) and 100% acetone, and subsequently embedded in Spurr's resin (Polysciences, Inc.). The blocks were cut with Reichert OmU<sub>2</sub> ultramicrotome. The ultra-thin sections were contrasted with uranyl acetate as well as lead citrate, and examined under JEM-1200 EX transmission electron microscope.

**Scanning electron microscopy (SEM).** The specimens for SEM were washed with phosphate buffered solution (PBS), fixed in 0.25 mol/l glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4) for 3h at 4°C. The material was washed again with the buffer and postfixed in 1% OsO<sub>4</sub>, dehydrated in ethyl alcohol (30 – 96%) and 100% acetone. The slides were dried with liquid CO<sub>2</sub> and covered with gold and palladium. The material was examined under a JEOLJSM-6100 scanning electron microscope.

**Table 2.** Parameters of immunohistochemical reaction to AR in the nuclei of epithelial columnar cells in lateral, dorsal and ventral lobes of prostate in control rats and in rats receiving MCP (mean  $\pm$ SD).

	Nuclei of epithelial columnar cells of rat prostate					
	Lateral lobe		Dorsal lobe		Ventral lobe	
	Control (n=575)	Experimental (n=611)	Control (n=575)	Experimental (n=611)	Control (n=598)	Experimental (n=603)
Integrated optical density (IOD)	490 (354-685)	551*** (438-720)	810 (554-1205)	763* (557-1027)	736 (559-974,5)	657* (519-882)
Mean optical density (MOD)	0,56 (0,51-0,87)	0,76*** (0,68-0,84)	0,84 (0,76-0,90)	0,81* (0,74-0,89)	0,97 (0,88-1,03)	0,94* (0,88-1)

\*\*\* –  $p < 0,001$  ; \* –  $p < 0,05$

**Immunohistochemistry.** The expression of AR was determined immunohistochemically in paraffin-embedded specimens fixed in 4% buffered formalin. The reaction was performed on deparaffinized and rehydrated sections (3  $\mu$ m). The radiation of microwave oven (250W) was applied for 30 min in citrate buffer. The endogenous peroxidase was inhibited with 3% H<sub>2</sub>O<sub>2</sub> in methanol for 10 min. The nonspecific binding sites were blocked with 3% normal goat serum (Sigma, USA) for 30 min. The sections were incubated overnight at 4°C with primary antibody: the polyclonal antibody against androgen receptor (NCL-ARp, 1:10, Novocastra Lab, Ltd Newcastle, UK) and subsequently, with secondary antibody, biotinylated goat anti-rabbit IgG (1:400, Vector Lab., Burlingame, CA, USA) for 60 min. In the next step, avidin-biotin-horseradish peroxidase complex (ABC/HRP; 1:100, Dako/AS, Denmark) was applied for 30 min. Diaminobenzidine (DAB) was used to visualize the immunohistochemical reaction. The sections were counterstained with Mayer's haematoxylin. In the control reaction, the primary antibody was replaced by normal goat serum. After each step, sections were rinsed with Tris-buffered saline (TBS). Finally, sections were examined with light microscope.

**Computer image analysis.** To enable the quantitative evaluation of the intensity of immunohistochemical reaction of AR, the optical density was measured and analyzed with the computer image analyzer (Quantimet 600 S, Leica, UK). The optical density of the immunohistochemical reaction product was related (matched) to the density of AR. The system was calibrated, light transmittance replacing grey levels (range 0 – 255) with the value of optical density in the range of 0 – 2. The microscopic image was analyzed at  $\times 400$  magnification and next transmitted as a monochromatic (grey) image to the computer system. For statistical analysis integrated optical density (IOD) and mean optical density (MOD) values were counted to estimate the intensity of immunohistochemical reaction (for AR) in nuclei of 600 columnar epithelial cells in each group.

**Statistical analysis.** Serum prolactin and testosterone concentrations were analyzed by the Cochran-Cox test. Parameters of integrated and mean optical density (IOD and MOD) were analyzed by the Mann-Whitney U-test. Statistical significance was accepted at  $p < 0,05$ .

## Results

In rats of the experimental group as compared to the control group, the mean PRL serum concentration was more than 2 times higher (28.6 $\pm$ 5.2 vs. 13.7 $\pm$ 3.4 ng/ml) whereas the mean T concentration

was about 2 times lower (1.35 $\pm$ 0.8 vs. 3.42 $\pm$ 1.9 ng/ml) (Table 1).

As compared to the control group light microscope (LM), TEM and SEM showed the following changes in morphology of epithelial cells of prostate lobes in rats with hyperprolactinemia.

In light microscopy (LM) we observed focal proliferation of epithelium in lateral lobe in rats with hyperprolactinemia. The columnar and cubical cells of epithelium were tall and they had irregular disposition in sites of hyperplasia (Fig. 1 A, B). In the epithelial cells of lateral lobe in rats with hyperprolactinemia we observed in TEM and SEM widened cysternae of the rough endoplasmic reticulum (RER) and Golgii apparatus, reduced number of dense granules in these cells, small numbers of microvilli and poor secretory material at the surface [28].

In LM we also observed focal proliferation of epithelial cells in dorsal lobe in rats with hyperprolactinemia. The columnar cells of epithelium were tall (Fig. 1 C, D). In the majority of columnar epithelial cells of prostate dorsal lobe we observed in TEM and SEM highly dilated RER cisternae, small number of microvilli at the surface but the apical blebs were observed in glandular lumen in both control as well as experimental animals [30].

In LM the epithelial cells of ventral lobe in rats with hyperprolactinemia was shorter than cells of ventral lobe of control rats. We observed only focal atrophy in the epithelium (Fig. 1 E, F). The columnar epithelial cells of ventral lobe did not show any changes as well as columnar epithelial cells of lateral and dorsal lobes in TEM and SEM.

Immunohistochemical study showed different expression of AR in the nuclei of columnar epithelial and stromal cells of lateral, dorsal and ventral prostate lobes in the experimental as well as in the control group (Fig. 2). In rats with hyperprolactinemia, the nuclei of epithelial cells of dorsal and ventral lobes revealed lower optical density of immunocytochemical reaction (weak expression) as compared to control



rats (strong expression). A significant decrease ( $p < 0.05$ ) in both IOD and MOD was noted in the experimental animals. In the lateral lobe we found higher optical density of immunocytochemical reaction in experimental rats (strong expression) as compared to control rats (weak expression). A significant increase ( $p < 0.001$ ) in both IOD and MOD was noted in the experimental animals (Table 2, Fig. 2).

## Discussion

In the study we compared the structure of the columnar cells as well as expression of androgen receptor in three lobes of rat prostate under conditions of hyperprolactinemia induced with metoclopramide.

We showed that intraperitoneal administration of MCP in dose 2.2 mg/kg body weight provoked hyperprolactinemia in rats. By immunoassay we found more than twofold increase in prolactin serum concentration in rats receiving MCP as compared to the control group. It is consistent with our previous results obtained with radioimmunoassay [34,36]. It is worth to notice the results obtained with immunoassay as well as those determined with radioimmunoassay in our previous study were comparable ( $28.6 \pm 5.2$  and  $23.5 \pm 6.1$ ). In the experimental study Amiri *et al.* [37] used even doubled dose of MCP than we were for 21 days and found 2.5 fold increase of PRL concentration comparing to control rats. The capability of MCP to induce hyperprolactinemia was reported by others too [38,40].

We measured also concentration of testosterone in serum and we noted a significant, more than twofold decrease of mean T serum concentration in animals treated with MCP when compared to control group. The similar results were reported in previous studies [34,36,39]. In another experimental model using different drug to induce hyperprolactinemia, reduced T serum concentration was also reported [41].

In our study we determined the AR immunorexpression in epithelial columnar cells and stromal cells of all three rat prostate lobes. However we did not observe AR in epithelial basal cells. The other authors made similar observations [5,9,10].

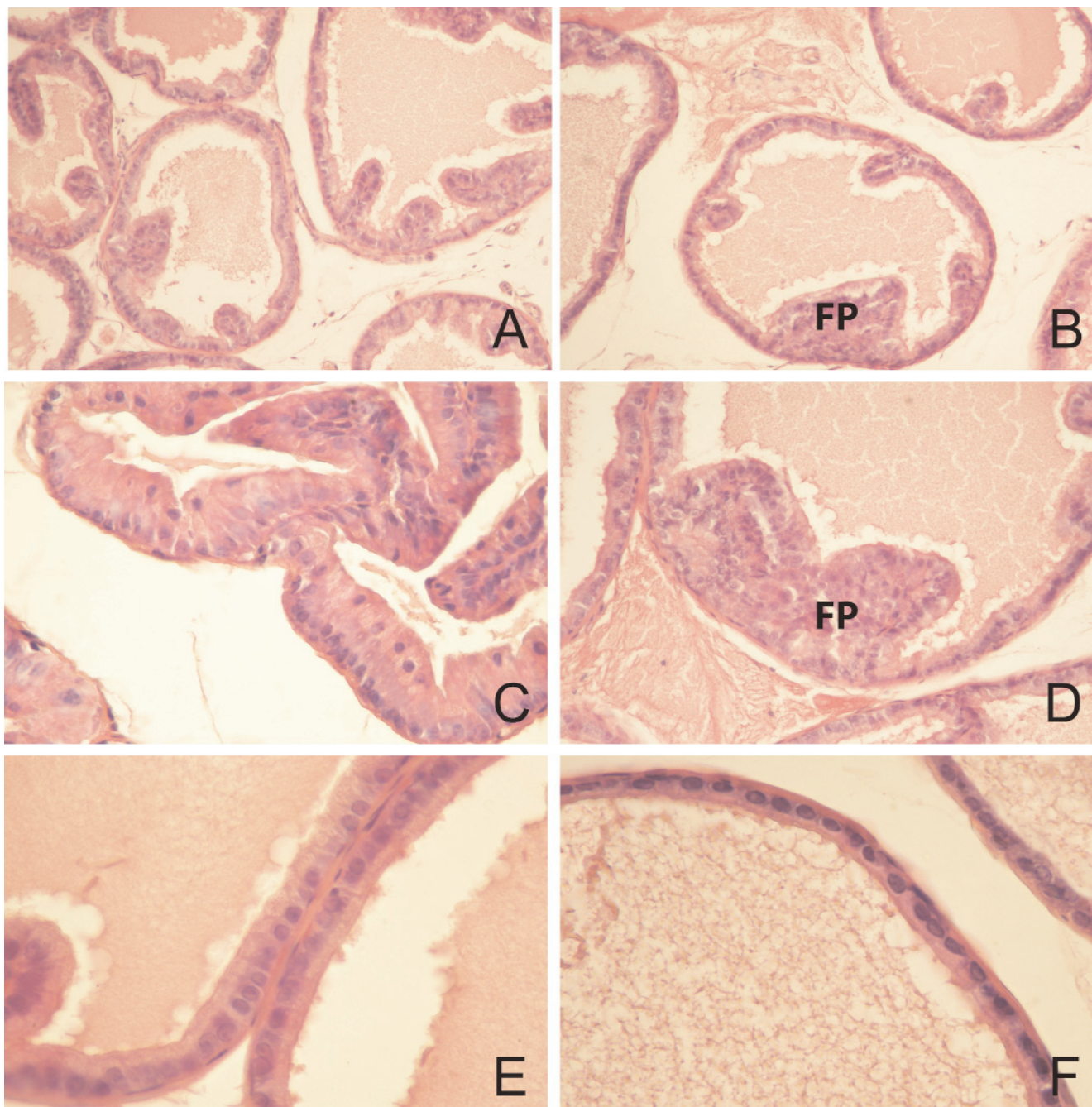
By computer image analysis using immunohistochemical reaction product IOD and MOD measurements were evaluated and subsequently AR expression in columnar epithelial cells of rat prostate lateral, dorsal and ventral lobes were compared between experimental and control groups. In the epithelial columnar cells of lateral lobe the higher immunorexpression of AR was found in rats with hyperprolactinemia comparing to control rats. On the other hand in dorsal and ventral lobes the AR expression was lower in experimental rats than in control ones. Higher AR expression in lateral lobe of hyperprolactinemic rats may be

explained by several mechanisms. Banerjee *et al.* [5] reported that AR expression in rat lateral lobe did not change following castration because it is not dependent on serum T concentration. Another research suggested that there is androgen independent regulation of AR expression in lateral lobe, dissimilar to others prostate lobes [6]. The experimental rat study of Prins [12] revealed that increased PRL causes up regulation of AR expression only in lateral lobe as the most prolactin dependent one. Moreover, the decrease of T serum level causes the lateral lobe is even more sensitive to prolactin [42]. The others also shown that the lateral lobe is the most sensitive to prolactin [15].

We also assessed the effect of hyperprolactinemia on morphology of columnar epithelial cells of rat prostate lateral, dorsal and ventral lobes using light as well as transmission and scanning electron microscopy.

In lateral lobe the epithelium was tall and ultrastructural changes of SER, RER, Golgi apparatus and the cell surface were observed only in some columnar cells in experimental group. Secretory granules containing electron dense material were found in both experimental and control rats. Since RER, Golgi apparatus and the cell surface are related to androgen dependent function of lateral lobe, the structural abnormalities of these organelles might be caused by reduced T serum level secondary to MCP administration. It is well known the androgens are responsible for development, maturation and function of the prostate [5,20,43,44]. The recent studies have shown that proteins synthesized in epithelial cells of prostate lateral lobe like probasin, SVSII or  $\alpha$ -microseminoprotein are androgen dependent [45]. It is possible the reduced testosterone serum level, despite higher AR expression, was not strong enough to maintain proper protein synthesis within the cells. On the other hand, the occurrence of secretory granules with electron dense material, reported previously as granules containing zinc [46], is likely associated with zinc accumulation rate. In the lateral lobe it is the highest and is controlled not only with testosterone but also with PRL [15,47].

The ultrastructural changes of rough endoplasmic reticulum were observed in prostate dorsal lobe of rats suffering hyperprolactinemia too. The epithelial columnar cells of this lobe were also tall. In some cells RER was strongly vacuolated and in the others the cisterns were bloated. Reduced serum testosterone level as well as lower AR expression in dorsal lobe epithelial columnar cells in rats suffering hyperprolactinemia might influence the structure of organelles associated with protein synthesis. It is known the synthesis of majority of proteins in dorsal lobe depends on androgens [48].



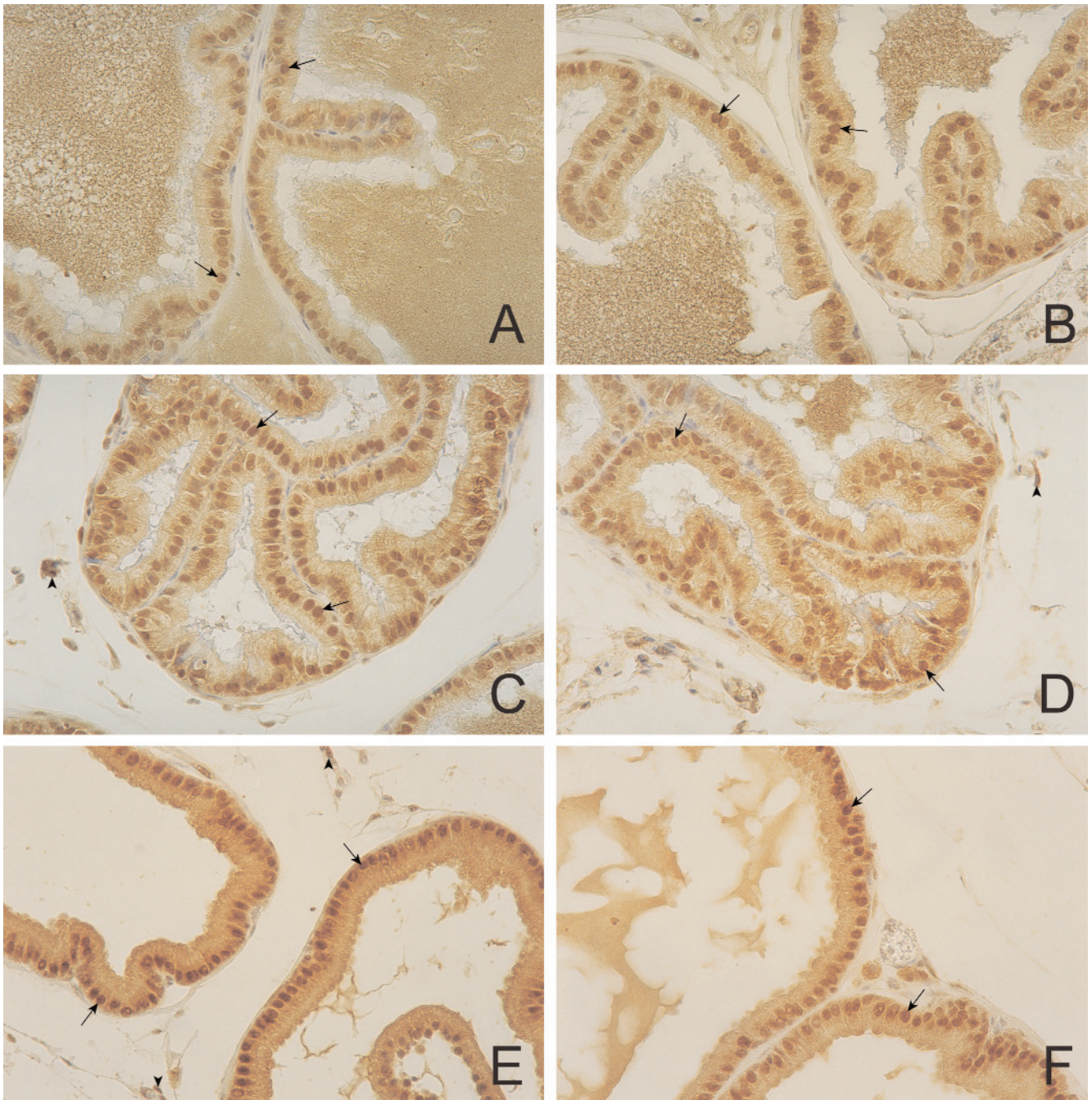
**Fig. 1.** The prostate lateral, dorsal and ventral lobes of control rat and rat receiving MCP. Epithelial cells of prostate lateral lobe of control rat (A) and rat receiving MCP (B) (magnification  $\times 100$ ). Epithelial cells of prostate dorsal lobe of control rat (C) (magnification  $\times 400$ ) and rat receiving MCP (D) (magnification  $\times 200$ ). Focal proliferation (FP) of epithelium of prostate lateral and dorsal lobe in rat receiving MCP (B, D). Epithelial cells of prostate ventral lobe of control rat (E) and rat receiving MCP (F). The shorter epithelium in ventral lobe of rat receiving MCP (F) than in epithelium of control rat (E) (magnification  $\times 400$ ). H+E staining.

In the ventral lobe however the shorter epithelial columnar cells and focal atrophy of the epithelium were observed in experimental group. It could be related to higher sensitivity of ventral lobe to decreased testosterone level. The ultrastructure of the cellular organelles was not markedly impaired. It is with agreement with the other authors that reported the elevated

PRL did not influenced the columnar cells of ventral lobe, which is highly sensitive to androgens [15,25,29].

It is well known so far that hyperprolactinemia causes morphological abnormalities in prostate cells [25,49]. Van Coppenolle *et al.* [25] determined the hyperprolactinemia induced with sulpirid caused





**Fig. 2.** Immunohistochemical nuclear localization of androgen receptor (AR) in the epithelial (arrow) and stromal (arrowhead) cells of three lobes of rat prostate (lateral, dorsal and ventral). Nuclear expression of AR in epithelial cells of prostate lateral lobe of control rat – week expression (A) and strong in rat receiving MCP (B); strong nuclear expression of AR in epithelial cells of prostate dorsal (C) and ventral (E) lobes of control rat and week nuclear expression of AR in epithelial cells of prostate dorsal (D) and ventral (F) lobes in rat receiving MCP (magnification  $\times 200$ ).

epithelial cells hyperplasia only in lateral lobe but not in dorsal and ventral ones. On the other hand Ahonen *et al.* [49] found the hyperprolactinemia caused hyperplasia in both lateral and dorsal lobes. Another group reported that in mice stimulated with PRL the hyperplasia of stromal and epithelial prostate cells occur independently on androgen level [50].

Based on our observations we can conclude that (I) experimental hyperprolactinemia induced with metoclopramide in rats caused structural changes in prostate cells expressing AR and (II) testosterone and prolactin serum levels regulate the AR expression in the prostate. Ultrastructural abnormalities in prostate epithelial columnar cells were found mostly in

organelles engaged in protein and glycoprotein synthesis or discharge and might be the reason of the infertility of the animals.

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