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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.**

**SBMB-D-17-00001**

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

Roberto Cosimo Melcangi<sup>1\*</sup>, Daniele Santi<sup>2</sup>, Roberto Spezzano<sup>1</sup>, Maria Grimoldi<sup>3</sup>, Tommaso Tabacchi<sup>4</sup>, Maria Letizia Fusco<sup>3</sup>, Silvia Diviccaro<sup>1</sup>, Silvia Giatti<sup>1</sup>, Giuseppe Carrà<sup>4</sup>, Donatella Caruso<sup>1</sup>, Manuela Simoni<sup>2</sup>, Guido Cavaletti<sup>3</sup>.

<sup>1</sup>Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy

<sup>2</sup>Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy

<sup>3</sup>Experimental Neurology Unit and Milan Center for Neuroscience, School of Medicine and Surgery, University of Milano Bicocca, Monza, Italy

<sup>4</sup>Department of Medicine and Surgery, University of Milano Bicocca, Monza, Italy

**Corresponding author:**

\*Roberto Cosimo Melcangi

Email: [roberto.melcangi@unimi.it](mailto:roberto.melcangi@unimi.it)

Tel. +39-02-50318238; Fax: +39-02-50318204.

**Abbreviations:** 3 $\alpha$ - hydroxysteroid oxidoreductase (3 $\alpha$ -HSOR); 3 $\beta$ -hydroxysteroid oxidoreductase (3 $\beta$ -HSOR); 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol (3 $\alpha$ -diol); 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol (3 $\beta$ -diol); 5 $\alpha$ -reductase (5 $\alpha$ -R); 17 $\beta$ -estradiol (17 $\beta$ -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; 5 $\alpha$ -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  $17\beta$ -estradiol ( $17\beta$ -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  $5\alpha$ -reductase ( $5\alpha$ -R), into dihydroprogesterone (DHP) and dihydrotestosterone (DHT) respectively. These neuroactive steroids are then further converted by the action of  $3\alpha$ - ( $3\alpha$ -HSOR) or  $3\beta$ -hydroxysteroid oxidoreductase ( $3\beta$ -HSOR) into further metabolites. In particular, DHP is converted into tetrahydroprogesterone (THP) and into isopregnanolone (Isopreg) while T is converted into  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol ( $3\alpha$ -diol) or  $5\alpha$ -androstane- $3\beta,17\beta$ -diol ( $3\beta$ -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  $5\alpha$ -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 5 $\alpha$ -reduced metabolites [8, 14]. Actually three 5 $\alpha$ -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 5 $\alpha$ -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, 3 $\alpha$ -diol, 3 $\beta$ -diol and 17 $\beta$ -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 ellipsoid: 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  $17\beta$ -Estradiol-2,3,4- $^{13}\text{C}_3$  (1ng/sample), PREG-20,21- $^{13}\text{C}_2$  (10ng/sample) and PROG-2,3,4- $^{13}\text{C}_3$  (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<sub>2</sub>O (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  $10.31 \pm 9.48$  (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,  $3\alpha$ -diol and  $17\beta$ -E were also affected in CSF of PFS patients. In particular, we reported a decrease in the levels of DHT and  $17\beta$ -E, associated with an increase in the  $3\alpha$ -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,  $3\alpha$ -diol and  $17\beta$ -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 5 $\alpha$ -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  $5\alpha$ -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  $17\beta$ -E as well as an increase in  $3\alpha$ -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  $5\alpha$ -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  $17\beta$ -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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## References

- [1] P. Micevych, K. Sinchak, Synthesis and function of hypothalamic neuroprogesterone in reproduction, *Endocrinology* 149(6) (2008) 2739-2742.
- [2] M. Frankfurt, V. Luine, The evolving role of dendritic spines and memory: Interaction(s) with estradiol, *Horm Behav* 74 (2015) 28-36.
- [3] M.A. Arevalo, I. Azcoitia, I. Gonzalez-Burgos, L.M. Garcia-Segura, Signaling mechanisms mediating the regulation of synaptic plasticity and memory by estradiol, *Horm Behav* 16 (2015) 17-29.
- [4] C. Guerra-Araiza, M.A. Amorim, I. Camacho-Arroyo, L.M. Garcia-Segura, Effects of progesterone and its reduced metabolites, dihydroprogesterone and tetrahydroprogesterone, on the expression and phosphorylation of glycogen synthase kinase-3 and the microtubule-associated protein tau in the rat cerebellum, *Dev Neurobiol* 67(4) (2007) 510-520.
- [5] D.A. Velazquez-Zamora, L.M. Garcia-Segura, I. Gonzalez-Burgos, Effects of selective estrogen receptor modulators on allocentric working memory performance and on dendritic spines in medial prefrontal cortex pyramidal neurons of ovariectomized rats, *Horm Behav* 61(4) (2012) 512-517.
- [6] M. Schumacher, R. Hussain, N. Gago, J.P. Oudinet, C. Mattern, A.M. Ghomari, Progesterone synthesis in the nervous system: implications for myelination and myelin repair, *Front Neurosci* 6 (2012) 10.
- [7] S. Giatti, L.M. Garcia-Segura, R.C. Melcangi, New steps forward in the neuroactive steroid field, *J Steroid Biochem Mol Biol* 153 (2015) 127-134.
- [8] R.C. Melcangi, S. Giatti, L.M. Garcia-Segura, Levels and actions of neuroactive steroids in the nervous system under physiological and pathological conditions: Sex-specific features, *Neurosci Biobehav Rev* 67 (2016) 25-40.



- [9] J.M. Wang, L. Liu, R.W. Irwin, S. Chen, R.D. Brinton, Regenerative potential of allopregnanolone, *Brain Res Rev* 57(2) (2008) 398-409.
- [10] J.M. Bowers, J. Waddell, M.M. McCarthy, A developmental sex difference in hippocampal neurogenesis is mediated by endogenous oestradiol, *Biol Sex Differ* 1(1) (2010) 8.
- [11] L.A. Galea, Gonadal hormone modulation of neurogenesis in the dentate gyrus of adult male and female rodents, *Brain Res Rev* 57(2) (2008) 332-341.
- [12] P. Celec, D. Ostatnikova, J. Hodosy, On the effects of testosterone on brain behavioral functions, *Front Neurosci* 9 (2015) 12.
- [13] R.C. Melcangi, L.M. Garcia-Segura, A.G. Mensah-Nyagan, Neuroactive steroids: state of the art and new perspectives, *Cell Mol Life Sci* 65(5) (2008) 777-797.
- [14] R.C. Melcangi, S. Giatti, D. Calabrese, M. Pesaresi, G. Cermenati, N. Mitro, B. Viviani, L.M. Garcia-Segura, D. Caruso, Levels and actions of progesterone and its metabolites in the nervous system during physiological and pathological conditions, *Prog Neurobiol* 113 (2014) 56-69.
- [15] A.M. Traish, 5alpha-reductases in human physiology: an unfolding story, *Endocr Pract* 18(6) (2012) 965-975.
- [16] V. Cantagrel, D.J. Lefeber, B.G. Ng, Z. Guan, J.L. Silhavy, S.L. Bielas, L. Lehle, H. Hombauer, M. Adamowicz, E. Swiezewska, A.P. De Brouwer, P. Blumel, J. Sykut-Cegielska, S. Houliston, D. Swistun, B.R. Ali, W.B. Dobyns, D. Babovic-Vuksanovic, H. van Bokhoven, R.A. Wevers, C.R. Raetz, H.H. Freeze, E. Morava, L. Al-Gazali, J.G. Gleeson, SRD5A3 is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder, *Cell* 142(2) (2010) 203-217.

- [17] D.A. Finn, A.S. Beadles-Bohling, E.H. Beckley, M.M. Ford, K.R. Gililand, R.E. Gorin-Meyer, K.M. Wiren, A new look at the 5alpha-reductase inhibitor finasteride, *CNS Drug Rev* 12(1) (2006) 53-76.
- [18] A.M. Traish, R.C. Melcangi, M. Bortolato, L.M. Garcia-Segura, M. Zitzmann, Adverse effects of 5alpha-reductase inhibitors: What do we know, don't know, and need to know?, *Rev Endocr Metab Disord* 16 (2015) 177-198.
- [19] M.S. Irwig, Safety concerns regarding 5alpha reductase inhibitors for the treatment of androgenetic alopecia, *Curr Opin Endocrinol Diabetes Obes* 22(3) (2015) 248-253.
- [20] E.A. Olsen, M. Hordinsky, D. Whiting, D. Stough, S. Hobbs, M.L. Ellis, T. Wilson, R.S. Rittmaster, The importance of dual 5alpha-reductase inhibition in the treatment of male pattern hair loss: results of a randomized placebo-controlled study of dutasteride versus finasteride, *J Am Acad Dermatol* 55(6) (2006) 1014-1023.
- [21] K.D. Kaufman, E.A. Olsen, D. Whiting, R. Savin, R. DeVillez, W. Bergfeld, V.H. Price, D. Van Neste, J.L. Roberts, M. Hordinsky, J. Shapiro, B. Binkowitz, G.J. Gormley, Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group, *J Am Acad Dermatol* 39(4 Pt 1) (1998) 578-589.
- [22] K.J. McClellan, A. Markham, Finasteride: a review of its use in male pattern hair loss, *Drugs* 57(1) (1999) 111-126.
- [23] A.K. Ali, B.S. Heran, M. Etminan, Persistent Sexual Dysfunction and Suicidal Ideation in Young Men Treated with Low-Dose Finasteride: A Pharmacovigilance Study, *Pharmacotherapy* 35(7) (2015) 687-695.

- [24] S.M. Belknap, I. Aslam, T. Kiguradze, W.H. Temps, P.R. Yarnold, J. Cashy, R.E. Brannigan, G. Micali, B. Nardone, D.P. West, Adverse Event Reporting in Clinical Trials of Finasteride for Androgenic Alopecia: A Meta-analysis, *JAMA Dermatol* 151(6) (2015) 600-606.
- [25] S. Gur, P.J. Kadowitz, W.J. Hellstrom, Effects of 5-alpha reductase inhibitors on erectile function, sexual desire and ejaculation, *Expert Opin Drug Saf* 12(1) (2013) 81-90.
- [26] G. Corona, G. Rastrelli, E. Maseroli, G. Balercia, A. Sforza, G. Forti, E. Mannucci, M. Maggi, Inhibitors of 5alpha-reductase-related side effects in patients seeking medical care for sexual dysfunction, *J Endocrinol Invest* 35(10) (2012) 915-920.
- [27] A.M. Traish, J. Hassani, A.T. Guay, M. Zitzmann, M.L. Hansen, Adverse side effects of 5alpha-reductase inhibitors therapy: persistent diminished libido and erectile dysfunction and depression in a subset of patients, *J Sex Med* 8(3) (2011) 872-884.
- [28] M.S. Irwig, S. Kolukula, Persistent sexual side effects of finasteride for male pattern hair loss, *J Sex Med* 8(6) (2011) 1747-1753.
- [29] M.S. Irwig, Persistent sexual side effects of finasteride: could they be permanent?, *J Sex Med* 9(11) (2012) 2927-2932.
- [30] G. Chiriaco, S. Cauci, G. Mazzon, C. Trombetta, An observational retrospective evaluation of 79 young men with long-term adverse effects after use of finasteride against androgenetic alopecia, *Andrology* 4(2) (2016) 245-250.
- [31] M.S. Irwig, Depressive symptoms and suicidal thoughts among former users of finasteride with persistent sexual side effects, *J Clin Psychiatry* 73(9) (2012) 1220-1223.

- [32] B. Rahimi-Ardabili, R. Pourandarjani, P. Habibollahi, A. Mualeki, Finasteride induced depression: a prospective study, *BMC Clin Pharmacol* 6 (2006) 7.
- [33] G. Altomare, G.L. Capella, Depression circumstantially related to the administration of finasteride for androgenetic alopecia, *J Dermatol* 29(10) (2002) 665-669.
- [34] C.A. Ganzer, A.R. Jacobs, F. Iqbal, Persistent Sexual, Emotional, and Cognitive Impairment Post-Finasteride: A Survey of Men Reporting Symptoms, *Am J Mens Health* 9 (2014) 222-228.
- [35] R.C. Melcangi, D. Caruso, F. Abbiati, S. Giatti, D. Calabrese, F. Piazza, G. Cavaletti, Neuroactive Steroid Levels are Modified in Cerebrospinal Fluid and Plasma of Post-Finasteride Patients Showing Persistent Sexual Side Effects and Anxious/Depressive Symptomatology, *J Sex Med* 10(10) (2013) 2598-2603.
- [36] D. Caruso, F. Abbiati, S. Giatti, S. Romano, L. Fusco, G. Cavaletti, R.C. Melcangi, Patients treated for male pattern hair with finasteride show, after discontinuation of the drug, altered levels of neuroactive steroids in cerebrospinal fluid and plasma, *J Steroid Biochem Mol Biol* 146 (2015) 74-79.
- [37] S. Basaria, R. Jasuja, G. Huang, W. Wharton, H. Pan, K. Pencina, Z. Li, T.G. Travison, J. Bhawan, R. Gonthier, F. Labrie, A.Y. Dury, C. Serra, A. Papazian, M. O'Leary, S. Amr, T.W. Storer, E. Stern, S. Bhasin, Characteristics of Men Who Report Persistent Sexual Symptoms after Finasteride Use for Hair Loss, *J Clin Endocrinol Metab* 101 (12) (2016) 4669-4680.
- [38] G. Carra, P. Sciarini, G. Segagni-Lusignani, M. Clerici, C. Montomoli, R.C. Kessler, Do they actually work across borders? Evaluation of two measures of

psychological distress as screening instruments in a non Anglo-Saxon country, *Eur Psychiatry* 26(2) (2011) 122-127.

[39] D.V. Sheehan, Y. Lecrubier, K.H. Sheehan, P. Amorim, J. Janavs, E. Weiller, T. Hergueta, R. Baker, G.C. Dunbar, The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10, *J Clin Psychiatry* 59 Suppl 20 (1998) 22-33;quiz 34-57.

[40] A.T. Beck, N. Epstein, G. Brown, R.A. Steer, An inventory for measuring clinical anxiety: psychometric properties, *J Consult Clin Psychol* 56(6) (1988) 893-897.

[41] T.A. Furukawa, Assessment of mood: guides for clinicians, *J Psychosom Res* 68(6) (2010) 581-589.

[42] S.R. Erickson, S. Guthrie, M. Vanetten-Lee, J. Himle, J. Hoffman, S.F. Santos, A.S. Janeck, K. Zivin, J.L. Abelson, Severity of anxiety and work-related outcomes of patients with anxiety disorders, *Depress Anxiety* 26(12) (2009) 1165-1171.

[43] R.C. Rosen, A. Riley, G. Wagner, I.H. Osterloh, J. Kirkpatrick, A. Mishra, The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction, *Urology* 49(6) (1997) 822-830.

[44] J.C. Cappelleri, R.C. Rosen, M.D. Smith, A. Mishra, I.H. Osterloh, Diagnostic evaluation of the erectile function domain of the International Index of Erectile Function, *Urology* 54(2) (1999) 346-351.

[45] T. Kaiser, W.H. Jost, J. Osterhage, H. Derouet, K. Schimrigk, Penile and perianal pudendal nerve somatosensory evoked potentials in the diagnosis of erectile dysfunction, *Int J Impot Res* 13(2) (2001) 89-92.

- [46] J.A. Munday, Instrumentation and electrode placement, *Respir Care Clin N Am* 11(4) (2005) 605-615, viii.
- [47] D. Caruso, M. Pesaresi, F. Abbiati, D. Calabrese, S. Giatti, L.M. Garcia-Segura, R.C. Melcangi, Comparison of plasma and cerebrospinal fluid levels of neuroactive steroids with their brain, spinal cord and peripheral nerve levels in male and female rats, *Psychoneuroendocrinology* 38(10) (2013) 2278-2290.
- [48] R.C. Rosen, J.C. Cappelleri, N. Gendrano, 3rd, The International Index of Erectile Function (IIEF): a state-of-the-science review, *Int J Impot Res* 14(4) (2002) 226-244.
- [49] M.J. Pagano, A. De Fazio, A. Levy, A. RoyChoudhury, P.J. Stahl, Age, Body Mass Index, and Frequency of Sexual Activity are Independent Predictors of Testosterone Deficiency in Men With Erectile Dysfunction, *Urology* 90 (2016) 112-118.
- [50] C.A. Podlasek, J. Mulhall, K. Davies, C.J. Wingard, J.L. Hannan, T.J. Bivalacqua, B. Musicki, M. Khera, N.F. Gonzalez-Cadavid, A.L. Burnett, 2nd, Translational Perspective on the Role of Testosterone in Sexual Function and Dysfunction, *J Sex Med* 13(8) (2016) 1183-1198.
- [51] C.V. Oztekin, S. Gur, N.A. Abdulkadir, U. Lokman, A.O. Akdemir, M. Cetinkaya, W.J. Hellstrom, Incomplete recovery of erectile function in rat after discontinuation of dual 5-alpha reductase inhibitor therapy, *J Sex Med* 9(7) (2012) 1773-1781.
- [52] R. Ball, R.A. Steer, Mean Beck Depression Inventory-II scores of outpatients with dysthymic or recurrent-episode major depressive disorders, *Psychol Rep* 93(2) (2003) 507-512.

- [53] C.F. Zorumski, S.M. Paul, Y. Izumi, D.F. Covey, S. Mennerick, Neurosteroids, stress and depression: Potential therapeutic opportunities, *Neurosci Biobehav Rev* 37(1) (2013) 109-122.
- [54] E. Romeo, A. Strohle, G. Spalletta, F. di Michele, B. Hermann, F. Holsboer, A. Pasini, R. Rupprecht, Effects of antidepressant treatment on neuroactive steroids in major depression, *Am J Psychiatry* 155(7) (1998) 910-913.
- [55] V. Uzunova, L. Sampson, D.P. Uzunov, Relevance of endogenous 3alpha-reduced neurosteroids to depression and antidepressant action, *Psychopharmacology (Berl)* 186(3) (2006) 351-361.
- [56] J. McHenry, N. Carrier, E. Hull, M. Kabbaj, Sex differences in anxiety and depression: Role of testosterone, *Front Neuroendocrinol* 35(1) (2014) 42-57.
- [57] M.M. Shores, V.M. Mocerri, K.L. Sloan, A.M. Matsumoto, D.R. Kivlahan, Low testosterone levels predict incident depressive illness in older men: effects of age and medical morbidity, *J Clin Psychiatry* 66(1) (2005) 7-14.
- [58] M.M. Shores, K.L. Sloan, A.M. Matsumoto, V.M. Mocerri, B. Felker, D.R. Kivlahan, Increased incidence of diagnosed depressive illness in hypogonadal older men, *Arch Gen Psychiatry* 61(2) (2004) 162-167.
- [59] F.A. Zarrouf, S. Artz, J. Griffith, C. Sirbu, M. Kommor, Testosterone and depression: systematic review and meta-analysis, *J Psychiatr Pract* 15(4) (2009) 289-305.
- [60] R.S. McIntyre, D. Mancini, B.S. Eisfeld, J.K. Soczynska, L. Grupp, J.Z. Konarski, S.H. Kennedy, Calculated bioavailable testosterone levels and depression in middle-aged men, *Psychoneuroendocrinology* 31(9) (2006) 1029-1035.

- [61] C. Di Loreto, F. La Marra, G. Mazzon, E. Belgrano, C. Trombetta, S. Cauci, Immunohistochemical evaluation of androgen receptor and nerve structure density in human prepuce from patients with persistent sexual side effects after finasteride use for androgenetic alopecia, *PLoS One* 9(6) (2014) e100237.
- [62] S. Giatti, B. Foglio, S. Romano, M. Pesaresi, G. Panzica, L.M. Garcia-Segura, D. Caruso, R.C. Melcangi, Effects of Subchronic Finasteride Treatment and Withdrawal on Neuroactive Steroid Levels and their Receptors in the Male Rat Brain, *Neuroendocrinology* 103(6) (2016) 746-757.
- [63] A. Soggiu, C. Piras, V. Greco, P. Devoto, A. Urbani, L. Calzetta, M. Bortolato, P. Roncada, Exploring the neural mechanisms of finasteride: a proteomic analysis in the nucleus accumbens, *Psychoneuroendocrinology* 74 (2016) 387-396.
- [64] I.G. Motofei, D.L. Rowland, S.R. Georgescu, M. Tampa, D. Baconi, E. Stefanescu, B.C. Baleanu, C. Balalau, V. Constantin, S. Paunica, Finasteride adverse effects in subjects with androgenic alopecia: A possible therapeutic approach according to the lateralization process of the brain, *J Dermatolog Treat* 27 (6) (2016) 495-497.
- [65] C.A. Ganzer, A.R. Jacobs, Emotional Consequences of Finasteride: Fool's Gold, *Am J Mens Health* (2016) pii: 1557988316631624. [Epub ahead of print].



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

Symptoms	Pre-Finasteride				During Finasteride				Post-Finasteride (during worst period)				Post-Finasteride (currently)			
	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 easily 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 worthlesn	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
Involuntary 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.**

<b>IIEF</b>	
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
<b>Testicular ultrasound</b>	
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
<b>Right varicocele n° (%)</b>	1 (6.25)
Grade 1	1 (100)
Grade 2	0
Grade 3	0
Grade 4	0
Grade 5	0
<b>Left varicocele n° (%)</b>	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	3 $\alpha$ -diol	3 $\beta$ -diol	17 $\beta$ -E
<b>CSF</b>											
CTRL (N=25)	0.31 $\pm$ 0.21	0.19 $\pm$ 0.14	2.83 $\pm$ 1.86	0.11 $\pm$ 0.03	2.01 $\pm$ 4.23	0.2 $\pm$ 0.11	0.13 $\pm$ 0.11	0.25 $\pm$ 0.21	UDL	UDL	0.07 $\pm$ 0.05
PFS (N=14)	0.12 $\pm$ 0.11 ***	UDL ***	0.56 $\pm$ 0.90 ***	UDL	0.18 $\pm$ 0.21	0.33 $\pm$ 0.07 ***	1.97 $\pm$ 1.99 ***	0.06 $\pm$ 0.01 ***	0.20 $\pm$ 0.43 *	UDL	UDL **
<b>PLASMA</b>											
CTRL (N=25)	0.96 $\pm$ 0.84	0.18 $\pm$ 0.09	0.73 $\pm$ 1.44	0.42 $\pm$ 1.56	0.29 $\pm$ 0.49	1.86 $\pm$ 3.19	5.70 $\pm$ 2.75	0.42 $\pm$ 0.39	0.12 $\pm$ 0.12	0.17 $\pm$ 0.25	UDL
PFS (N=14)	2.48 $\pm$ 2.02 **	0.20 $\pm$ 0.23	0.26 $\pm$ 0.04 *	0.26 $\pm$ 0.41	UDL **	8.79 $\pm$ 7.42 **	12.3 $\pm$ 3.99 ***	0.49 $\pm$ 0.21	0.18 $\pm$ 0.13	0.12 $\pm$ 0.16	UDL

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