# The impact in the agnors expression and apoptosis in the prostate of hamster-*mesocricetus auratus* (H*MA*) submitted to finasteride application

## *O impacto na expressão* agnors *e apoptose na próstata do hamster-* mesocricetus auratus (*H*MA) *submetido à aplicação de finasterida*

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A B S T R A C T

**Objective**: To evaluate the impact on the AgNORs expression and apoptosis in the prostate of the hamster-*Mesocricetus auratus* (HMA) submitted to the application of finasteride. **Methods**: Twenty male rodents of the species HMA (n = 20) were randomly assigned to groups of ten animals: Finasteride group (n = 10) and the Control group (n = 10). In the finasteride group 7.14 ng / mL finasteride was subcutaneously (SC) administered on the back of the animals three times a week for 90 days. AgNOR expression was evaluated as a marker of cell proliferation and apoptosis as a marker of cell death. **Results**: The expression of AgNORs was lower in the finasteride group, 2.846 ± 0.877 vs. 3.68 ± 1.07 argyrophilic regions per square micrometer (µm2) in the control group, p = <0.0001. Apoptosis was more frequent in the finasteride group, 53.62 ± 1.389 versus 14.76 ± 2.13 per µm2 in the control group, p = 0.0408. **Conclusion**: We observed decreased expression of AgNORs and promotion of apoptosis in the prostate of rodents treated with finasteride.

**Key words**: Finasteride. Prostate. Mesocricetus

## INTRODUCTION

Recent studies have shown benefits of finasteride on prostate proliferative processes [1-3]. These studies are based on four clinical trials[4]. Investigations in animals with markers of proliferation and cell death may be of interest to corroborate these results.

The anatomy, histology and physiology of the prostate of the hamster – *Mesocricetus auratus* (HMA) – were described by Toma *et al.*[5], allowing the realization of experiments with these animals with medications that can act in this gland.

Vidigal *et al.*[6] studied the prostates of the HMA, when they had the opportunity to know the anatomy and histology of this gland. Combining theory with practice, they realized that these rodents were suitable for the development of research as experimental animals.

The objective of this study therefore is to assess the impact on AgNOR expression (*Argyrophilic Nuclear Organizer Regions*), a marker of cell proliferation, apoptosis and cell death in the prostate of HMA submitted to finasteride treatment.

## METHODS

### Experimental design

This work was performed in accordance with the recommendations of the International Protection of Animals and the Brazilian Code on Animal Experimentation (1988) and it has also been approved by the Ethics Committee (CEP), President Antonio Carlos University (UNIPAC), Barbacena, Minas Gerais, Brazil under No. 129/06.

We studied 20 adult HMA rodents randomly selected from those available in a vivarium, aged one year, divided into two groups: finasteride group, consisting of ten animals (n = 10); and control group, also formed by ten animals (n = 10).

The hamsters were purchased from the animal house of the Institute of Biological Sciences, Federal University of Minas Gerais (ICB-UFMG), Belo Horizonte, Minas Gerais, Brazil. The finasteride group weighed 145 ± 15.27 g, and control group 129 ± 18.82 g.

The animals were housed in plastic cages measuring 40cmx60cmx20cm (two animals per unit) and lined with wood shavings. They were fed ad libitum with chow prepared for hamsters and sunflower seeds, peanuts

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and corn. Water was offered to the animals in suction fountains. They were exposed to indirect light from 6 am to 18h and darkness for twelve hours at a temperature of 21° C.

The finasteride group received 0.5 mL of injectable solution containing 7.14 ng of finasteride subcutaneously in the dorsal region; the solution was prepared at a concentration of 14.28 ng/mL by the laboratory Citopharma Special Drugs Ltd®.

After 90 days the hamsters were anesthetized with ketamine hydrochloride for veterinary use in the dosage of 200mg/kg and diazepam at doses of 2.5 mg / kg, intraperitoneally[6], in different syringes. After being anesthetized, they were killed by hypovolemia.

On laparotomy, the prostates (ventral and dorsal) were removed *en bloc* along with the genitourinary system[6]. The glands were then dissected, fixed in 10% formaldehyde solution and sent for histological processing.

The prostates, ventral and dorsal, were chosen at random for examination; at first, they were submitted to histological analysis in an optical light microscope, and then sent to morphometric analysis at the Laboratory of Apoptosis, Department of General Pathology of ICB-UFMG.

### Parameters and morphometric strategy

From each of the slides investigated, we analyzed the expression of AgNORs as a marker of cell proliferation and activation and *in situ* identification of the fragmentation of the genome as a marker of apoptosis (*TUNEL – Terminal Deoxinucleotidil Transferase Uracil Nick End Labeling* reaction). The technique combines principles of TUNEL histochemical and immunohistochemical studies to mark the fragmented DNA of apoptotic cells *in situ*. This technique was applied to paraffin sections using histological 4mm thick slices. The nuclei obtained in the TUNEL reaction were counted manually.

Five hundred acinar nuclei were evaluated per slide for the count of argyrophilic lumps. Each animal had its respective slide examined and the amount of argyrophilic lumps found represented its own expression of AgNORs.

### Statistical treatment

We constructed frequency distributions and calculated values of mean, median (md), standard deviations (SD) and the relative proportions of the variables of the study: AgNOR and apoptosis displayed by finasteride and control groups.

The groups were compared in RXC-type contingency tables for frequency analysis and ANOVA table as for averages.

The statistical significance of differences between proportions was determined by Fisher's exact test (for both ends of the distribution) and the "t" Student test for comparison of mean values.

When the variances of the compared means were not similar ($p < 0.05$) in the Bartlett test, the significance of differences between groups was assessed by the Mann-Whitney test and the Wilcoxon test. The statistical significance level used in the analysis was 5%. Logistic regression tests were used to assess whether age and weight affected the results.

## RESULTS

The finasteride group was aged 17.7 ± 0.67 months and the control group 15.2 ± 1.13 months. The value of "t" in the comparison of mean ages between the two groups was 5.98, p=0.001. The average weight of the finasteride group was 145 ± 15.27 g and of the control group 129 ± 18.82 g. The comparison of means between the two groups in terms of weight revealed t = 2.08, p=0.0514.

Figure 1 shows the expression of AgNORs. Animals in the finasteride group had an average of 2.846 ± 0.87 argyrophilic lumps per ìm$^2$ and median of 3 argyrophils/ìm$^2$. The control animals had a mean of 3.68 ± 1.07 argyrophilic lumps per ìm$^2$ and a median of 4 argyrophils/ìm$^2$, the difference between the medians by Mann-Whitney test presenting p<0.0001.

Figure 2 shows the result of apoptosis between the finasteride and control groups. The finasteride group had an average of apoptosis (number of labeled cells in the TUNEL test) of 53.62 ± 1.389 and median of 54.34, p=0.0408. The control group showed an apoptosis mean of 14.76 ± 2.137 and median of 12.50. Apoptosis was more evident in animals that were treated with finasteride.

## DISCUSSION

The comparison between the two groups as for weight and age showed them to be different in age and the difference between the weight was at the limit of significance level. Tests were performed to determine the influence of these two factors in the effects of finasteride on the outcome variables studied and showed that the difference between age and weight of the animals did not affect the comparability of the two groups regarding the effects of finasteride on the outcome of the study of AgNORs and apoptosis.

The control group was not subjected to any treatment, even the administration of placebo, thus avoiding the effect of stress on handling, aiming to provide information that could serve as a parameter with the results obtained in the finasteride group.

Since the medication is clinically used continuously for the treatment of benign prostatic hyperplasia (BPH), based on what is inferred, there would be no need to add new groups, because the goal was whether the animal, such as men, would be subject to finasteride drug action.

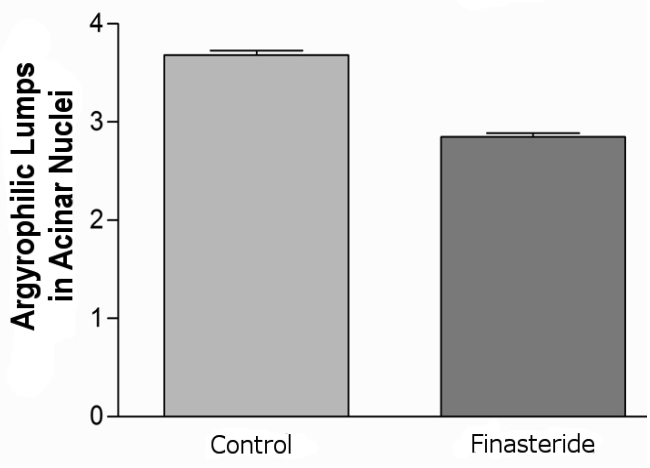


***Figure 1*** - *Values of AgNORs expression, control group versus finasteride group.*

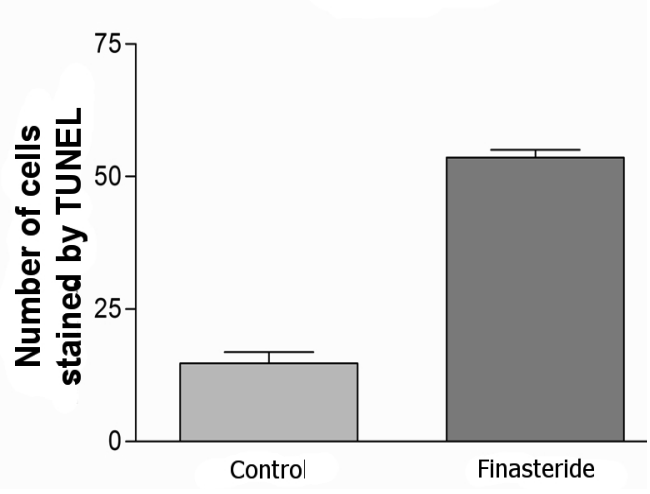


***Figure 2*** - *Results of apoptosis in the control group versus finasteride group.*

In the animals of this study we used a dose of finasteride a thousand times smaller than the one indicated for the treatment of BPH in an adult man of 70 kg, at intervals of three times a week, showing the impact on the prostate of these animals. Due to technical difficulties, it was not possible to inject finasteride every day in the animals. We chose to use the medication three times a week.

The cellular mechanism of finasteride effect in the prostate is not well understood[7]. It has been proven the action of this drug in androgen receptors for type-2 alpha-5-reductase in the prostate of men[8]. This study suggests that prostate androgen receptors of the HMA are also type 2.

In an experimental study using gerbil[9] – *Meriones unguiculatus* – finasteride at a dose of 10mg/kg per day was administered associated with letrozole (an aromatase inhibitor) 1mg/Kg daily for a period of 21 days. At the end of treatment, significant changes were observed in prostate cells, suggestive of apoptosis.

Studies in rats with high dose finasteride showed atrophy of the prostate gland of animals[10-12], and there is evidence that the prostatic glandular atrophy with finasteride is secondary to blockage of apoptosis promoted by 5áR[12]. These findings and those presented in this study show that blocking the formation of intracellular dihydrotestosterone (DHT) causes prostate atrophy by apoptosis.

Dysfunctions in apoptotic mechanisms are involved in the development and progression of proliferative processes of the prostate[13]. It has also been shown that finasteride leads to induction of apoptosis and an anti-angiogenesis effect in the gland[14]. Changes in human homeostasis between Growth Factor (GF) and apoptosis are involved in the pathogenesis of BPH[15].

In this study there was also decreased AgNORs in animals treated with finasteride when compared to the control group. The AgNORs are important markers of cell proliferation[16].

The findings in the literature[17-19], and also here, showed that finasteride protects the prostate from proliferative events, placing it as a promising drug for the treatment and prevention of these diseases.

In this study, finasteride was found to be a drug with beneficial effects on prostate acini of the HMA, although we cannot yet say that the findings presented here have the same effect on the human prostate, due to genetic peculiarities differing *Homo sapiens* from *Mesocricetus auratus*, but surely a science-based reference has emerged.

This study concluded that finasteride decreases the expression of AgNORs and promotes apoptosis in prostate acini of the HMA.

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## R E S U M O

***Objetivo:*** *Avaliar o impacto na expressão* AgNORs *e apoptose na próstata do hamster-*Mesocricetus auratus *(h*Ma*) submetido à aplicação de finasterida.* ***Métodos:*** *Vinte roedores da espécie h*Ma *(n=20), machos foram separados aleatoriamente em grupos de dez animais: grupo-Finasterida (n=10) e grupo-Controle (n=10). No grupo-finasterida foi administrado 7,14 ng/mL de finasterida, subcutâneo (SC), no dorso, três vezes por semana, por 90 dias. Foi avaliada a expressão* AgNORs *como marcador de proliferação celular e a apoptose como marcador de morte celular.* ***Resultados:*** *A expressão de* AgNORs *foi menor no grupo-finasterida, 2,846±0,877* versus *3,68 ±1,07 grumos argilófilos por micrômetro ao quadrado (μm²) no grupo-controle, p= < 0,0001. A apoptose foi mais frequente no grupo-finasterida, 53,62±1,389* versus *14,76 ± 2,137 μm² no grupo-controle, p= 0,0408.* ***Conclusão:*** *Observou-se diminuição da expressão de* AgNORs *e promoção da apoptose na próstata dos roedores em estudo, que foram submetidos à aplicação de finasterida.*

***Descritores:*** *Finasterida. Próstata. Mesocricetus.*

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