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# The effects of Finasteride on the expression of *Dazl, Tsga10, Sycp3, Prm2* genes during spermatogenesis in testes of NMRI mice

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**Abstract. – OBJECTIVE: In this study, the impact of Finasteride was assessed on the expression of four biomarkers of the spermatogenesis process, namely *Dazl, Tsga10, Sycp3*, and *Prm2* using the Real-Time PCR technique.**

**MATERIALS AND METHODS: The experimental protocol was carried out on male NMRI mice for 35 days in which three animal groups received three different doses of Finasteride (1, 5, and 20 mg/body weight).**

**RESULTS: The results showed that the expression levels of both *Dazl* and *Prm2* genes were significantly decreased only at a dose of 20 mg/body weight, but at doses of 5 and 20 mg/body weight, the expression levels of *Sycp3* and *Tsga10* genes were significantly reduced.**

**CONCLUSIONS: It seems that Finasteride, at a dose of 5 mg/body weight or higher, may have adverse effects on male spermatogenesis and fertility.**

*Key Words:*
Finasteride, Spermatogenesis, Dihydrotestosterone, 5α-reductase, Gene.

## Introduction

The 5α-reductase enzyme converts testosterone (T) into dihydrotestosterone (DHT) in the body. It has been shown that Finasteride is useful for the treatment of androgenic alopecia (1 mg/day) and benign prostatic hyperplasia (5 mg/day) by inhibiting the 5α-reductase enzyme[1]. DHT plays an essential role in the regulation of the GnRH and gonadotropin feedback loop. Many genes are responsible for the regulation of the spermatogenesis process, a few of which are very significant and known as biomarkers for spermatogenesis. Studies have indicated that *Tsga10*, *Sycp3*, *Dazl*, and *Prm2* could be considered the indices of spermatogenesis. The current study aimed to assess the effect of Finasteride on spermatogenesis through the assessment of the expression levels of four genes, which are considered the biomarkers of spermatogenesis.

## Materials and Methods

The experimental procedures were carried out on 60 male NMRI mice. The animals were divided into five groups as follows: the control group, the sham group, and three experimental groups that received Finasteride at doses of 1, 5, and 20 mg/body weight for 35 days. After the end of the treatment period, isolated removed testes were placed in liquid nitrogen for the genetic analyses[2].

The present research was approved by the Ethics Committee with the Ethical Code: IR.IAU.SRB.REC.1397.132. All of the experimental procedures were carried out based on the Ethics Guidelines of the Local Committee.

Total RNA was extracted and purified with the Hybrid-R kit according to the manufacturer's instructions. Samples with purified RNA were utilized for the synthesis of cDNA by means of the HyperScript First-strand Synthesis Kit. The specific primers for the analysis of the four genes, along with the reference gene (beta-actin), were designed by the Blast software[3] (Table I). The synthesized cDNA was amplified using specific primers by the Real-Time PCR technique. All PCR reactions were performed on a thermal cycler using the Taq DNA polymerase 2 x master mix RED kit (Amplicon) in a final volume of 20 ul, including master mix, forward and reverse primer, distilled water, and DNA templates, based on the following program: an initial denaturation step at 95°C for 15 min, followed by 40 cycles of 95°C for 30 secs, 60°C for 30 secs, and 72°C for 30 sec. A final extension step was carried out for 30 secs at 72°C[4].



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**Table I.** Primers used in Real-Time PCR detection.

| Gene symbol | Primers |
|---|---|
| Actin beta *(Actb)* | F: GTCCCTCACCCTCCCAAAAG<br>R: CTCAGACCTGGGCCATTCAG |
| Protamine 2 *(Prm2)* | F: CTCCCTCCTCCTCCAATCCAG<br>R: CCATAGTCCTCTACGCGCTC |
| Synaptonemal complex protein 3 *(Sycp3)* | F: TGGAGCTGACATCAACAAAGC<br>R: CCCACTGCTGCAACACATTC |
| Testis specific 10 *(Tsga10)* | F: TCCATGCAGAATCTCGAGGC<br>R: TCTGAACTGTGCCACGTCTC |
| Deleted in azoospermia-like *(Dazl)* | F: TAACTACCAGATGCCACCGC<br>R: CACAGACTTCTTTTGCGGGC |

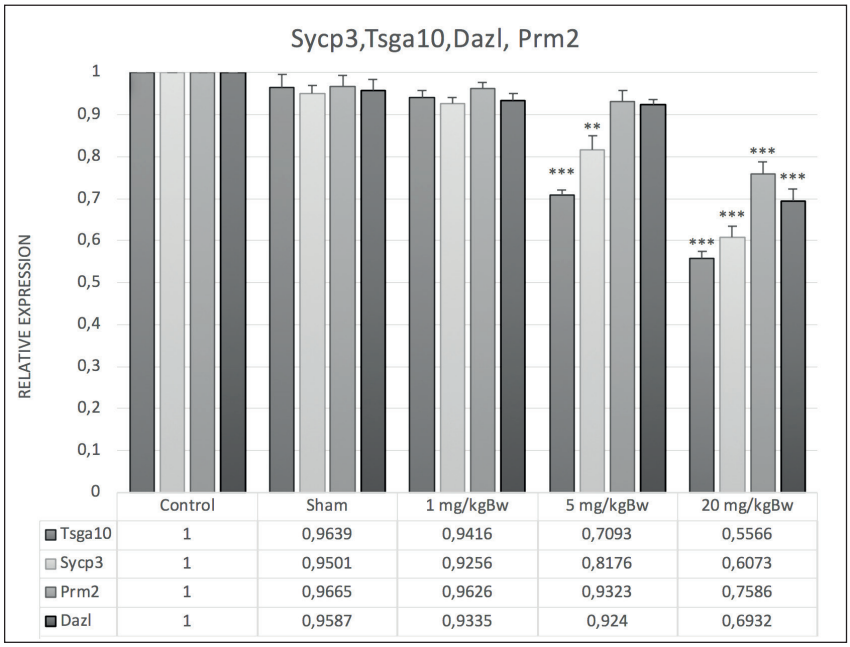
After obtaining the results of the Real-Time PCR method, including the Ct values, all data were analyzed by the REST software[4]. The expression levels of genes were analyzed by the SPSS software (SPSS Inc., Chicago, IL, USA). The difference between the experimental groups was analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test[2].

## Results

The expression levels of *Dazl* and *Prm2* genes in response to two doses of 1 and 5 mg/body weight were not significantly altered compared with the control group. However, there was a significant decrease in the expression of these two genes in response to a dose of 20 mg/body weight when compared with the control group (Figure 1). The *Tsga10* gene was not significantly changed in mice receiving 1 mg/body weight Finasteride, but at doses of 5 and 20 mg/body weight, the expression rate of *Tsga10* was markedly reduced in comparison with the control group (Figure 1). The *Sycp3* gene was not significantly altered in mice receiving a dose of 1 mg/body weight, but at doses of 5 and 10 mg/body weight, the expression of this gene was considerably diminished when compared with the control group (Figure 1).

## Discussion

Finasteride can cause a decrease in the concentration of DHT concentration, along with an increase in the level of testosterone by blocking




| | Control | Sham | 1 mg/kgBw | 5 mg/kgBw | 20 mg/kgBw |
|---|---|---|---|---|---|
| Tsga10 | 1 | 0,9639 | 0,9416 | 0,7093 | 0,5566 |
| Sycp3 | 1 | 0,9501 | 0,9256 | 0,8176 | 0,6073 |
| Prm2 | 1 | 0,9665 | 0,9626 | 0,9323 | 0,7586 |
| Dazl | 1 | 0,9587 | 0,9335 | 0,924 | 0,6932 |

**Figure 1.** Effect of finasteride on Sycp3, Tsga10, Dazl, Prm2 genes expression compared to the control group in real-time PCR. (*: $p<0.05$), (**: $p<0.01$), (***: $p<0.001$).

the activity of the enzyme 5-alpha reductase, leading to a reduction in the number of androgenic receptors in the brain.

The results showed that the expression levels of both *Dazl* and *Prm2* genes were significantly decreased only at a dose of 20 mg/body weight, but at doses of 5 and 20 mg/body weight, the expression levels of *Sycp3* and *Tsga10* genes were significantly reduced when compared with the control group.

Aslani et al[3] investigated the expression of some specific genes in adult spermatozoa and indicated that those are associated with fertility potential in men. Their research showed the importance of *TSGA10*, *DAZL*, *SYCP3*, *PRM2* genes in spermatogenesis.

Seven lines of evidence[5] have proposed that the expression of *Dazl* is necessary for the regulation of meiotic progression in the male gender by complex regulation of mRNAs translation. Studies reported that the expression of claudin-11 depends on the synthesis of DHT, playing a role in tight junction integrity of the blood-testis barrier, consisting of Sertoli cells. A decrease in DHT can lead to an immunologic attack on haploid cells in testes[6]. The *Dazl* gene, known as one of the criticalgenes that it is involved in the initiation of the development of spermatogonial cells, was analyzed. At doses of 1 and 5 mg/body weight, the expression of the *Dazl* gene did no change, but at a dose of 20 mg/body weight, the expression of the gene was significantly altered.

The *Prm2* gene is essential for normal sperm function. Its functionality has been reported at all stages of spermatogenesis. One of the most significant roles of this gene in the sperm genome is the compression and packaging of the genome contents by histones during spermiogenesis. The inactivity and improper expression of this gene could also cause DNA abnormalities in spermatozoa[4]. The expression of the *Prm2* gene was decreased in response to all doses of Finasteride, but at a dose of 20 mg/body weight, the difference was statistically significant.

Syrjänen et al[7] reported that decreased expression of the *SYCP3* gene (synaptonemal complex protein3) had adverse effects on fertility and spermatogenesis in humans. The *SYCP3* gene is expressed during the maturation of germinal cells when they are at the stage of spermatocytes. The *Sycp3* gene plays an essential role at all stages of meiosis during spermatogenesis, especially in the transition of primary spermatocytes to secondary spermatocytes and spermatids. In this study, the expression of this gene was remarkably reduced. At both doses of 5 and 20 mg/body weight, the change in the expression of the *Sycp3* gene was significant.

A group of studies has shown that testis-specific gene antigen (*TSGA10*) has a critical role in dividing the cells during spermatogenesis. Also, the *TSGA10* protein has been detected in the fibrous sheath of the tail of spermatozoa[8]. The *Tsga10* gene contributes to the development of spermatozoa, sperm maturation, and sperm motility. In this research, the expression of this gene was also significant at doses of 5 and 20 mg/body weight.

## Conclusions

These results showed that Finasteride, at a dose of 5 mg/body weight or higher, may have severe effects on male spermatogenesis and fertility, especially at various stages of spermatogenesis. Hence, it would be plausible that the long-term use of Finasteride would have more severe effects on the fertility potential of adult men. It is suggested that further investigations should be performed to determine the impact of Finasteride on spermatogenesis.

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### Conflict of Interests

The authors reported no potential conflict of interest.

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