Morphometric-stereological and functional epididymal alterations and a decrease in fertility in rats treated with finasteride and after a 30-day post-treatment recovery period

Patrick Vianna Garcia, M.Sc.,a Mainara Ferreira Barbieri, B.Sc.,a Juliana Elaine Perobelli, M.Sc.,b Silvio Roberto Consonni, M.Sc.,a Suzana de Fátima Paccola Mesquita, Ph.D.,c Wilma de Grava Kempinas, Ph.D.,b and Luis Antonio Violin Pereira, M.D., Ph.D.a

Department of Histology and Embryology, Institute of Biology, State University of Campinas (UNICAMP), Campinas, São Paulo; b Department of Morphology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo; and c Department of Biology, Institute of Biological Sciences, State University of Londrina, Londrina, Paraná, Brazil

Objective: To evaluate morphometric-stereological changes in the epididymal caput, sperm quality, and fertility parameters in rats treated with finasteride and after a 30-day post-treatment recovery period.

Design: Experimental study in a research laboratory.

Setting: Reproductive biology research laboratory.

Animal(s): Male and female Sprague Dawley rats.

Intervention(s): Treatment with finasteride (5 mg/kg/day) for 56 days followed by 30 days without treatment.

Main Outcome Measure(s): Serum hormone analyses, morphometric-stereological and ultrastructural evaluation of the epididymal caput, sperm transit time, natural mating, in utero insemination, sperm membrane integrity, and fertility parameters.

Result(s): Serum dihydrotestosterone levels in the finasteride group decreased by ~40% compared with that of control rats. Ultrastructural analysis revealed significant reductions in several morphometric-stereological parameters of the epididymal caput. All parameters recovered significantly in the post-treatment period. There was no alteration in daily sperm production in the finasteride group. However, significant reductions in sperm transit time, motility, sperm membrane integrity, and fertility parameters were observed in rats treated with finasteride.

Conclusion(s): Treatment with finasteride caused morphometric-stereological and functional changes in the epididymis and in sperm function that led to a reduction in fertility parameters. A 30-day post-treatment recovery period was insufficient to restore normal sperm motility, sperm transit time, and some fertility parameters. (Fertil Steril 2012;97:1444–51. ©2012 by American Society for Reproductive Medicine.)

Key Words: Finasteride, epididymis, sperm and fertility

In men, testosterone (T) is produced by Leydig cells in the testis and converted into dihydrotestosterone (DHT) by the enzymatic action of 5α-reductase in the skin, liver, and urogenital tissues such as the prostate and epididymis. DHT participates in the differentiation of external male genitalia, the growth of reproductive organs, and the maintenance of secondary male sexual characteristics (1–3).

However, high DHT levels in adulthood can lead to various disorders such as androgenic alopecia (4–6). Androgenic alopecia, the most frequent type of hair loss, decreases the number of hair follicles and is one of the most common dermatological conditions to affect both genders (7–9); this condition affects about 30% of the male population above 30 years of age and about 50% of men above 50 years of age (10–12).

Many medications, such as finasteride, were produced to inhibit the action of 5α-reductase (13). For the treatment of androgenic alopecia, finasteride (1 mg/day) reduces serum DHT levels by 60%–70% and significantly attenuates hair loss (6, 8, 14). Indeed, the early use of finasteride by young people in an attempt to prevent and minimize androgenic alopecia has become common (12, 14). Many of the

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Reprint requests: Luis Antonio Violin Pereira, M.D., Ph.D., Department of Histology and Embryology, Institute of Biology, State University of Campinas (UNICAMP), P.O. Box 6109, Campinas, São Paulo, 13083-970, Brazil (E-mail: lviolin@unicamp.br).

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The effects of finasteride are not fully understood, especially those related to organs of the male reproductive system such as the epididymis (15, 16). Approximately 75% of the detected proteins that participate in epididymal maturation are secreted by epithelial cells of the initial epididymal segment and caput, and 48% of these proteins are dependent on DHT (17–20).

Studies in humans have reported cases of male factor infertility associated with the chronic use of finasteride at a dose of 1 mg/day (21). These studies reported a reduction in ejaculate volume, abnormal sperm concentration and motility (22), a reduction in sperm quality, sperm DNA damage (5, 6, 23), and persistent sexual dysfunction in young men (16). Moreover, in rats, finasteride significantly reduces the weight of the epididymis (13, 24–26), which results in significant phenotypic changes (27, 28) and reduces fertility parameters (29, 30). However, no investigation has examined whether changes in sperm quality are correlated with epididymal maturation.

The aim of this study was therefore to evaluate the morphometric-stereological and functional alterations in the epididymal caput, sperm quality, and fertility parameters in rats treated with finasteride and those treated with this drug followed by a 30-day post-treatment recovery period.

**MATERIALS AND METHODS**

**Animals**

Male and female Sprague Dawley rats were obtained from the Multidisciplinary Center for Biological Investigation at the State University of Campinas (UNICAMP) and were housed at 23°C ± 2°C on a 12-hour light/dark cycle with free access to pelleted rodent chow and water.

The male rats (60 days old) were randomly allocated to three groups: group I (control group) consisted of rats that were treated by gavage with the vehicle solution of finasteride (0.5% aqueous methyl cellulose) for 56 days (n = 25), group II (finasteride group) consisted of rats that were treated with finasteride (5 mg/kg/day) by gavage for 56 days (n = 25), and group III (post-treatment group) consisted of rats that were treated with finasteride (5 mg/kg/day) by gavage for 56 days followed by a 30-day post-treatment recovery period (n = 25). Sixty-three female rats were used to determine the fertility parameters after natural mating and in utero insemination.

This study was approved by an institutional Committee for Ethics in Animal Experimentation (CEUA/UNICAMP, protocol number 1622–1). The National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* were followed.

**Treatment**

For experiments involving serum hormone and morphometric-stereological analyses, finasteride was provided in pure form by Merck Sharp & Dohme Corp. For other analyses, finasteride was purchased commercially upon presentation of a certificate of the manufacturer’s quality. Finasteride was diluted in an aqueous solution containing 0.5% methyl cellulose (Sigma) and administered once a day (5 mg/kg/day) by gavage (24, 25). This dose was chosen to mimic the dose used clinically to treat androgenic alopecia (in humans, this dose reduces the concentration of T to 60%–70% of the normal values). In the control and finasteride groups, all analyses were done 24 hours after the final dose, whereas in the post-treatment group all analyses were done 30 days after terminating the treatment.

**Serum Hormone Analyses**

Five rats per group were anesthetized and sacrificed by decapitation. An ACTIVE DSL T kit (Diagnostic Systems Laboratories Inc.) was used to determine the serum T levels, and an ACTIVE DHT radioimmunoassay kit S/E DSL 96100 (without extraction; Diagnostic Systems Laboratories) was used to determine the serum DHT levels. All of the samples were assayed in duplicate, and the intra- and interassay coefficients of variation were, respectively, ≤8.5% and ≤8.7% for T and ≤4.3% and ≤6.4% for DHT.

**Tissue Collection and Processing for Morphometric-Stereological Analysis and Electron Microscopy**

Five rats per group were weighed, anesthetized, and perfused with 4% paraformaldehyde (Sigma) and 2% glutaraldehyde (Electron Microscope Science) in 0.1 M sodium cacodylate buffer at pH 7.4 (Electron Microscope Science) that contained 0.3% tannic acid (Riedel-de Haen). One epididymis from each animal was processed for morphometric-stereological analysis, and the other was processed for transmission electron microscopy. The epididymides were weighed, and the epididymal weight-to-body weight ratio and total epididymal volume (V) were determined as described elsewhere (31). The epididymides were then postfixed with perfusion solution for 4 hours.

The epididymal caput was sectioned stereomicroscopically (Leica Microsystems CMS GmbH, Leica) into four anatomically distinct regions based on connective tissue septation (32): region 1 (initial segment), region 2 (proximal caput), region 3 (medial caput), and region 4 (distal caput). These regions were used for morphometric-stereological and ultrastructural analyses (Fig. 1A).

For morphometric-stereological analysis, the tissues were dehydrated in a graded ethanol series, embedded in historesin (Leica), sectioned (2 μm thick), and stained with hematoxylin-floxin (Sigma) (33). For transmission electron microscopy, the tissues were washed in 0.1 M cacodylate buffer and postfixed in 1% osmium tetroxide (Electron Microscope Science) for 1 hour at 4°C. The samples were then dehydrated in a graded ethanol series and embedded in epoxy resin Epon 812 (Electron Microscope Science). Ultrathin sections were collected on copper grids and stained with uranyl acetate and lead citrate (Electron Microscope Science) (34).

**Morphometric-Stereological Analysis**

Morphometric-stereological analysis was done on 30 random, histological cross-sections of the four regions of epididymal caput from each animal. The total duct diameter and area,
luminal area, and epithelial height and area were measured using a ×10 objective lens connected to a computer image analysis system (Image Pro Plus 4.5, Media Cybernetics, Inc.). The total duct, epithelial, luminal, and interstitial volumes were calculated (31).

Ultrastructural Analysis

Ultrastructural analysis was done by transmission electron microscopy (LEO 906, Electron Microscope Ltd.). The ultrastructural analysis involved qualitatively observing the location, number, and conservation of cytoplasmic organelles, as well as the presence of cell junctions and the integrity of the epithelial and stromal tissue.

Daily Sperm Production and Transit Time in the Epididymis

The right testis and epididymis were removed from five rats per group. The homogenization-resistant testicular spermatids (i.e., stage 19 of spermiogenesis) and sperm in the caput-corpus and cauda epididymis were counted as described elsewhere (35, 36). The daily sperm production in the testis (DSP) was estimated by dividing the number of spermatids at stage 19 by 6.1 (i.e., the number of days these spermatids are present in the seminiferous epithelium). The sperm transit time through the caput/corpus and cauda epididymis was determined by dividing the number of sperm in each portion by the DSP.

Sperm Motility and Assessment of Epididymal Necrospermia

A suspension of sperm was prepared from sperm isolated from the distal cauda of the left epididymis from five rats per group. The number of sperm in a 2.5-µL aliquot of this suspension was counted, and the sperm were classified as mobile or immobile (36). To assess epididymal necrospermia, 10-µL aliquots of sperm suspension were stained with eosin-Y and also processed for the hypoosmotic solution (HOS) test (37).

Natural Mating

Females (n = 10 per group) in natural estrus were housed with male rats (n = 5 per group). Vaginal smears were collected, and the day of initial sperm detection was determined to be day 0 of gestation (36, 38).

In Utero Insemination

Females in estrus were paired with sexually experienced, vasectomized males for 1 hour. Receptive females were selected for insemination (n = 11 females per group). Five male rats per group were used for insemination and sperm isolation as described elsewhere (36, 39). Briefly, the sperm were released from the proximal epididymal cauda by nicking the duct and collecting the sperm in 2 mL of modified human tubular fluid (HTF) medium (Irvine Scientific). After a 10-fold dilution, the sperm were counted and each uterine horn was injected with a volume containing 5 × 10⁶ sperm (36, 37).
Fertility Parameters
On the twentieth day of gestation, the females used for natural mating and in utero artificial insemination were euthanized. The number of corpora lutea, implants, resorptions, and dead and live fetuses was recorded.

For females subjected to in utero insemination, the gestation rate (number of pregnant females/number of inseminated females × 100) and fertility potential (implantation sites/corda lutea × 100) were calculated as described elsewhere (36, 37). For females subjected to natural mating, an additional three parameters were analyzed, namely, the fecundity index (number of males siring at least one viable fetus/number of males exposed for mating × 100) and the pre-implantation losses (number of implantation sites/number of corpora lutea × 100) and post-implantation losses (number of implantation sites/number of corpora lutea × 100) (31, 36, 37).

Statistical Analysis
The results were expressed as the mean ± SD. The normal distribution of the data was checked with the Kolmogorov–Smirnov test. One-way analysis of variance (ANOVA) followed by Dunn’s post hoc test was used for nonparametric data, Kruskal Wallis analysis followed by Dunn’s post hoc test was used for nonparametric data, and the χ²-test was used for categorical dependent variables. P<.05 was considered statistically significant. All data analyses were done using GraphPad Prism software v.5 (GraphPad Inc.).

RESULTS
Serum Hormone Levels
The serum DHT levels in the finasteride group were significantly lower than in the control and post-treatment groups (the reduction was ~40% when compared with the control group), but there was no significant difference between the control and post-treatment groups (Fig. 1B). There was a significant increase in the serum T levels of the finasteride group compared with the control group but no significant difference between the control and post-treatment groups (Fig. 1C).

Morphometric-Stereological Analyses
Morphometric-stereological analyses showed alterations only in the proximal epididymal caput (region 2) of the finasteride group when compared with the control and post-treatment groups.

The epididymal–body weight ratio, epithelial height (Fig. 2A–2C), and epididymal and epithelial duct area were significantly lower in the finasteride group compared with in the control and post-treatment groups. Additionally, there was a significant increase in the lumen epididymal duct area in the finasteride and post-treatment groups compared with in the control group (Table 1).

The epididymal, epididymal caput, and epithelial duct volumes were significantly lower in the finasteride group than in the control and post-treatment groups (Table 1).

However, the lumen duct volume was significantly higher than in the control and post-treatment groups. There was no significant difference in the epididymal duct and interstitial tissue volumes (Table 1).

Ultrastructural Analyses
Ultrastructural analyses showed a reduction in epithelial height only in the proximal caput (region 2) in the finasteride group (Fig. 2D–2F). There were no alterations in the location, number, and conservation of cytoplasmic organelles (Fig. 2G–2I) or in the integrity of the cell junction complex in the four regions of the epididymal caput in any of the groups (Fig. 2J–2L).

Daily Sperm Production and Transit Time in the Epididymis
There was no significant difference in the daily sperm production in any of the groups (Table 2). However, there was a significant decrease in sperm transit time in the caput-corpus and cauda in the finasteride and post-treatment groups, indicating an increased rate of sperm passage in these segments. There was a significant difference in sperm transit time between the finasteride and post-treatment groups in the caput-corpus segment but no significant difference between the finasteride and post-treatment groups in the cauda segment (Table 2).

Sperm Motility
There was a significant reduction in sperm motility in the finasteride and post-treatment groups compared with in the control group. However, there was no significant difference between the finasteride and post-treatment groups (Table 2).

Epididymal Necrospermia
Eosin-Y staining revealed a significant reduction in the percentage of live sperm in the finasteride and post-treatment groups compared with in the control group. There was a significant difference between the finasteride and post-treatment groups (Table 2). Similarly, the HOS test revealed a significant reduction in the percentage of live sperm in the finasteride and post-treatment groups compared with in the control group. There was significant difference between the finasteride and post-treatment groups (Table 2).

Fertility Parameters in Natural Mating and In Utero Insemination
In natural mating, there was no significant difference in the gestational rate and fecundity index of all groups. There was a significant reduction (by ~32%) in the fertility potential of the finasteride group compared with in the control group, but there was no significant difference between the control and post-treatment groups (Table 2). There was no significant difference in the preimplantation loss among the groups, although there was an evident increase in this rate in the finasteride and post-treatment groups. There was a significant increase in the postimplantation loss in the finasteride and...
post-treatment groups compared with in the control group, but there was no significant difference between the finasteride and post-treatment groups (Table 2).

In in utero insemination, there was no significant difference in the gestational rate among the groups, but there was an evident reduction in this rate in the finasteride and post-treatment groups. There was a significant reduction in the fertility potential of the finasteride and post-treatment groups compared with the control group, but there was no significant difference between the finasteride and post-treatment groups (Table 2).

DISCUSSION

Many studies have shown that DHT is responsible for most androgen-stimulated responses in the epididymis and that this hormone has an important role in maintaining the epididymal microenvironment (40–44). In the present study, the percentage reduction in serum DHT in the finasteride group corresponded to that seen in men undergoing androgenic alopecia treatment (i.e., DHT levels were reduced to 60%–70% of the control) (8, 14). This finding indicated that the rats were exposed to the same DHT variations that
has been also observed in some gene expression profiles. The regional in the height, area, and volume of the epididymal studies. These regions appear to be functional units within the epididymis and more susceptible to changes in the micro-environment (46–49).

Many studies have shown that finasteride reduces prostate size by decreasing the intraprostatic levels of DHT. This reduction involves a decrease in the stimulatory effect of this androgen on the cellular replacement of the secretory epithelium, which in turn diminishes the protective effect of these cells against apoptosis and results in a progressive decrease in the functionality of epithelial cells and an increase in the rate of cell death (49). Our morphometric–stereological

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<td><strong>Morphometric-stereological analysis of the control, finasteride, and 30 days post-treatment groups.</strong></td>
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<td><strong>Group</strong></td>
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<td>Epidermal duct area, log µm²**</td>
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<td>Lumenal duct area, log µm²**</td>
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<td>Interstitial tissue volume, %**</td>
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**Note:** The data are expressed as the mean ± SD. The epithelial height, epididymic duct area, lumenal duct area, epididymal duct volume, epithelial duct volume, lumenal duct volume, and interstitial tissue volume were calculated for region 2 (proximal caput) of the epididymal caput. NS = not significant.

**TABLE 2 |
| **Sperm counts, motility, epididymidal necrospermia, and fertility parameters in natural mating and in utero insemination in the control, finasteride, and 30 days post-treatment groups.** |
| **Group** | Control (n = 5) | Finasteride (n = 5) | Post-treatment (n = 5) | P value |
| Daily sperm production, ×10⁶/testis/day** | | | |
| Sperm transit time in the caput/corpus epididymis, days* | | | |
| Sperm transit time in the cauda epididymis, days* | | | |
| Motility, %** | | | |
| Eosin-Y staining, live sperm, %* | | | |
| HOS test, live sperm, %* | | | |
| Gestational rate, NM %**** | | | |
| Fecundity index, NM %*** | | | |
| Fertility potential, NM %*** | | | |
| Preimplantation loss, NM %*** | | | |
| Postimplantation loss, NM %** | | | |
| Gestational rate, IUI %**** | | | |
| Fertility potential, IUI %** | | | |

**Note:** The data are expressed as the mean ± SD. IUI = in utero insemination; NM = natural mating; NS = not significant.

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and ultrastructural results on the epididymis caput agree with the alterations seen in the prostate upon DHT depletion.

The reduction in serum DHT levels and consequent decrease of this hormone in the intraepididymal compartment in the finasteride group could explain the epididymal structural alterations and the decrease in sperm transit time, despite the unaltered daily sperm production in any of the groups studied. Local factors such as endothelin-1 (50), cGMP, androgens (51, 52), and prostaglandins (53) can affect peristalsis in the epididymal duct by modulating the activity of epididymal muscle.

Sympathetic innervation controls smooth muscle contractions in all regions of the epididymal duct, and T, E2, and DHT can interfere with this sympathetic control to modulate epididymal contractile activity, thereby influencing sperm transit time through this organ (36, 54, 55). The acceleration of sperm passage in the epididymal duct, such as seen in the finasteride and post-treatment groups, reduces the time that sperm are available for epididymal maturation and interferes with sperm quality and some fertility parameters (31, 37, 56).

The efficiency of implantation, that is, fertility potential, was clearly compromised in rats treated with finasteride and may indicate sperm genotoxicity or a problem in sperm recognition by the oocyte. A decrease in fertility potential is frequently seen as an increase in postimplantation loss (57). Although there were no significant differences in the preimplantation loss, the gestational rate, and fecundity index of the finasteride and post-treatment groups, these data should nevertheless be considered when evaluating patterns of fertility.

Changes in the fertility parameters of the finasteride and post-treatment groups cannot be explained solely by a decrease in secretion by the coagulation gland and the absence of vaginal plug formation (29, 30), particularly since in in utero insemination all of the females that were previously mated with vasectomized males formed vaginal plugs. Even in this condition, the fertility potential in the finasteride and post-treatment groups was lower than in the controls. Since rats produce and ejaculate an excess of qualitatively normal sperm, insemination in utero of a fixed, critical number of sperm can increase the probability of detecting a decrease in sperm quality in this species (56).

The changes in fertility described here agree with the recent finding that human male infertility and an increase in pregnancy loss may be associated with the continuous administration of small doses of finasteride, possibly because of a negative influence on sperm quality and DNA integrity (23). In addition, the interruption of treatment with finasteride does not ensure immediate recovery from the side effects of this compound. Although the cessation of treatment with finasteride immediately increases semen volume, the sperm concentration remains below normal for up to 4 months after treatment (6). A similar improvement in semen volume has been observed in two other cases of azospermia and severe oligospermia 6 months after stopping treatment with finasteride (5).

Our findings indicate that the finasteride-stimulated acceleration of sperm transport through the epididymis compromises sperm maturation, thereby affecting sperm motility, sperm membrane intactness, and fertility potential, and that a 30-day post-treatment recovery period is insufficient for the restoration of all parameters. Further studies are required to establish the ideal post-treatment recovery period for the restoration of fertility parameters and to determine whether these parameters can indeed be fully restored. Our findings also suggest that young men who use finasteride as a preventive and palliative method against androgenic alopecia may be at risk of impaired fertility.

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