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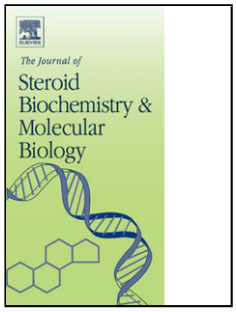

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HIGHLIGHTS

- **In patients treated with finasteride for male pattern hair loss, persistent side effects may occur**
- **Erectile dysfunction and abnormal somatosensory evoked potentials of the pudendal nerve were reported**
- **Major depressive disorder and altered levels of neuroactive steroids were observed.**

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NEUROACTIVE STEROID LEVELS AND PSYCHIATRIC AND ANDROLOGICAL FEATURES IN POST-FINASTERIDE PATIENTS.

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Abbreviations: 3α- hydroxysteroid oxidoreductase (3α-HSOR); 3β-hydroxysteroid oxidoreductase (3β-HSOR); 5alpha-androstane-3alpha,17beta-diol (3α-diol); 5alpha-androstane-3beta,17beta-diol (3β-diol); 5alpha-reductase (5α-R); 17β-estradiol (17β-E); androgenetic alopecia (AGA); cerebrospinal fluid (CSF); dehydroepiandrosterone (DHEA); dihydroprogesterone (DHP);

dihydrotestosterone (DHT); DSM-IV major depressive disorder (MDD); erectile dysfunction (ED); isopregnanolone (Isopreg); liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS); pelvic somatosensory evoked potentials of the pudendal nerve (PN_SEPs); post-finasteride syndrome (PFS); pregnenolone (PREG); progesterone (PROG); testosterone (T); tetrahydroprogesterone (THP).

ABSTRACT

Recent reports show that, in patients treated with finasteride for male pattern hair loss, persistent side effects including sexual side effects, depression, anxiety and cognitive complaints may occur. We here explored the psychiatric and andrological features of patients affected by post-finasteride syndrome (PFS) and verified whether the cerebrospinal fluid (CSF) and plasma levels of neuroactive steroids (i.e., important regulators of nervous function) are modified. We found that eight out of sixteen PFS male patients considered suffered from a DSM-IV major depressive disorder (MDD). In addition, all PFS patients showed erectile dysfunction (ED); in particular, ten patients showed a severe and six a mild-moderate ED. We also reported abnormal somatosensory evoked potentials of the pudendal nerve in PFS patients with severe ED, the first objective evidence of a neuropathy involving peripheral neurogenic control of erection. Testicular volume by ultrasonography was normal in PFS patients. Data obtained on neuroactive steroid levels also indicate interesting features. Indeed, decreased levels of pregnenolone, progesterone and its metabolite (i.e., dihydroprogesterone), dihydrotestosterone and 17beta-estradiol and increased levels of dehydroepiandrosterone, testosterone and 5alpha-androstane-3alpha,17beta-diol were observed in CSF of PFS patients. Neuroactive steroid levels were also altered in plasma of PFS patients, however these changes did not reflect exactly what occurs in CSF. Finally, finasteride did not only affect, as expected, the levels of 5alpha-reduced metabolites of progesterone and testosterone, but also the further metabolites and precursors suggesting that this drug has broad consequence on neuroactive steroid levels of PFS patients.

Keywords: major depressive disorder; erectile dysfunction; 5α-reductase; pregnenolone; progesterone; testosterone.

INTRODUCTION

Neuroactive steroids include both hormonal steroids synthesized in the peripheral glands and acting in nervous system, as well as neurosteroids locally synthesized. Among these, pregnenolone (i.e., PREG, the first neuroactive steroid formed from cholesterol), dehydroepiandrosterone (DHEA), progesterone (PROG), testosterone (T) and 17β-estradiol (17β-E) exert a wide range of important physiological effects regulating nervous functions. These include neuroendocrine control of reproduction and sex behavior [1], synaptic plasticity [2, 3], cytoskeletal proteins and the morphology of neurons and astrocytes [4, 5], myelin compartment [6-8], adult neurogenesis [9-11], and cognition-related functions [2, 3, 5, 12]. Some of these effects are due to the final active metabolites of neuroactive steroids. Indeed, also in the nervous system PROG and T are metabolized by the enzyme 5α-reductase (5α-R), into dihydroprogesterone (DHP) and dihydrotestosterone (DHT) respectively. These neuroactive steroids are then further converted by the action of 3α- (3α-HSOR) or 3β-hydroxysteroid oxidoreductase (3β-HSOR) into further metabolites. In particular, DHP is converted into tetrahydroprogesterone (THP) and into isopregnanolone (Isopreg) while T is converted into 5α-androstane-3α,17β-diol (3α-diol) or 5α-androstane-3β,17β-diol (3β-diol) [13]. These metabolic pathways have an important impact in the mechanism of action of steroid substrates. Indeed, neuroactive steroids so metabolized, may exert their effects not only by classical (e.g., androgen, progesterone, estrogen receptors) but also non-classical steroid receptors (e.g., GABA-A receptor)[13]. Thus, the enzyme 5α-R exerts a crucial role in the mechanism of action of neuroactive steroids. In agreement with these concepts, as demonstrated in several experimental models and in clinical studies,

neurodegeneration as well as psychiatric disorders show altered levels of neuroactive steroids, including 5α-reduced metabolites [8, 14]. Actually three 5α-R isozymes, defined as type 1, 2 and 3 have been identified in the brain [15], albeit the physiological role of type 3 remains to be further explored [16].

Finasteride (Propecia or Proscar) is an inhibitor of 5α-R type 1 and 2, although it has higher affinity for the type 2 [17, 18]. Finasteride administration was approved for the treatment of benign prostatic hyperplasia and androgenetic alopecia (AGA). However, recent studies reported serious, adverse side effects during and after drug administration in patients treated for male pattern hair loss [15, 19-24]. These persistent side effects include sexual side effects (i.e., low libido, erectile dysfunction, decreased arousal and difficulty in reaching orgasm) [25-30], depression, anxiety and cognitive complaints [31-34]. Our recent observations performed in three [35] and seven [36] patients treated with finasteride for AGA, indicated, after drug discontinuation, persistent altered neuroactive steroid levels in cerebrospinal fluid (CSF) and plasma. In addition, recent observation performed in a higher number of patients affected by post-finasteride syndrome (PFS) reported impaired sexual function and higher depression scores, without any significant changes in sex steroids plasma levels [37].

The aim of this study was the description of the CSF and plasma neuroactive steroid pattern in patients with PFS, comprehensively evaluated in their psychiatric and andrological features. Moreover, to better understand the neuropsychological basis of the sexual dysfunction, the neurogenic control of erection was evaluated.

METHODS

Study design and sample preparation

A multicentric, prospective, longitudinal, case-control clinical trial was carried out. PFS patients were recruited through the *Italian network finasteride side effects*. Healthy men, aged 22-44 years who reported persistent sexual and mental health side effects after the use of 1-1.25 mg daily of finasteride (i.e., Propecia, Proscar or generic finasteride) for androgenetic alopecia were considered in the case group. Only subjects who had discontinued finasteride at least 3 months earlier, did not use drugs known to potentially interfere with neuroactive steroid levels and did not report depression or sexual dysfunction before finasteride use were included. A questionnaire was used to evaluate the absence of PFS signs and symptoms before the finasteride treatment, as well as the presence of this accompanying signs and symptoms during and after the drug treatment. Although not validated, it represents the only available tool to systematically collect information on patient conditions and to assess the features of PFS (Table 1). The questionnaire was filled by the patient himself only after the description of the study design to the patient, in order to limit selection and recall bias. The study procedure was approved by the Ethics Committee of the San Gerardo Hospital (approval n.142/2012), Monza-Italy and the participating subjects provided their written informed consent before enrolment. Through the screening procedure we recruited for psychiatric and andrological assessment 16 PFS patients. Among these, two patients refused to undergo CSF sampling and we analysed in the remaining 14 PFS patients the paired CSF and plasma levels of 11 different neuroactive steroids (i.e., PREG,

DHEA, PROG, DHP, THP, Isopreg, T, DHT, 3α-diol, 3β-diol and 17β-E) by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

In order to obtain reliable normal control values, paired CSF and plasma samples were collected from 25 subjects who underwent spinal anesthesia for planned orthopedic surgery of the lower limb at the San Gerardo Hospital of Monza. These subjects were otherwise healthy, were carefully screened for the absence of any neurological or psychiatric disorder in their personal or family history. After written informed consent was given, CSF and plasma were collected. Usually, CSF was collected in these patients to verify the correct position of the spinal needle, according to the procedure approved by the Ethics Committee of the S. Gerardo Hospital in Monza. The mean age of healthy controls (33 years old) was not significantly different from PFS patients (p=0.791).

Mental health assessment

Consecutive subjects with PFS were screened by K-10 [38] in order to evaluate the likely presence of major mental disorders. If screened positive, they were assessed using the Mini-International Neuropsychiatric Interview (M.I.N.I.), a validated diagnostic interview for DSM-IV with a coding system for diagnosing major depressive disorder [39]. In addition, the Beck Depression and Anxiety Inventories (BDI, BAI) were administered [40]. Depression scores are classified by BDI, as follows: (i) minimal (BDI score = 0 -13), (ii) mild (BDI score = 14 – 19), (iii) moderate (BDI score = 20 -28), and (iv) severe (BDI score = 28 - 63) [41]. Anxiety scores are classified by BAI, as follows: (i) minimal (BAI score = 0 -9), (ii) mild (BAI score = 10 – 16), (iii) moderate (BAI score = 17 – 29), and (iv) severe (BAI score = 29 - 63) [42].

Erectile function evaluation

All patients received the validated Italian version of the International Index of Erectile Function (IIEF)-15 self-administered questionnaire to assess erectile dysfunction (ED) [43]. A cut-off score below or equal to 25 of the erectile function domain was used to diagnose ED as previously validated by Rosen et al. [43]. According to Cappelleri et al. [44], the score of the erectile function domain was classified into the following four diagnostic categories: (i) no ED (EF score = 26 – 30); (ii) mild ED (EF score = 17 – 25); (iii) moderate ED (EF score = 11 – 16); and (iv) severe ED (EF = 6 – 10). For the purpose of our analysis, the mild and mild-moderate categories of Cappelleri et al. [44] were combined into a single category of mild ED (EF score = 17 – 25).

Ultrasonography scan

Ultrasonography (US) scan of testes was performed by a single operator using an Esaote® My Lab25 Gold equipment (Esaote North America Inc., Indianapolis, 10 MegaHertz-linear scanner, B mode). US volume is currently considered the most reliable method of determining testicular volume, calculated by using axial and longitudinal scans. Testicular volume was calculated for each testis, estimated as elipsoid: length (cm) x width (cm) x depth (cm) x 0.479.

Pelvic somatosensory evoked potentials of the pudendal nerve

Patients were assessed for the presence of any neurological impairment through careful personal and family history assessment and physical examination performed by a certified neurologist. Particular attention was placed on the

search for peripheral nerve diseases before recording of pelvic somatosensory evoked potentials of the pudendal nerve (PN_SEPs). To this aim prior medical history and laboratory screening for metabolic, toxic (e.g. alcohol abuse), or inherited disease known to be associated with peripheral nervous system damage was performed.

PN_SEPs were performed by stimulating bipolar penile ring electrodes placed at the base of the penis (cathode) and distally on the penile shaft (anode). The potentials were recorded with intradermal needle electrodes from Cz and Fz (reference electrode) placed according to the 10-20 system [45, 46]. A ground electrode was placed between the site of stimulation and the recording site, in order to decrease stimulus artefacts. During the recording phase, the patient was in supine position on the examination table with dimmed room lights. Intensity of the stimuli in average were 3-4 times the threshold level. Threshold was considered the intensity at which the patient was first able to perceive the stimulus. Once the sensory threshold was determined, the stimulation was progressively increased up to the maximally tolerable intensities and with a frequency of 5 Hz. Sampling was performed averaging 500 responses. Measurements were repeated at least 2 times in order to ensure reproducibility of the response potentials.

Latency of P1 wave was defined as the first positive deflection of the averaged cortical waveform wave form and it was considered normal if < 45.0 ms [45, 46]. If the response could not be reproduced at least twice or if the cortical response could not be clearly identified, the P1 was classified as not evocable.

Quantitative analysis of neuroactive steroids by LC-MS/MS

Extraction and purification of the samples were performed according to Caruso et al. [47].

Briefly, samples were spiked with 17β-Estradiol-2,3,4-$^{13}C_3$ (1ng/sample), PREG-20,21-$^{13}C_2$ (10ng/sample) and PROG-2,3,4-$^{13}C_3$ (0.4ng/sample), as internal standards (IS) and homogenized in MeOH/acetic acid (99:1 v/v) using a tissue lyser (Qiagen, Italy). After an overnight extraction at 4°C, samples were centrifuged at 12000 rpm for 5 min and the pellet was extracted twice with 1 ml of MeOH/acetic acid (99:1 v/v). The organic residues were resuspended with 3 ml of MeOH/H_20 (10:90 v/v) and passed through a SPE cartridges, the steroids were eluted in MeOH, concentrated and transferred in autosampler vials before the LC-MS/MS analysis. Quantitative analysis of neuroactive steroids was performed on the basis of calibration curves daily prepared and analysed as previously described [47]. Linear least-square regression analysis was performed and in addition, a blank (non-spiked sample) and a zero sample (only spiked with IS) were run to demonstrate the absence of interferences at the retention times and m/z corresponding to all the analytes. Moreover, the precision of the assay, inter-assay accuracy, precision and reproducibility are calculated as described in [47] and are within tolerance range for all the neuroactive steroids.

Instrumental conditions

Positive atmospheric pressure chemical ionization (APCI+) experiments were performed with a linear ion trap - mass spectrometer (LTQ, ThermoElectron Co,

San Jose, CA, USA) using nitrogen as sheath, auxiliary and sweep gas. The instrument was equipped with a Surveyor liquid chromatography (LC) Pump Plus and a Surveyor Autosampler Plus (ThermoElectron Co, San Jose, CA, USA). The mass spectrometer (MS) was employed in tandem mode (MS/MS) using helium as collision gas.

The LC mobile phases, the analytical conditions and the transition used were described by Caruso et al. [47].

Statistical analysis

Considering the not-normal distribution of parameters collected, group differences were tested using Mann–Whitney U test. Differences among categorical variables were evaluated using Fisher's exact test. The linearity of the standard curve (r^2) and all the validation parameters of the method were judged by GraphPad4 PRISM (version 5). A p-value of less than 0.05 was considered significant.

RESULTS

General data of the PFS patients at the clinical evaluation.

Sixteen men were evaluated. Mean age was 32 years old; mean of treatment duration was 1037 days. The interval between finasteride withdrawal and clinical evaluation was very wide (range 451-4697 days, median 1970).

Mental health assessment

All consecutive subjects with PFS were screened by K-10, and nine out sixteen screened positive. Eight out of sixteen subjects (50%) suffered from a DSM-IV major depressive disorder (MDD) as diagnosed by MINI. BDI and BAI of subjects with MDD, as compared with those without this disorder, are shown in table 2 showing significant higher levels for those with MDD.

Andrological assessment

All patients (100%) showed some degree of ED, with a mean score at erectile function domain of 10.31±9.48 (Table 3). In particular, we found 10 men with severe ED (62.50%) and six with mild-moderate forms (37.50%). Although a clear cut-off for normal values was not proposed in the literature for other IIEF-15 domains, our patients showed a low score also for orgasmic function, sexual desire and overall satisfaction domains, compared to general population [48] (Table 3). No differences were found in the incidence of MDD and ED (p=0.296). Normal testicular volume was found in all patients considered, without alterations in ejaculatory ducts (Table 3).

Assessment of neurogenic control of erection

Patients had no evidence of neurological disease by objective examination or personal and family history. However, while 12 patients (75%) had normal PN_SEPs results, in four cases (25%) the results were abnormal: in 3 cases no reproducible response was evoked, while 1 patient had increased P1 wave latency. Significant abnormal PN_SEP results were found in patients with severe ED, compared to men with mild-moderate ED (p=0.032).

Neuroactive steroid levels in PFS patients and healthy controls.

As reported in Table 4, the levels of some neuroactive steroids analysed in CSF of PFS patients were significantly different versus those in healthy controls. In particular, the levels of PREG, as well as of its further metabolites, PROG and DHP, were significantly decreased in CSF of PFS patients. On the contrary, the levels of DHEA and T were significantly increased. The levels of metabolites of T, such as DHT, 3α-diol and 17β-E were also affected in CSF of PFS patients. In particular, we reported a decrease in the levels of DHT and 17β-E, associated with an increase in the 3α-diol levels. Assessment of the levels of neuroactive steroids in plasma of PFS patients showed similarities and dissimilarities in comparison to what observed in CSF. Thus, the pattern in plasma did not exactly reflect what observed in CSF. In particular, at variance to what observed in CSF, the plasma levels of PREG were significantly increased. In addition, the levels of PROG and T metabolites, such as DHT, 3α-diol and 17β-E, were unaffected in plasma of PFS patients. Furthermore, the levels of THP that were unaffected in CSF, showed a significant decreased in plasma. In agreement to what observed in

CSF, the plasma levels of DHEA and T showed a significant increase and those of DHP a significant decrease.

DISCUSSION

After approval for treatment of AGA in 1997, warnings of persistent adverse sexual effects of finasteride were made by Swedish Medical Products Agency in 2008 and by Medicines and Healthcare Products Regulatory Agency of UK in 2009. In 2012, the Food and Drug Administration (FDA) required the finasteride labels to include multiple persistent side effects. Up to now several reports highlighted the presence of such persistent sexual effects [25-30], depression and anxiety [31-34]. However, these observations were mainly based on self-reporting of the symptomatology by the patients. Indeed, few papers have rigorously investigated these aspects. Here, we confirm the presence of persistent ED and MDD in PFS patients, confirming the recent results of Basaria and colleagues who found functional MRI abnormalities in brain regions of PFS patients targeted by the dopamine system (e.g. nucleus accumbens and prefrontal cortex) that are critical for normal erectile function and overlap with abnormalities seen in MDD [37]. In particular, all patients enrolled in our study showed ED, of severe degree in 62.50% and mild-moderate form in 37.50%. As expected, based on the findings of normal androgen levels in PFS patients by Basaria et al. [37], persistent ED in PFS patients is neither associated with altered testicular volume nor with alterations in ejaculatory ducts. Interestingly, as here observed, the incidence of severe ED is not related to MDD, but only to abnormal PN_SEPs results.

Recently, Traish et al. confirmed the negative effect of 5α-R inhibitors on erectile function [18]. In particular, these authors described, after chronic administration for benign prostatic hyperplasia, a negative impact of finasteride, but not tamsulosin, on erectile function, throughout a reduction in T serum levels [18].

These results confirm the known relationship between T and sexual function [49, 50]. Here we describe self-reported occurrence of persistent ED after finasteride withdrawal in young patients treated for AGA who had no prior history of ED before finasteride treatment. However, contrary to previous results, we do not find a decrease in T plasma levels, suggesting that other neuroactive steroids could be responsible for ED.

Besides clinical and andrological examination, comprehensive neuro-urological diagnostic evaluation of erectile dysfunction requires also neuro-physiological evaluation. In this study, we limited our assessment to PN_SEPs in order to limit patients discomfort and in view of previous observations showing that P1 latencies results were not significantly different adding other assessments, such as perianal stimulation [45, 46]. We have presented the first objective evidence in PFS patients of peripheral neuropathy of the pudendal nerve which is critical for normal neurogenic control of erection. PN_SEP abnormalities were found in 25% of PFS patients, in spite of normal neurological examination and no prior history of neurological disease. Moreover, no evidence of metabolic, toxic (e.g. alcohol abuse), or inherited disease known to be associated with peripheral nervous system damage which might be correlated with PN_SEPs alterations was detected. On the other hand, our observations may be supported by data in rat. In this model, altered neurogenic control of erection (i.e., cholinergic and adrenergic contractile responses) was observed after discontinuation of the 5α-R inhibitor, dutasteride [51].

MDD was present only in 50% of our group of PFS patients. Thus, the ED detected in our group seems to be not associated only to depressive symptomatology, which was consistently rated, similarly to anxiety, as high as

expected in subjects with MDD [52]. Although depression could be characterized by alteration in neuroactive steroid levels [53-55], the correlation between these molecules and ED is not demonstrated so far. Our research unit previously reported altered plasma and CSF levels of neuroactive steroids, but neither andrological nor psychiatric assessment was performed [35, 36]. In the plasma of PFS patients, we here reported a decrease of THP levels. A decrease of the plasma levels of this neuroactive steroid is a common feature of anxious/depressive symptomatology and this disequilibrium may be corrected by antidepressant [53-55]. A link between T levels and depression was already investigated [56], showing that low T plasma levels are observed in young as well as aged hypogonadal men with anxiety/depressive symptomatology [57-60]. However, in our group of PFS patients, an increase in T levels were detected both in plasma and in CSF. In this context it is important to highlight that, the CSF levels of the active metabolite of T, DHT (i.e., a neuroactive steroid showing, in comparison to T, higher affinity vs androgen receptor) were significantly decreased in all PFS patients. Thus, we consider that T levels may not be predictive of ED and MDD, while its metabolite seems to be related to these conditions. Moreover, an involvement of androgen receptor (AR) in PFS effects has been already proposed. In fact, an upregulation of AR expression in the prepuce of PFS patients [61] and in the nervous system of male rats [62] one month after end of treatment with finasteride (i.e., withdrawal period) was observed. Thus, a combination between reduction in DHT and upregulation of AR could be proposed as pathogenetic mechanism underlying PFS.

Our reported changes in neuroactive steroid levels occurring in CSF represent a different proposed pathogenetic mechanism. A larger number of neuroactive

steroids are altered in CSF in comparison to what is observed in plasma. Indeed, only the levels of DHEA and T are increased and DHP decreased in both fluids. On the contrary, PREG decreased in CSF but increased in plasma. Moreover, a decrease in the levels of PROG, DHT and 17β-E as well as an increase in 3α-diol levels was observed only in CSF of PFS patients. A different pattern between plasma and CSF is not surprising. Indeed, as demonstrated in various physiological or pathological conditions in several experimental models, changes occurring in plasma do not reflect exactly what occurs in CSF and in the nervous system [8, 14, 47]. In particular, this different pattern may be observed in our preliminary studies performed in a smaller number of PFS patients [35, 36] as well as one month after withdrawal of the treatment with finasteride in the nervous system of male rats [62].

It is important to highlight that, as mentioned above, a significant decrease in the levels of PROG was observed in the CSF of PFS patients. This may suggest a possible association between this neuroactive steroid and MDD symptomatology. Indeed, a role of PROG in depressive symptomatology associated to different pathologies has been already proposed [53]. Larger studies are warranted to further evaluate the role of CSF PROG levels in PFS patients with MDD.

Another important finding here reported is that the effect of finasteride on neuroactive steroid levels do not only affect the levels of 5α-reduced metabolites of PROG and T (i.e., DHP and DHT respectively) and further metabolites (i.e., THP), but also PROG and T themselves, as well as their precursors (i.e., PREG and DHEA). Thus, finasteride treatment has broad consequences on neuroactive steroid levels. Finally, it is also important to note that the assessment of neuroactive steroids here performed in a high number of patients show also

some small differences to what previously reported in a low number and different PFS patients [35, 36]. For instance, in CSF we here report decrease in PREG and 17β-E levels, unchanged THP levels and increase in DHEA levels suggesting that neuroactive steroid changes in PFS patients remain heterogeneous. Therefore, to analyse in future studies the neuroactive steroid levels and psychiatric and andrological features in a larger number of PFS patients and to compare these parameters in an age-matched healthy control group and asymptomatic former users of comparable finasteride dose ranges will be important.

In conclusion, PFS patients show altered levels of important physiological regulators of brain function, such as neuroactive steroids. This could explain the andrological and psychiatric features observed in PFS patients. However, even if the present observations add another piece of information to what has been so far proposed by others, such as an alteration of dopaminergic signalling in the nucleus accumbens (i.e., a brain region that is critical for normal libido and mood regulation) [63], lateralization process of the brain [64] or pre-existing familial mental health condition [65] a clear demonstration of the pathogenic mechanism underlying the PFS is not yet understood.

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Table 1. Symptoms reported by PFS patients at the moment of the clinical evaluation.

	Pre-Finasteride				During Finasteride				Post-Finasteride (during worst period)				Post-Finasteride (currently)			
Symptoms	**never**	**sometimes**	**often**	**always**	**never**	**sometimes**	**often**	**always**	**never**	**sometimes**	**often**	**always**	**never**	**sometimes**	**often**	**always**
Decreased self-confidence	9	6	1	0	6	6	3	1	1	5	2	8	2	7	5	2
Decline of emotional verve, initiative and desire t	8	8	0	0	4	9	1	2	0	2	8	6	0	5	7	4
Difficulty concentrating and focusing (brain fog)	8	7	1	0	7	7	1	1	1	4	5	6	1	5	5	5
Mental confusion	13	3	0	0	10	6	0	0	4	3	4	5	5	4	5	2
Forgetfulness or loss of short-term memory	11	5	0	0	8	8	0	0	4	3	7	2	4	4	6	2
Losing train of thought or reasoning	15	1	0	0	11	3	1	1	6	3	5	2	7	3	5	1
Slurred speech or stumbling over words	12	4	0	0	9	5	1	1	5	6	2	2	5	6	4	1
Irritability or easly flying into a rage	7	7	2	0	4	6	5	1	3	5	4	4	3	9	2	2
Nervousness, agitation, inner restlessness	6	8	2	0	4	7	4	1	2	1	7	6	4	2	5	5
Depression, hopelessness, feelings of worthlessn	10	6	0	0	5	5	5	1	3	1	4	8	3	4	3	6
Suicidal thoughts	15	1	0	0	12	3	0	1	5	4	4	3	7	4	3	2
Anxiety	5	9	2	0	3	6	6	1	3	4	4	5	4	6	2	4
Panic attacks	15	0	1	0	11	1	4	0	8	4	2	2	8	4	3	1
Sleep problems	9	5	2	0	7	5	2	2	2	2	4	8	3	2	5	6
Loss of libido and sexual desire	15	1	0	0	3	8	3	2	0	0	4	12	0	2	4	10
Difficulty in achieving an erection	14	1	1	0	6	5	3	2	0	0	4	12	0	3	4	9
Feeling of lack connection brain-penis	16	0	0	0	5	6	1	4	0	1	2	13	0	3	3	10
Genital numbness	16	0	0	0	10	2	3	1	2	1	2	11	2	4	3	7
Feeling tinglings or pinpricks	15	1	0	0	15	1	0	0	10	1	3	2	10	3	2	1
Tics, muscle spasms and fasciculations	13	2	1	0	11	2	3	0	8	2	1	5	8	4	2	2
Tremors (body, limbs, hands, neck, head, etc.)	14	1	1	0	12	2	1	1	11	2	0	3	11	2	1	2
Involontary muscle tension and contraction	15	1	0	0	12	2	1	1	10	1	2	3	10	2	3	1
Dizziness	13	3	0	0	12	2	2	0	12	2	1	1	13	2	0	1
Headache, migraine, head pressure	14	2	0	0	13	3	0	0	8	5	1	2	8	7	0	1
Chronic fatigue, weakness, ataxia	12	3	1	0	6	6	4	0	2	1	5	8	4	1	7	4
Joint pain and muscular ache	14	2	0	0	13	2	1	0	5	4	5	2	5	4	4	3
Decreased body temperature	15	1	0	0	13	2	0	1	8	2	2	4	8	2	1	5
Photophobia and other visual problems	13	1	1	1	14	0	1	1	8	1	3	4	8	2	2	4

In the table are represented the number of patients reporting the frequency of specific symptoms.

Table 2. Anxiety and depression levels by DSM IV Major Depressive Disorder.

		Beck Anxiety Inventory	Beck Depression Inventory
PFS patients	MDD (N=8)	21.50±10.76 **	20.87±11.62 *
	NON-MDD (N=8)	7.50±5.55	7.62±6.48

MDD: major depressive disorder. Data are expressed as mean± SD. * p<0.05; ** p< 0.01 vs PFS non-MDD with Mann-Whitney U test.

Table 3. International Index of Erectile Function (IIEF)-15 scores and testicular volume.

IIEF	
Erective function	10.31±9.48
Orgasmic Function	4.12±3.77
Sexual Desire	4.44±2.22
Intercourse Satisfaction	2.87±3.90
Overall Satisfaction	3.37±1.86
Testicular ultrasound	
Right testis volume (mL)	17.77±5.06
Left testis volume (mL)	17.13±4.69
Right epididymal head (mm)	9.61±2.14
Left epididymal head (mm)	8.82±1.80
Right varicocele n° (%)	1 (6.25)
Grade 1	1 (100)
Grade 2	0
Grade 3	0
Grade 4	0
Grade 5	0
Left varicocele n° (%)	8 (50.00)
Grade 1	2 (25.00)
Grade 2	6 (75.00)
Grade 3	0
Grade 4	0
Grade 5	0

Data are expressed as mean+SD.

TABLE 4. Neuroactive steroid levels in cerebrospinal fluid and plasma in controls and PFS patients

	PREG	PROG	DHP	Isopreg	THP	DHEA	T	DHT	3α-diol	3β-diol	17β-E
CSF											
CTRL (N=25)	0.31±0.21	0.19±0.14	2.83±1.86	0.11±0.03	2.01±4.23	0.2±0.11	0.13±0.11	0.25±0.21	UDL	UDL	0.07±0.05
PFS (N=14)	0.12±0.11 ***	UDL ***	0.56±0.90 ***	UDL	0.18±0.21	0.33±0.07 ***	1.97±1.99 ***	0.06±0.01 ***	0.20±0.43 *	UDL	UDL **
PLASMA											
CTRL (N=25)	0.96±0.84	0.18±0.09	0.73±1.44	0.42±1.56	0.29±0.49	1.86±3.19	5.70±2.75	0.42±0.39	0.12±0.12	0.17±0.25	UDL
PFS (N=14)	2.48±2.02 **	0.20±0.23	0.26±0.04 *	0.26±0.41	UDL **	8.79±7.42 **	12.3±3.99 ***	0.49±0.21	0.18±0.13	0.12±0.16	UDL

PREG=pregnenolone, PROG=progesterone, DHP=dihydroprogesterone, Isopreg=isopregnanolone, THP=tetrahydroprogesterone, DHEA=dehydroepiandrosterone, T=testosterone, DHT=dihydrotestosterone, 3α-diol=5α-androstane-3α,17β-diol, 3β-diol=5α-androstane-3β,17β-diol, 17β-E=17β-estradiol. UDL=under detection limit. Detection limit was 0.1 pg/µL for Isopreg and THP, 0.05 pg/µL for PROG, 3α-diol and 3β-diol, 0.02 pg/µL for 17β-E. Data are expressed as pg/µL (mean±SD). * $p<0.05$; ** $p< 0.01$ and *** $p<0.001$ vs CTRL by Mann-Whitney U test.