Pregnenolone and Dehydroepiandrosterone as an Adjunctive Treatment in Schizophrenia and Schizoaffective Disorder: An 8-Week, Double-Blind, Randomized, Controlled, 2-Center, Parallel-Group Trial

Michael S. Ritsner, MD, PhD; Anatoly Gibel, MD; Tatyana Shleifer, MD; Igor Boguslavsky, MD; Ahmad Zayed, MD; Rachel Maayan, PhD; Abraham Weizman, MD; and Vladimir Lerner, MD, PhD

Objective: Pregnenolone (PREG) and dehydroepiandrosterone (DHEA) are reported to have a modulatory effect on neuronal excitability, synaptic plasticity, and response to stress; they are associated with mood regulation and cognitive performance. We investigated the influence of PREG and DHEA on psychotic symptoms and cognitive functioning as an add-on to ongoing antipsychotic treatment of patients with chronic schizophrenia or schizoaffective disorder.

Method: This 8-week, double-blind, randomized, placebo-controlled, 2-center study compared 30 mg/d of PREG (PREG-30), 200 mg/d of PREG (PREG-200), 400 mg/d of DHEA, and placebo as an adjunctive treatment of 58 chronic schizophrenia or schizoaffective disorder patients (*DSM-IV*). The data were collected from February 2005 until June 2007. The outcome measures were symptomatic and neurocognitive changes, functioning, and tolerability as assessed primarily by the Clinical Global Impressions-Severity of Illness scale and the Positive and Negative Syndrome Scale. Analyses are presented for 44 patients who completed 8 weeks of treatment and for 14 noncompleters.

Results: Compared with subjects who received placebo, those administered PREG-30 had significant reductions in positive symptom scores and extrapyramidal side effects (EPS) and improvement in attention and working memory performance, whereas subjects treated with PREG-200 did not differ on outcome variable scores for the study period. The general psychopathology severity and general functioning of patients receiving placebo and PREG-30 improved more than that of those subjects treated with DHEA, while EPS improved more in subjects treated with DHEA than in patients receiving placebo. Negative symptoms and akathisia were not significantly benefited by any treatment. The administration of PREG and DHEA was well tolerated.

Conclusions: Low-dose PREG augmentation demonstrated significant amelioration of positive symptoms and EPS and improvement in attention and working memory performance of schizophrenia and schizoaffective disorder patients. Further double-blind controlled studies are needed to investigate the clinical benefit of pregnenolone augmentation.

Trial Registration: clinicaltrials.gov Identifier: NCT00174889

J Clin Psychiatry 2010;71(10):1351–1362

Submitted: *January 2, 2009; accepted April 23, 2009.*
Online ahead of print: *June 15, 2010 (doi:10.4088/JCP.09m05031yel).*
Corresponding author: *Michael S. Ritsner, MD, PhD, Acute Department, Sha'ar Menashe Mental Health Center, Mobile Post Hefer 38814, Hadera, Israel (ritsner@sm.health.gov.il).*

Despite the effectiveness of antipsychotic medications in a substantial proportion of schizophrenia patients, the overall clinical response remains insufficient and incomplete. Consequently, the development of more effective treatments is an important research goal. One promising direction is the use of neurosteroids (substances produced in the brain) and neuroactive steroids (produced in the adrenal glands and gonads), both of which affect the brain.

Neurosteroids such as pregnenolone (PREG) and dehydroepiandrosterone (DHEA) and their sulfates (PREGS and DHEAS) are reported to have a modulatory effect on neuronal excitability and synaptic plasticity.[1,2] These neurosteroids may be suitable candidates in the treatment of schizophrenia and schizoaffective disorder patients, since PREG and DHEA have many functions associated with response to stress, mood regulation, and cognitive performance.[3,4] In particular, they may act as potential signaling molecules for neocortical organization during brain development, regulating the neuronal function by affecting the neuronal excitability through prominent modulatory effects on the γ-aminobutyric acid type A ($GABA_A$), *N*-methyl-D-aspartate (NMDA), sigma-1,[5–9] cholinergic,[10,11] and dopamine systems.[12] These modulations may lead to important changes for neuronal excitability. Neurosteroids demonstrate cognitive-enhancing effects and they have been reported to improve memory.[11,13] In preclinical studies, memory-enhancing effects of PREGS and DHEAS have been attributed to their NMDA-agonistic properties.[14,15] Finally, PREG may be relevant to the beneficial effects of antipsychotic agents, since both clozapine and olanzapine elevate PREG in rat hippocampus, cerebral cortex, and serum.[16] These findings, however, need to be replicated.

The main effect of PREG and DHEA and their sulfates is neuroprotective.[17–20] The specific mechanisms by which PREG and DHEA exert their neuroprotective actions are just beginning to be clarified. In particular, they may regulate the growth of neurons and cerebral brain-derived neurotrophic factor levels and enhance myelinization and synaptogenesis in the central nervous system. Pregnenolone and DHEA have shown a dose-dependent protective effect of hilar neurons against kainic acid.[21] Pregnenolone has neuroprotective

effects against both glutamate and amyloid β protein neuropathology, and it also prevents glucocorticoid receptor localization to the nucleus, which may be involved in the observed neuroprotective effects of PREG against glutamate neurotoxicity.[17]

There is accumulating evidence that neurosteroids may be involved in the pathophysiology of schizophrenia, mood disorders, dementia, and substance abuse.[2,22,23] Previous clinical studies have demonstrated low circulating levels of PREG in the elderly, including those with dementia,[24] and in individuals with schizophrenia.[25]

Early human trials with PREG conducted on healthy volunteers under stressful conditions demonstrated significant improvements in mood, general well-being, psychomotor performance, and learning.[26–28] Low-dose oral PREG (30 mg/d) was generally well-tolerated in 17 healthy volunteers receiving PREG for 4 weeks.[29] It has been suggested that PREG might antagonize acute benzodiazepine effects by enhancing arousal via its $GABA_A$ receptor antagonism.[29] To the best of our knowledge, no clinical trials examining the use of PREG as an add-on to ongoing antipsychotic treatment in schizophrenic patients have been published. In addition, there are conflicting data concerning the potential utility of DHEA augmentation (50–200 mg/d) for a period of 1 to 12 weeks in the treatment of schizophrenia patients.[30–33]

In the first clinical trial, 27 adult schizophrenia patients were drawn from an inpatient population and were randomly assigned to receive either DHEA daily (n = 15) or a placebo (n = 12) in addition to a constant dosage of antipsychotic agents for the 6-week trial period.[32] The starting dose was 25 mg/d, which was increased to 50 mg/d for the next 2 weeks and then to 100 mg/d for the remainder of the trial. The authors noted a decrease in anxiety, depression, and negative symptoms among the DHEA-treated patients.

In a second study, Nachshoni et al[30] investigated the effect of DHEA administration during a period of only 7 days on medication-induced extrapyramidal side effects (EPS) among inpatients with schizophrenia or schizoaffective disorder. Patients were randomly assigned in double-blind fashion to receive either 100 mg DHEA or placebo in addition to a constant dosage of antipsychotic medications. Analysis was performed on 30 patients (15 patients in each arm). The authors reported that DHEA caused a significant favorable effect on parkinsonism.

A third study[33] performed by the same research group included 40 patients with chronic schizophrenia stabilized on olanzapine therapy. The subjects were randomly assigned in double-blind fashion to receive either DHEA (150 mg/d) or placebo augmentation for a period of 12 weeks. Sixteen patients receiving DHEA and 15 patients receiving placebo completed the study. DHEA augmentation was not superior to placebo in improving scores on the Scale for the Assessment of Negative Symptoms (SANS), the Positive and Negative Syndrome Scale (PANSS), measures of side effects (the Simpson-Angus Scale [SAS] for extrapyramidal side effects, the Barnes Akathisia Scale [BAS], and the Abnormal Involuntary Movements Scale [AIMS]), cognitive performance (Mindstreams battery), and aggressive behavior (the Life History of Aggression scale). These cross-sectional DHEA trials[30,32,33] did not replicate one another in terms of depressive and anxiety symptoms and in medication-induced adverse side effects. The trials did not show a consistent and unequivocal significant favorable effect for DHEA administration on negative symptoms compared to placebo.

In order to resolve some of the concerns that have arisen in the cross-sectional trials, Ritsner et al[31] conducted a randomized, double-blind, placebo-controlled crossover study in 2 mental health centers. During this trial, 55 patients received either DHEA (200 mg/d) or placebo in identical capsules for 6 weeks, following which they were switched to either placebo or DHEA for a further 6 weeks. Patients continued to receive their regular treatment with daily doses of antipsychotic medication kept constant for at least 2 weeks prior to entering the study and throughout the study period. The crossover analysis revealed no statistically significant treatment effect of DHEA on severity of illness symptoms (PANSS), side effects (AIMS and ESRS), or quality of life measures compared with placebo treatment. However, this investigation, while preliminary, does support some improvement noted in visual sustained attention and visual and motor skills due to DHEA administration.

Here we report for the first time the results from a randomized, placebo-controlled, double-blind comparative trial with 2 neurosteroids (PREG or DHEA) added to ongoing antipsychotic treatment in patients with schizophrenia and schizoaffective disorder for 8 weeks. Given the potential neuroprotective roles of PREG, we hypothesized that PREG augmentation to ongoing and unchanged antipsychotic therapy would improve psychotic symptoms and cognitive performance in chronic schizophrenia and schizoaffective disorder patients compared to DHEA and placebo administration.

METHOD

Study Design

This was an 8-week, controlled, double-blind, randomized, 2-center, parallel-group trial with 30 mg/d and 200 mg/d dosages of PREG and 400 mg/d DHEA augmentation to ongoing antipsychotic treatment. Since it is unknown if higher or lower doses produce greater or lesser therapeutic effects, 2 daily doses of PREG were used in this study. The low dose of PREG (30 mg/d) was chosen in order to increase the patient's circulating PREG levels to at least the range of the typical physiologic peak in young, healthy subjects. The high dose of PREG (200 mg/d) was chosen because PREG's behavioral effects in humans and animals may be more apparent at larger doses.[13,28] In previous studies with DHEA administrations of 90–450 mg/d, an improvement was demonstrated in depressive mood in humans.[34–36] The effect of high dose (400 mg/d) DHEA was examined in the current trial, since the use of 50–200 mg/d of DHEA had not previously revealed conclusive findings in the treatment of schizophrenia patients.[30–33]

© Copyright 2010 Physicians Postgraduate Press, Inc.

Eligible patients had had schizophrenia or schizoaffective disorder (according to *DSM-IV* criteria) for longer than 2 years. The subjects' ages varied from 18 to 60 years, and they were able and willing to sign an informed consent. Major exclusion criteria included an unstable medical condition, any significant medical (including prostate illness) or neurologic illness, pregnancy, or receiving mood stabilizers or any steroid or hormonal supplement (eg, estrogen). The absence of medical or neurologic illnesses was verified by means of a routine laboratory investigation (including prostate-specific antigen level in men older than 50 years), a physical and neurologic examination, reports of the treating physician, and medical records. No change in the doses of antipsychotic medications or addition of any other psychoactive medication was permitted before study entry or throughout the study period. Prior to the study entry, all subjects provided written informed consent after receiving a full explanation regarding the nature of the study and the potential risks and benefits of study participation. The study was approved by the Institutional Review Boards of the Sha'ar Menashe Mental Health Center (Hadera, Israel) and the Be'er-Sheva Mental Health Center (Be'er-Sheva, Israel) and the National Ministry of Health Ethical Review Board.

Procedure

Data were collected from February 2005 until June 2007. An initial evaluation of the patients included a review of medical and psychiatric history and current medications; blood was collected for laboratory assessment, and written informed consent was obtained for participation. Eligible patients were examined by senior psychiatrists (M.S.R., A.G., T.S., and V.L.) who conducted a confirmatory psychiatric evaluation. After screening and baseline assessments, patients were randomly assigned (by means of random number generation) to receive 30 or 200 mg/d of PREG (PREG-30 and PREG-200, respectively), 400 mg/d of DHEA, or placebo in identical capsules (Biosynergy, Boise, Idaho), each for 8 weeks in a double-blind manner. The randomization procedure was performed using the Random Allocation Software, version 1.0 (M. Saghaei, MD, Department of Anesthesia, Isfahan University of Medical Sciences, Isfahan, Iran: http://mahmoodsaghaei.tripod.com/Softwares/randalloc.html). The pharmacist conducted randomization of participants by using a random and equal block size for placebo, DHEA, and PREG-30 arms (with ratio 1.5:1 for PREG-30 and PREG-200 arms, respectively) and conducted the blinding of the trial. The patient allocation details were coded and kept confidential until the trial was completed.

The outcome measures, except neurocognitive tasks, and serum samples of hormones were collected over 5 visits: at baseline before starting therapy, and then after 2, 4, 6, and 8 weeks. All observed or self-reported adverse events that appeared during the study or exacerbations of preexisting illnesses were recorded. Adverse events were evaluated for severity, duration, and possible relation to the drug under study. When a trial was discontinued, for any reason, the patients who did not complete the trial ("discontinued") were observed according to the protocol until the end of the study.

Participants

We screened 70 outpatients with ongoing residual symptoms in 2 large state referral institutions: the Sha'ar Menashe Mental Health Center (Hadera, Israel) and the Be'er-Sheva Mental Health Center (Be'er-Sheva, Israel). Of all screened subjects, 12 patients did not enter the study. Two subjects who were excluded had organic brain damage, 4 patients had comorbidity with substance abuse, 1 patient had a serious medical illness (hepatitis), 2 patients had low comprehension skills, and 3 patients declined to participate. Thus, a total of 58 patients were randomly assigned to treatment.

The study sample consisted of 13 women and 45 men, with a mean age of 35.8 years (SD = 8.3; range, 23–55) and a mean duration of education of 11.0 years (SD = 2.0; range, 6–15). Of the participants, 15.5% were married, 61% were single, and 23.5% were divorced or widowed. Mean age at illness onset was 23.6 years (SD = 6.1; range, 12–45), mean duration of disorder was 12.2 years (SD = 7.5; range, 2–28), and mean number of lifetime hospitalizations was 7.0 (SD = 7.5; range, 1–20). Of the total of 58 patients, 43 subjects met *DSM-IV* criteria for schizophrenia, paranoid type, and 15 met *DSM-IV* criteria for schizoaffective disorder. At baseline, 26 patients were treated with first-generation antipsychotics (FGAs): chlorpromazine, haloperidol, haloperidol decanoate, perphenazine, zuclopenthixol, zuclopenthixol decanoate, or fluphenazine decanoate; 23 patients were treated with second-generation antipsychotics (SGAs): clozapine, risperidone, olanzapine, quetiapine, ziprasidone, or amisulpride; and 9 patients received both types of antipsychotic medications (combined therapy). Mean ± SD chlorpromazine (CPZ) equivalents were 556 ± 106 mg/d for FGAs, 470 ± 64 mg/d for SGAs, and 481 ± 102 mg/d for combined therapy. In addition to the antipsychotic medications, the patients continued to take anticholinergics and benzodiazepines that they had received prior to the study recruitment.

Outcome Measures

At the screening visit, the investigators collected background and demographic data, family and personal histories, details about the present illness, medications, and psychiatric and general medical history, conducted a physical examination, and obtained samples for laboratory analysis. Senior psychiatrists at each site enrolled patients and established diagnoses according to *DSM-IV* criteria.

The primary rating instruments were the Clinical Global Impressions—Severity of Illness scale (CGI-S)[37] and the Positive and Negative Syndrome Scale (PANSS).[38] Secondary outcome measures included the Global Assessment of Functioning scale (GAF),[37] the Extrapyramidal Symptom Rating Scale (ESRS),[39,40] and the Barnes Akathisia Rating Scale (BARS).[41] Raters were trained before the study to produce acceptable levels of interrater reliability, estimated by intraclass correlation coefficient (ICC), for the primary

diagnosis and CGI-S, PANSS, GAF, ESRS, and BARS scores (ICC = 0.89, 0.90, 0.87, 0.92, 0.88, and 0.90, respectively).

Neuropsychological assessment was conducted with the computerized Cambridge Automated Neuropsychological Test Battery (CANTAB).[42,43] Measures were collected at the baseline visit and at the end of the study (or at discontinuation of treatment). The CANTAB battery tests, which run on an IBM-compatible personal computer with a touch-sensitive screen, are grouped into the following cognitive domains: *attention*, *memory*, and *executive functions*. In particular, the Matching to Sample Visual Search (MTS) is a speed/accuracy tradeoff task, testing the subject's ability to match visual samples. The Delayed Matching to Sample (DMS) is a test of perceptual matching and immediate and delayed visual memory in a 4-choice simultaneous and delayed recognition memory paradigm. Other components of the CANTAB include Pattern Recognition Memory (PRM); Rapid Visual Information Processing sustained attention (RVP A′); and the Stockings of Cambridge (SOC). The nonverbal nature of the CANTAB tests makes them largely language-independent and culturally blind. Performance on neurocognitive tests was presented using the standard *z* score, which is the number of SDs from the mean performance computed relative to an extensive database of raw scores for healthy adult subjects matched by age and sex. The CANTAB program calculated *z* scores on the basis of the extensive normative database included in CANTAB. A negative value of the *z* score indicates poorer than average performance. (For a description of the nature of these tests, the performance measures used, and how the test scores are derived, see http://www.camcog.com/science/default.asp.)

Laboratory Testing

Blood samples were collected between 8:00 and 9:00 AM after 20 minutes of rest, and serum was separated. Subjects were instructed to abstain from unusual physical activity or stress for a period of 24 hours prior to blood sampling. Hormone levels in all samples were measured simultaneously to avoid interassay variability.

Pregnenolone was evaluated using Immune Biological Laboratories direct ELISA kit DB52031 (Immune Biologic Laboratories, Hamburg, Germany), DHEA was evaluated using DHEA-DSL 9000 Active, DHEAS was evaluated using DHEA-S-DSL-3500 Active (Diagnostic Products Corporation, Los Angeles, California), progesterone was evaluated using Progesterone RIA DSL-3900 Active, 17α-OH progesterone was evaluated using 17α-OH progesterone DSL-5000 Active, androstenedione was evaluated using Androstenedione DSL-3800 Active, 3α–androstanediol glucuronide (3α–androstane-3α–17β–diol-glucuronide; 3α–Diol G) was evaluated using DSL 9200 Active, testosterone was evaluated using DSL 4000 Active, and estradiol (E_2) was evaluated using DSL 4300 Active. All these kits were coated-tube-radioimmunoassay (RIA) kits (Diagnostic Systems Laboratories, Webster, Texas). Cortisol was evaluated using Cortisol-TKCO1 Coat-A-Count coated tube RIA kit (Diagnostic Products Corporation, Los Angeles, California). The sensitivity of the commercial RIA kits was 0.05 ng/mL for pregnenolone, 0.07 nmol/L for DHEA, 46 nmol/L for DHEAS, 0.12 ng/mL for progesterone, 0.01 ng/mL for 17α-OH progesterone, 0.1 nmol/L for androstenedione, 0.4 ng/mL for testosterone, 0.08 ng/mL for 3α–Diol G, 6.5 pg/mL for estradiol, and 5.5 nmol/L for cortisol. The cross-reactivity of PREG with progesterone was 6.0%, with DHEA was 5.2%, and with all other steroids was less than 1.0%; the cross-reactivity of DHEA with DHEAS was 0.88% and with all others was negligible; the cross-reactivity of DHEAS with DHEA was 41% and with all others was negligible; the cross-reactivity of progesterone with similar hormones was negligible; the cross-reactivity of 17α-OH progesterone with similar hormones was negligible; the cross-reactivity of androstenedione with similar hormones was less than 0.4%; the cross-reactivity of 3α–Diol G with similar hormones was 2%–10%; the cross-reactivity of testosterone with similar hormones was less than 5.8%; the cross-reactivity of estradiol with similar hormones was negligible; the cross-reactivity of cortisol with prednisolone was 76%, with 11-deoxycortisol was 11.4%, with prednisone was 2.3%, with cortisone and corticosterone was 1%, and with all others was < 0.3%.

Specificity (assay variability) was 9.6%–14.5% for pregnenolone, 10.2% for DHEA, 10% for DHEAS, 4.8%–8.0% for progesterone, 8.1%–9.5% for 17α-OH progesterone, 6.0%–9.8% for androstenedione, 6.4%–7.2% for testosterone, 8.4%–9.1% for 3α–Diol G, 4.9%–9.4% for estradiol, and 4.0%–6.4% for cortisol, between runs, and 7.8%–10.6% for pregnenolone, 5.6%–10.6% for DHEA, 6.3%–9.4% for DHEAS, 9.2%–13.1% for progesterone, 6.3%–10.8% for 17α-OH progesterone, 2.8%–5.6% for androstenedione, 4.9%–5.7% for testosterone, 7.8%–9.6% for 3α–Diol G, 5.3%–19.2% for estradiol, and 3.0%–4.8% for cortisol, within runs according to level.

Statistical Analysis

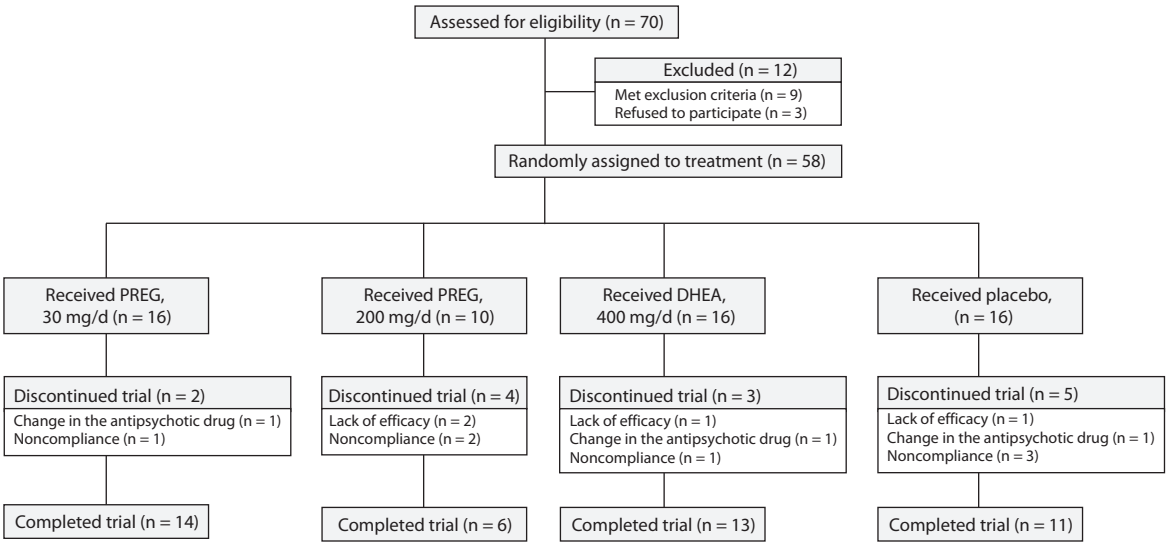
Patients who completed the study (completers) were included in the statistical analysis. The LOCF procedure was used to analyze those subjects who completed at least 4 weeks (selected a priori) but failed to complete all 8 weeks of the study (noncompleters).

Effects of PREG and DHEA administration on the outcome measures were determined by analysis of covariance (ANCOVA) with 2 kinds of variables: (1) rating scale scores at all time points (at baseline, weeks 2, 4, 6, and 8, *time* factor); and (2) the changes of the outcome measures during the study period, which were calculated as an absolute difference value for each subject (2, 4, 6, and 8 weeks minus baseline) controlling the baseline scores of each outcome measure, and duration of illness (years). Post hoc analyses were carried out in cases of significant outcomes, using the Tukey-Kramer method and the Bonferroni correction for multiple comparisons.

In particular, rating scales data were analyzed by using repeated-measures ANCOVA model 2 [or 3, or 4] × 5 × 2 with a main factor of *treatment* (separately 2, 3, or 4 treatment conditions) by *time* factor with comparison between completers and discontinued patients controlling the baseline scores

© Copyright 2010 Physicians Postgraduate Press, Inc.

Figure 1. A Flow Diagram of the Study Population



Abbreviations: DHEA = dehydroepiandrosterone, PREG = pregnenolone.

Table 1. Baseline Characteristics of 44 Participants Who Completed The Trial

	Placebo (n = 11)		PREG-30 (n = 14)		PREG-200 (n = 6)		DHEA (n = 13)		ANOVA (df = 3,40)	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	P
Age, y	34.6	5.3	38.3	9.2	34.3	9.9	35.5	9.2	0.5	.66
Education, y	11.4	1.9	11.0	2.4	11.0	1.5	10.3	2.1	0.5	.66
Age at onset, y	23.5	5.3	23.6	8.4	22.7	5.5	25.3	4.4	0.3	.81
Admissions, no.	6.8	4.7	9.4	8.3	10.0	4.5	4.2	2.8	2.3	.090
Duration of illness, y	11.1	6.5	15.1	8.0	11.7	7.7	10.3	7.3	1.1	.37
Body mass index, kg/m^2	26.8	6.0	25.1	2.7	26.2	6.5	24.4	2.9	0.6	.18
Daily dose, CPZ equivalents, mg/d)	621.3	455.3	476.4	337.6	585.0	704.3	441.1	276.2	0.4	.70
Sex (male/female)	8/3		9/5		5/1		10/3		χ^2_3 = 0.95, P = .81	
Married/single	1/10		4/10		0/6		1/12		χ^2_3 = 4.2, P = .24	
DSM-IV diagnosis, n										
Schizophrenia	9		10		5		10		χ^2_3 = 5.3, P = .91	
Schizoaffective disorder	2		4		1		3			

Abbreviations: CPZ = chlorpromazine, DHEA = dehydroepiandrosterone (400 mg/d), PREG-30 = pregnenolone (30 mg/d), PREG-200 = pregnenolone (200 mg/d).

of each outcome measure, and the duration of the illness (years). Other ANCOVAs models applied, with either sex or drug treatment (FGAs, SGAs, combined therapy controlling daily dose as CPZ equivalent, mg/d as covariate) or *DSM-IV* diagnosis (schizophrenia versus schizoaffective disorder) being added to the models.

Changes in the performance of the CANTAB tasks (z scores) from baseline to endpoint were analyzed with ANCOVA model 4 × 2 × 2 (without and with the sex effect or type of antipsychotic agents) controlling education (years) and a daily dose of antipsychotics (CPZ equivalent, mg/d). Distribution of the types of antipsychotic agents (FGAs/SGAs/combined therapy patients) in the groups was placebo (8/6/2), PREG-30 (5/8/3), PREG-200 (8/2/0), and DHEA (5/7/4).

Finally, an ANCOVA model with 3 main factors (*treatment group*, *time,* and *sex*) controlling for age (years) and daily dose (CPZ equivalent, mg/d) was performed for comparison of the serum hormonal concentrations throughout the study period.

Effect sizes (Cohen d with CI) were calculated for changes in outcome variables from baseline to endpoint examination by the estimated pooled standard deviation, with a small effect size defined as 0.2, a moderate effect size as 0.5, and a large effect size as 0.8.[44] Effect size is simply a way of quantifying the effectiveness of a particular intervention relative to some comparison. As the name suggests, an effect size estimate can place an easily interpretable value on the direction and magnitude of an effect of a treatment, a difference between 2 treatment groups, or any other numerical comparison or contrast.

Baseline values of rating scales and cognitive tasks among 4 treatment groups of patients were compared by a multivariate analysis of variance (MANOVA) with Hotelling test.

Continuous variables were compared using the 2-tailed t test or the Wilcoxon signed rank test (z) for assessing the difference in medians. Differences in the frequency of categorical variables were examined with the χ^2 test. For all analyses, the level of statistical significance was defined as an α less than .05. We performed the statistical analysis using the Number Cruncher Statistical Systems (Kaysville, Utah).[45]

RESULTS

Sample Composition

Figure 1 presents a flow diagram of the study population. Fifty-eight enrolled patients were randomly assigned to receive 30 mg/d of PREG (n = 16), 200 mg/d of PREG (n = 10), 400 mg/d of DHEA (n = 16), or placebo (n = 16).

The allocation into the experimental groups was independent of the antipsychotic drugs used by the subjects (classified as FGA, SGA, and combined therapy; $\chi^2_6 = 8.3$, $P = .21$). There were no notable imbalances among 4 treatment groups in age, sex ($\chi^2_3 = 1.8$, $P = .62$), marital status ($\chi^2_6 = 7.5$, $P = .28$), body mass index (BMI), age at illness onset, number of hospital admissions, duration of disease, or the distribution of diagnoses (schizophrenia or schizoaffective disorder, $\chi^2_3 = 1.7$, $P = .63$).

Of the 58 patients randomly assigned to this trial, 14 patients dropped out. Discontinuation of the treatment occurred in 2 patients in the PREG-30, 4 patients in the PREG-200, 3 patients in the DHEA groups, and 5 patients assigned which received placebo. More specifically 1, 1, 2, and 3 patients assigned to receive PREG-30, PREG-200, DHEA, and placebo, respectively, dropped out between 4 and 6 weeks, and 7 patients dropped out between 6 and 8 weeks. The treatment was discontinued due to reasons not related to the neurosteroids: lack of efficacy (n = 4), change in the antipsychotic drugs (n = 3), loss to follow-up (n = 5), and noncompliance (n = 2).

Forty-four patients (12 of 13 women and 32 of 45 men) completed the trial. Ten patients met *DSM-IV* criteria for schizoaffective disorders; all other subjects met *DSM-IV* criteria for schizophrenia. The baseline characteristics of the 44 subjects who completed the study are listed in Table 1. As can be seen, there were no notable differences in the completers among the 4 treatment groups in age, sex, marital status, BMI, age at illness onset, number of hospital admissions, duration of disease, the distribution of diagnoses (schizophrenia or schizoaffective disorder), or antipsychotic medication daily dose.

In this study, 7 discontinued patients were examined at the end of the trial; the missing data of the other 7 discontinued patients were imputed using LOCF. Among patients who were randomly assigned to receive PREG-30, PREG-200, DHEA, or placebo and who completed this study, there were no significant between-group differences in baseline mean scores on the CGI-S scale, PANSS subscales, GAF scale, ESRS, and BARS (MANOVA, Hotelling test, $F_{21,98} = 0.71$, $P = .75$). Outcome measures are described in Figure 2 and Tables 2 and 3.

Effectiveness

Neurosteroids compared to placebo. Compared with placebo, PREG-30 augmentation significantly improved PANSS positive symptom scores ($F_{1,148} = 6.8$, $P = .010$), whereas no significant effects were observed on the other PANSS subscales, the CGI-S scale, or GAF scale scores (Table 2). A mean reduction on the PANSS positive subscale

Table 2. Efficacy and Safety Ratings of 44 Participants Who Completed the Trial

Variable	Placebo (n = 11)				PREG-30 (n = 14)				PREG-200 (n = 6)				DHEA (n = 13)				Significance for Main Effect of Neurosteroid Augmentation on Rating Scales for 8-Week Trial (ANCOVA)[a]						
	Baseline		After 8-Week Treatment		Baseline		After 8-Week Treatment		Baseline		After 8-Week Treatment		Baseline		After 8-Week Treatment		PREG-30 vs Placebo (*df* = 1,148)		PREG-200 vs Placebo (*df* = 1,115)		DHEA vs Placebo (*df* = 1,148)		PREG-30 vs PGEG-200 vs DHEA (*df* = 2,191)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*	*P*	*F*	*P*	*F*	*P*	*F*	P
Illness severity, CGI-S scale score	3.8	0.7	3.4	0.8	4.0	1.4	3.6	0.8	4.0	0.9	3.5	0.8	4.2	0.9	4.3	1.2	0.02	.88	0.8	.36	7.2	.008[c]	7.6	.028[f]
PANSS Positive subscale score	16.0	5.2	13.7	5.0	17.6	5.6	14.9	6.8	20.5	9.2	16.7	5.6	19.2	5.1	17.3	7.2	6.8	.010[b]	2.2	.14	0.4	.54	6.2	.002[e,g]
PANSS Negative subscale score	22.8	5.8	20.0	6.0	23.4	7.2	20.9	6.8	25.8	2.0	23.5	3.1	25.8	6.1	24.3	7.1	0.8	.35	0.08	.77[g]	1.2	.27	4.0	.018[f]
PANSS General Psychopathology subscale score	37.1	8.8	31.5	8.8	39.9	12.0	34.6	12.5	47.7	7.6	39.7	8.1	46.2	11.1	42.8	14.9	0.6	.44	2.1	.15[g]	4.9	.028[c]	5.0	.007[f,g]
General functioning, GAF score	61.4	13.4	63.2	14.1	57.5	11.7	60.7	13.1	57.5	14.0	60.3	13.9	56.4	12.8	55.8	16.9	1.1	.30	0.4	.53	8.2	.005[c]	7.0	.001[d]
Extrapyramidal side effects, ESRS score	2.3	4.3	1.7	4.1	2.6	5.5	0.1	0.4	0.7	1.2	0.5	0.8	7.8	10.8	2.9	4.8	3.9	.049[b,g]	2.8	.092[g]	3.9	.049[b,g]	0.4	.70
Akathisia, BARS score	0.09	0.3	0.18	0.4	0.21	0.6	0	0	0.17	0.4	0	0	0.38	0.8	0.23	0.6	3.2	.23	2.1	.15	1.5	.63	0.1	.88

[a]Three-way ANCOVA compares scores of the outcome measures among the treatment groups by time (2, 4, 6, and 8 weeks) with comparison between the completers and the discontinued patients controlling for the baseline scores of each outcome measure, and duration of illness (years). The ratings of the discontinued patients were determined with LOCF procedure. Post hoc analysis with the Tukey-Kramer *t* test ($P < .05$).
[b]Superior to placebo.
[c]Placebo is superior to DHEA.
[d]PREG-30 and PREG-200 are superior to DHEA.
[e]PREG-30 is superior to DHEA and PREG-200.
[f]PREG-30 is superior to DHEA.
[g]Completers are superior to discontinued patients.
Abbreviations: ANCOVA = analysis of covariance, BARS = Barnes Akathisia Rating Scale, CGI-S = Clinical Global Impressions-Severity of Illness scale, DHEA = dehydroepiandrosterone, ESRS = Extrapyramidal Symptom Rating Scale, GAF = Global Assessment of Functioning scale, PANSS = Positive and Negative Syndrome Scale, PREG = pregnenolone.

© Copyright 2010 Physicians Postgraduate Press, Inc.

score was –2.6 among PREG-30 subjects compared with –0.9 among placebo subjects with a small effect size ($F_{3,219} = 5.9$, $P = .006$, power = 0.95; $d = 0.28$; CI, –1.7 to 0.51) (Figure 2A). Patients who were treated with PREG-200 did not differ on the outcome variable scores compared to placebo for the study period (all P values > .05).

The condition of patients receiving placebo improved more than DHEA-treated subjects as measured in the reduction on CGI-S scores ($F_{1,148} = 7.2$, $P = .008$; $d = 0.61$; CI, –0.20 to 1.42) and on PANSS general psychopathology scores ($F_{1,148} = 4.9$, $P = .028$; $d = 0.24$; CI, –0.56 to 1.05), and in an increase on GAF scores ($F_{1,148} = 8.2$, $P = .005$, $d = 0.41$; CI, –1.22 to 0.40; Table 2). Since 4 comparisons were significant (see Figure 2), Bonferroni correction for 4 tests was applied ($P = .05/4 = .0125$). After Bonferroni correction, improvement in the PANSS positive and general psychopathology subscales remained significant ($P < .05$).

PREG compared to DHEA. An ANCOVA model yielded a significant reduction in the CGI-S, PANSS positive, PANSS negative, and PANSS general psychopathology scores and an increase in GAF scores among patients receiving PREG-30 compared to those receiving DHEA (Table 2, Figure 2B). PREG-30 augmentation was significantly superior for ameliorating PANSS positive symptoms compared to PREG-200 augmentation ($F_{2,191} = 6.2$, $P = .002$, power = 0.89). The treatment with PREG-30 and PREG-200 significantly improved GAF scores compared to DHEA augmentations ($P = .001$, power = 0.92); the differences between PREG-30 and PREG-200 groups were not significant. When multiple comparisons between the groups of patients who received any augmentation were analyzed, 5 comparisons were significant (Table 2, far right column), Bonferroni correction for 5 tests was applied ($P = .05/5 = .01$). After Bonferroni correction, improvement in PANSS positive and general psychopathology subscales and GAF scores remained significant ($P < .05$).

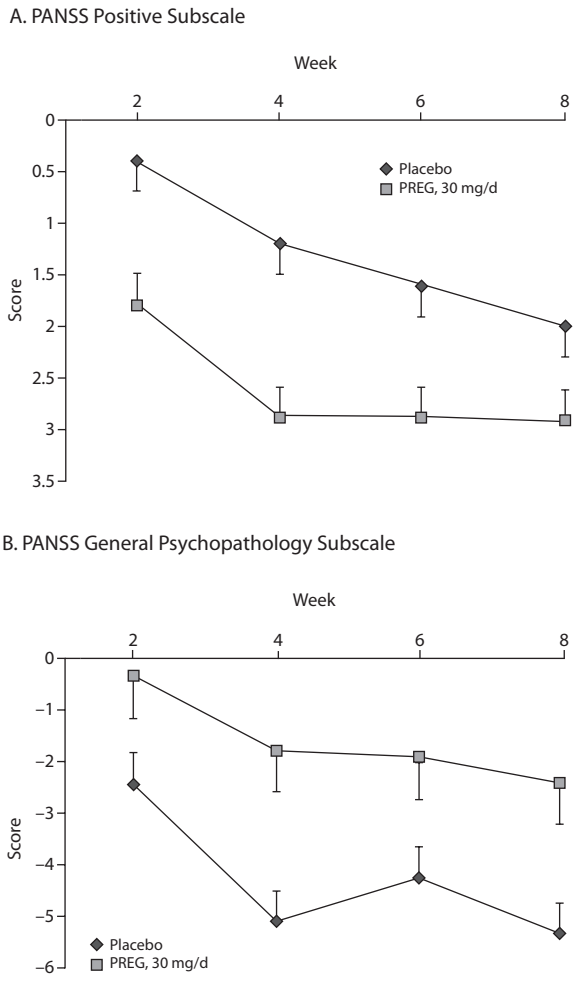
Tolerability and Safety

A significantly favorable effect on EPS compared to the placebo group was observed among subjects treated with PREG-30 and DHEA (for both groups $F_{1,148} = 3.9$, $P = .049$, power = 0.74; Table 2). As indicated by changes in the ESRS total scores from baseline to week 8, PREG-30 ($d = 0.44$; CI, –1.24 to 0.36) and DHEA ($d = 0.59$; CI, –1.41 to 0.23) patients who completed the study had significantly lower ESRS total scores than patients receiving placebo ($F_{3,219} = 3.2$, $P = .024$, Table 3). No significant interactions between group × time, or group × type of antipsychotics were found (all P values > .13). With regard to the analysis of the BARS scores, no differences between the 4 groups were noted. During the trial, no significant adverse events (such as oily skin, acne, voice deepening, or hirsutism) were observed. Thus, the administration of PREG and DHEA was well tolerated.

Analysis of Covariates

No significant main effect of the type of antipsychotics or type of antipsychotics × time interaction on CGI-S, PANSS subscale, GAF, ESRS, and BARS scores was observed for

Figure 2. Changes in Mean Scores on the PANSS Subscales for 8-Week Trial

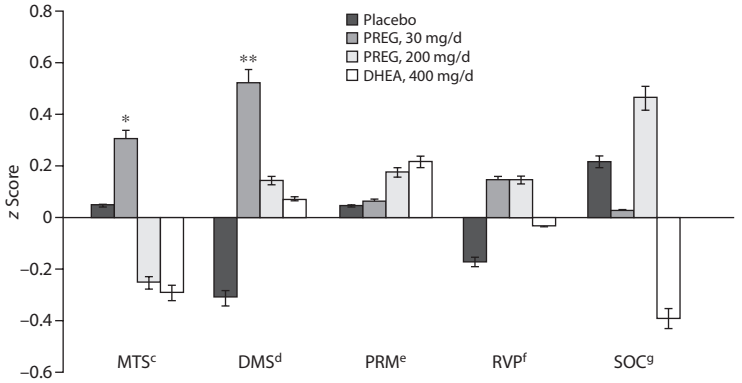


Mean scores and SEs are shown.

Significance for Changes in Rating Scale Scores for 8-Week Trial (ANCOVA)[a]	Treatment Condition ($df = 3,219$)		Completers vs Discontinued Patients ($df = 1,219$)	
	F	*P*	*F*	*P*
PANSS Positive subscale	5.9	.006[b]	5.4	.021[d]
PANSS Negative subscale	2.2	.091	2.3	.13
PANSS General Psychopathology subscale	4.3	.006[c]	7.9	.005[d]

[a]Three-way ANCOVA compares the changes in the outcome measures between 4 treatment groups by time factor (from baseline to 2, 4, 6, and 8 weeks) with comparison between the completers and the discontinued patients, controlling for the baseline scores of each outcome measure and duration of illness (years). The ratings of the discontinued patients were determined with the LOCF procedure. Post hoc analysis with the Tukey-Kramer *t* test ($P < .05$).
[b]PREG-30 is superior to placebo and to DHEA.
[c]PREG-30 is superior to DHEA.
[d]The completers improved more than the discontinued patients. Since 4 comparisons were significant, Bonferroni correction for 4 tests was applied ($P = .05/4 = 0.0125$). After Bonferroni correction, improvement in PANSS positive and general psychopathology subscales remained significant ($P < .05$).
Abbreviations: ANCOVA = analysis of covariance, DHEA = dehydroepiandrosterone, LOCF = last observation carried forward, PANSS = Positive and Negative Syndrome Scale, PREG = pregnenolone.

Figure 3. Changes in Cognitive Functioning From Baseline to Endpoint Among Completers (CANTAB tasks, *z* scores)[a,b]



[a]Mean *z* scores are shown. Error bars indicate SEs.
[b]The standard *z* score is given as the number of SDs from the mean performance computed relative to an extensive CANTAB database of raw scores for healthy adult subjects matched by age and sex. A positive value indicates performance better than average in healthy control subjects. In the test descriptions that follow, outcome measures appear in parentheses.
[c]The MTS (percentage correct) is a speed/accuracy trade-off task, testing the subject's ability to match visual samples. Reaction time is measured on the basis of the release of the press-pad.
[d]The DMS (percentage correct) is a test of perceptual matching, immediate and delayed visual memory, in a 4-choice simultaneous and delayed recognition memory paradigm. Subjects must recognize a previously presented stimulus item from among 4 very similar stimuli after a delay of either 0, 4, or 12 s.
[e]The PRM (percentage correct).
[f]The RVP (sustained attention, RVP A').
[g]The SOC (mean initial thinking time).
*$F_{1,25} = 5.0$, $P = .035$.
**$F_{1,25} = 4.3$, $P = .049$.
Abbreviations: CANTAB = Cambridge Automated Neuropsychological Test Battery, DMS = Delayed Matching to Sample, MTS = Matching to Sample Visual Search, PRM = Pattern Recognition Memory, RVP = Rapid Visual Information Processing, SOC = Stockings of Cambridge.

patients receiving PREG-30, PREG-200, DHEA, or placebo (all P values > .05). No relation was found between the diagnosis and any differences between treatment arms in the changes on the CGI-S, PANSS subscale, GAF, ESRS, and BARS scores (all P values > .05). The analysis of between- and within-group gender differences did not show any significant main effect on the outcome variables.

Neurocognitive Functioning

No significant between-group differences in the performance of CANTAB attention, memory, and executive function tasks (MTS, DMS, PRM, RVP, and SOC) at the baseline assessment were observed (MANOVA, $F_{15,290} = 1.45$, $P = .12$). At the end of the trial, PREG-30 augmentation significantly improved *z* scores compared to placebo in the DMS ($F_{1,25} = 4.3$, $P = .049$; $d = 0.79$; CI, 0.04–1.69) and in the MTS ($F_{1,32} = 5.0$, $P = .035$, $d = 0.75$; CI, 1.57–0.07) tasks; Figure 3). None of the treatment effects, in pairwise comparisons on other cognitive tasks, were significant (all P values > .15), and no significant differences between the groups in the cognitive performance were detected when only 3 treatment groups (PREG-30, PREG-200, and DHEA) were compared (ANCOVA, $F_{2,42} = 0.2–1.5$, all P values > .05). The type of the antipsychotic agents and sex did not have a significant effect on cognitive performance.

Completers Versus Noncompleters

In this clinical trial, there were no significant differences between the discontinued patients and completers in the mean values for age, education, age at onset, number of admissions, duration of illness, BMI, and CPZ equivalent daily dose (2-way MANOVA; $F_{7,47} = 0.47$, $P = .85$ for completers vs discontinued patients by 4 treatment groups: $F_{21,137} = 1.09$, $P = .36$). Baseline CGI-S, PANSS subscale, GAF, ESRS, and BARS scores did not differ between 44 completers and 14 discontinued patients (2-way MANOVA; $F_{7,47} = 0.68$, $P = .69$) across 4 treatment groups ($F_{21,137} = 0.71$, $P = .81$). However, after the treatment had begun, completers had significantly lower PANSS positive, PANSS general psychopathology, and ESRS scores than patients who discontinued prior to the end of the study (Tables 2 and 3, Figure 2). The completers also showed a significant improvement on GAF scores compared to the discontinued subjects ($F_{1,219} = 8.2$, $P = .005$, Table 3). There were no significant differences between the completers and discontinued patients in the performance of CANTAB tasks (MTS, DMS, PRM, RVP, and SOC; MANOVA, $F_{5,94} = 0.76$, $P = .58$).

Blood Hormone Levels

No significant difference was observed among the groups in the baseline values of the tested hormones (MANOVA, $F_{27,95} = 1.1$, $P = .35$). Figure 4 presents blood concentrations of hormones throughout the study period.

According to an ANCOVA ($F_{3,204} = 12.4$, $P < .001$), serum PREG level was significantly higher among the patients treated with PREG-30, PREG-200, and DHEA compared to the placebo group ($P < .05$). Blood PREG level was also higher among the PREG-200 group compared to the PREG-30 and DHEA groups ($P < .05$; Figure 4A). The main time effect of augmentation with PREG and DHEA on blood PREG levels was observed by the fourth week ($F_{4,204} = 4.4$, $P = .014$).

Serum levels of other hormones among subjects treated with both PREG-30 and PREG-200 were similar to placebo throughout the study period (all P values > .05). The DHEA treatment revealed a significant elevation of the blood levels of DHEA ($F_{3,240} = 28.0$, $P < .001$), DHEAS ($F_{3,240} = 54.6$, $P < .001$), androstenedione ($F_{3,204} = 31.9$, $P < .001$), 3α-androstanediol glucuronide ($F_{3,240} = 56.5$, $P < .001$), and estradiol ($F_{3,240} = 10.3$, $P < .001$) compared to PREG-30, PREG-200, and placebo conditions (Figure 4B–4E). There was no significant between-group difference in levels of progesterone ($F_{3,201} = 0.5$, $P = .66$), 17-OH-progesterone ($F_{3,240} = 1.0$, $P = .39$), testosterone ($F_{3,204} = 2.4$, $P = .073$), and cortisol ($F_{3,204} = 0.8$, $P = .50$).

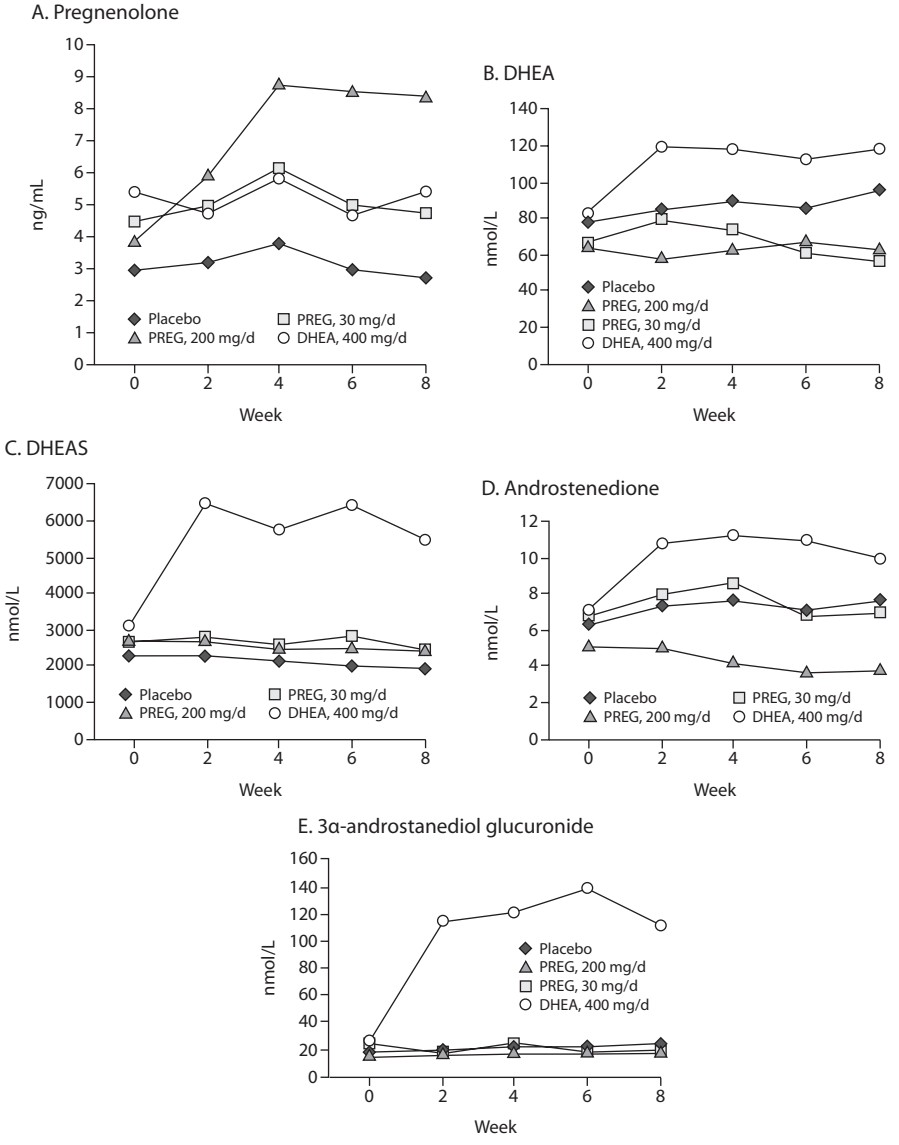
A sex effect was noticeable in the blood levels of pregnenolone ($F_{1,2046} = 25.9$, $P < .001$; females > males), 3α-androstanediol glucuronide ($F_{1,240} = 5.6$, $P = .019$; males > females), testosterone ($F_{1,204} = 210.8$, $P < .001$; males >

Table 3. Analysis of Changes in CGI-S, GAF, ESRS, and BARS Scores Among 44 Participants Who Completed the 8-Week Trial

	Placebo (n = 11)		PREG-30 (n = 14)		PREG-200 (n = 6)		DHEA (n = 13)		Significance of Changes in Rating Scale Scores for 8-Week Trial (ANCOVA)[a]			
									Treatment Condition (*df* = 3,219)		Completers vs Noncompleters (*df* = 1,219)	
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*F*	*P*	*F*	*P*
Illness severity, CGI-S scale score	−0.5	0.08	−0.5	0.08	−0.3	0.1	−0.2	0.08	3.4	.009[b]	3.6	.058
General functioning, GAF score	3.3	0.8	5.2	0.8	4.1	1.0	0.7	0.7	6.0	<.001[c]	8.2	.005[e]
Extrapyramidal side effects, ESRS score	0.04	0.4	−1.7	0.4	−1.4	0.5	−1.4	0.5	3.2	.024[d]	11.3	<.001[e]
Akathisia, BARS score	−0.07	0.04	−0.2	0.04	−0.2	0.06	−0.09	0.04	1.7	.16	0.01	.93

[a]Three-way ANCOVA compares the changes in the outcome measures between 4 treatment groups by time factor (from baseline to 2, 4, 6, and 8 weeks) with comparison between completers and discontinued patients controlling for the baseline scores of each outcome measure and duration of illness (years). The ratings of the discontinued patients were determined with the LOCF procedure. Post hoc analysis with the Tukey-Kramer *t* test ($P < .05$).
[b]PREG-30 is superior to DHEA.
[c]PREG-30 and PREG-200 are superior to DHEA.
[d]PREG-30 is superior to placebo.
[e]Completers are superior to discontinued patients: Mean ± SE GAF scores = 4.8 ± 0.9 vs 1.8 ± 0.4, respectively; Mean ± SE ESRS scores = −2.1 ± 0.2 vs −0.1 ± 0.5, respectively.
Abbreviations: ANCOVA = analysis of covariance, BARS = Barnes Akathisia Rating Scale, DHEA = dehydroepiandrosterone, ESRS = Extrapyramidal Symptoms Rating Scale, GAF = Global Assessment of Functioning, LOCF = last observation carried forward, PREG = pregnenolone.

Figure 4. Concentration of Serum Neurosteroid Levels Through the Clinical Trial



Abbreviations: DHEA = dehydroepiandrosterone, DHEAS = dehydroepiandrosterone sulfate.

females), estradiol ($F_{1,240} = 5.5$, $P = .004$; females > males), progesterone ($F_{1,201} = 15.3$, $P < .001$; females > males), and 17-OH-progesterone ($F_{1,240} = 36.8$, $P < .001$; males > females), but not in levels of DHEA, DHEAS, and androstenedione (all P values > .05).

DISCUSSION

In this randomized double-blind, placebo-controlled trial, we report for the first time that, compared with placebo, an 8-week low-dose PREG (30 mg/d) augmentation of an ongoing antipsychotic treatment showed a statistically significant reduction in positive symptoms and improvement in attention, memory, and EPS in chronic schizophrenia and schizoaffective disorder patients, whereas subjects who were treated with PREG-200 did not differ on outcome variable scores for the study period. Cohen *d* effect sizes demonstrated slight beneficial effects for PREG-30 versus placebo in positive symptoms ($d = 0.28$) and EPS scores ($d = 0.44$). This is especially noteworthy considering the fact that the baseline scores of the outcome measures, duration of illness, and education were controlled. Since there was no overall significant effect of diagnosis, sex, or type of antipsychotic drug on the therapeutic effects of PREG-30, the results of the covariance analyses suggest that the favorable therapeutic effects of PREG-30 are achieved, at least partially, due to direct effects of the augmentation.

Interestingly, a significant effect of DHEA augmentation was observed with 50–150 mg/d[32,33] but not with 200 mg/d[31] or 400 mg/d in the present trial. Moreover, the augmentation of 400 mg/d of DHEA resulted in significantly less improvement on CGI-S and PANSS general psychopathology scale scores compared to placebo. Therefore, we suggest an inverted-U clinical response with a daily dose of PREG and DHEA augmentations (although there could be other reasons for the inconsistent results: methodological issues, different sample characteristics, different baseline severity of illness, and varying durations of combination treatment).

The present study showed a slight improvement in cognitive performance in the MTS (Cohen $d = 0.75$), and in the DMS (Cohen $d = 0.79$) tasks of CANTAB among the patients receiving a low dose of PREG augmentation compared with placebo. It should be noted that the MTS is a speed/accuracy trade-off task testing the subject's ability to match visual samples, while the DMS measures the ability to remember a target stimulus (a complex, multicolored pattern). Theoretical analysis suggests that strategic ability, attention, and working memory are critical to the performance of these tasks.[46] No significant improvement on CANTAB tasks was observed among patients receiving 200 mg/d of PREG or 400 mg/d of DHEA compared with placebo.

The mechanisms by which neurosteroids influence cognitive functioning have been studied in various species. The beneficial effect of PREG and PREGS on the cognitive functioning of animals has been shown in several preclinical studies. Experiments with animals show enhancement of the posttraining memory processes after the injection of low doses of PREG or PREGS.[13,47,48] In particular, the memory-enhancing effects of PREGS and DHEAS in preclinical studies have been attributed to their NMDA-agonistic properties[14,15] or cholinergic activity.[10,11] Furthermore, a systemic or intracerebral administration of PREG and PREGS enhances memory in rodents by increasing the animal's natural performance or antagonizing pharmacologically induced amnesia.[49] PREG treatment of rats during the neonatal period influences the cortical dopaminergic and adenosinergic systems and behavioral responses.[50]

While the precise mechanisms of action of PREG that result in the improvement of positive symptoms remain unclear, some researchers ascribe them to the stimulation of the hippocampal dopaminergic system,[12,51] mixed modulatory effects (positive and negative) on the $GABA_A$ receptors,[8,52] and neuromodulatory effects on NMDA[53–55] and sigma-1[6] receptors. It should also be noted that PREG and its sulfate demonstrate potent neuroprotective qualities and play an important role in neurodevelopment.[17,56] Although we do not have any definitive explanation for the mechanisms underlying the beneficial effects of the addition of neurosteroids to antipsychotics, the neuromodulatory effects of PREG may be relevant to the clinical activity, as has been demonstrated to be the case with clozapine.[16]

The findings of this study indicate a significant beneficial effect of low daily doses of PREG administration on EPS but not on akathisia. The beneficial effect of DHEA on EPS is consistent with a previous preliminary trial[30] but not with a crossover study.[31] Mechanisms by which PREG might have an EPS-lowering effect remain unclear, but several intriguing possibilities exist. One such possibility is that PREG's positive modulatory influence on dopamine neurotransmission[50,51] may counter the dopamine-blocking effect of antipsychotic-induced EPS as has been suggested for the favorable effect of DHEA on neuroleptic-induced EPS.[32]

As recommended by the CONSORT guidelines,[57] we developed ANCOVA models with comparisons between 44 completers and 14 noncompleters using the LOCF method. This approach provides a more valid assessment of the treatment effectiveness, because it relates to actual clinical practice.[58,59] Although there was no significant difference in the baseline scores between these subgroups of participants, they were followed throughout the study. In particular, the patients who discontinued the study early appeared to have less improvement on the PANSS positive, PANSS general psychopathology, ESRS, and GAF scales than did completers, whereas no significant differences between these subgroups were found in the performance of the CANTAB tasks.

Circulatory PREG was found to be significantly higher among the patients treated by either neurosteroid compared to the placebo group; however, it was significantly higher among those receiving 200 mg/d of PREG compared to the PREG-30 and DHEA groups. This study demonstrates no effects of PREG administration on the other hormones measured in this trial, while the treatment with DHEA significantly elevated blood levels of PREG (but to a lesser extent than PREG 200 mg/d) and DHEA, DHEAS, androstenedione,

3α-androstanediol glucuronide, testosterone, and estradiol compared to PREG-30, PREG-200, and placebo. No differences between groups in the levels of progesterone, 17-OH-progesterone, and cortisol were demonstrated. Our findings that circulatory DHEA and DHEAS increase after DHEA administration (400 mg/d) are consistent with a previous report,[31] although among the patients treated for a period of 12 weeks with DHEA (150 mg/d) blood DHEA and DHEAS levels failed to reach significance compared to the placebo group.[33] Blood levels of 3α-androstanediol glucuronide, testosterone, and 17-OH-progesterone were increased in men, while blood levels of pregnenolone, estradiol, and progesterone were increased among women. No sex differences were indicated in levels of DHEA, DHEAS, or androstenedione. Thus, the patients receiving PREG are not at risk for elevation of androgenic metabolites, like DHEA, which may in turn potentially predispose to various problems such as prostatic hypertrophy in men and hirsutism in women. The treatment effects of PREG cannot be explained by an impact of their neuroactive metabolites like DHEA, DHEAS, androstenedione, 3α-androstanediol glucuronide, testosterone, and estradiol. Considering the lack of any significant effect of PREG on the measured hormonal profile in this study, the therapeutic effects of PREG noted here may be mediated by other mechanisms, including further potential hormonal influences not investigated in this study. In addition, PREG's effect may be mediated by its direct neuromodulatory effects on the $GABA_A$, NMDA, sigma-1, dopaminergic, cholinergic, or neurotrophic systems.

Last, since no persistent new adverse effects or unfavorable drug interactions were observed, the combination of these neurosteroids with antipsychotic drugs appears to be safe and well tolerated, confirming the results of previous trials with PREG[26,29] and DHEA[31,32,60] as additions to ongoing antipsychotic treatment.

Limitations of this study include the relatively small sample size and the relatively short duration of the study. Long-term, large-scale studies are required to obtain greater statistical significance and more confident clinical generalization. In addition, it would be important in such larger sample sizes to investigate whether any interaction exists between PREG and any specific medication and to compare responses to PREG and DHEA in those receiving typical and atypical antipsychotics within the context of a standardized medication regimen.

In conclusion, the present data, although based on a relatively small sample size, suggest that low-dose PREG treatment for 8 weeks, used as an adjunct to antipsychotics, has the valuable effect of ameliorating positive symptoms and attention and memory impairments and antipsychotic-induced EPS in chronic schizophrenia and schizoaffective disorder patients. Although the results of this study are notable, it is crucial to replicate the trial with a larger sample of chronic and nonchronic schizophrenia or schizoaffective patients for a longer duration of treatment. Further double-blind controlled studies are needed in order to investigate the clinically significant benefits of PREG augmentation.

Drug names: clozapine (Clozaril, FazaClo, and others), haloperidol (Haldol and others), olanzapine (Zyprexa), quetiapine (Seroquel), risperidone (Risperdal and others), ziprasidone (Geodon).

Author affiliations: Department of Psychiatry, The Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, Haifa (Dr Ritsner); Sha'ar Menashe Mental Health Center, Hadera (Drs Ritsner, Gibel, Boguslavsky, and Zayed); Division of Psychiatry, Faculty of Health Sciences Ben-Gurion University of the Negev, and Ministry of Health Mental Health Center, Be'er-Sheva (Drs Shleifer and Lerner); Laboratory of Biological Psychiatry, Felsenstein Medical Research Center, Beilinson Campus, Petah Tikva (Drs Maayan and Weizman); and Research Unit, Geha Mental Health Center, and Sackler Faculty of Medicine, Tel Aviv University (Dr Weizman), Israel.

Potential conflicts of interest: None reported.

Funding/support: None reported.

Author contributions: Dr Ritsner contributed to study design, data analysis, and, with Dr Lerner, oversaw data collection. Drs Gibel, Shleifer, Boguslavsky, Zayed, and Lerner contributed to data collection. Drs Maayan and Weizman contributed to biologic testing. Dr Ritsner was primarily responsible for manuscript preparation, with contributions from Drs Weizman, Maayan, and Lerner. All authors contributed to and have approved the final version of the manuscript.

REFERENCES

1. Mellon SH. Neurosteroid regulation of central nervous system development. *Pharmacol Ther*. 2007;116(1):107–124.
2. Ritsner MS, Gibel A, Ratner Y, et al. Dehydroepiandrosterone and pregnenolone alterations in schizophrenia. In: Ritsner MS, Weizman A, eds. *Neuroactive Steroids in Brain Function, Behavior and Neuropsychiatric Disorders: Novel Strategies for Research and Treatment*. Bazel, Belgium: Springer Science & Business Media; 2008:251–298.
3. Morrow AL. Recent developments in the significance and therapeutic relevance of neuroactive steroids—Introduction to the special issue. *Pharmacol Ther*. 2007;116(1):1–6.
4. Tagawa N, Sugimoto Y, Yamada J, et al. Strain differences of neurosteroid levels in mouse brain. *Steroids*. 2006;71(9):776–784.
5. Baulieu EE. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. *Recent Prog Horm Res*. 1997;52:1–32.
6. Bergeron R, de Montigny C, Debonnel G. Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. *J Neurosci*. 1996;16(3):1193–1202.
7. Holsboer F, Grasser A, Friess E, et al. Steroid effects on central neurons and implications for psychiatric and neurological disorders. *Ann N Y Acad Sci*. 1994;746:345–359, discussion 359–361.
8. Majewska MD. Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. *Prog Neurobiol*. 1992;38(4):379–395.
9. Rupprecht R. The neuropsychopharmacological potential of neuroactive steroids. *J Psychiatr Res*. 1997;31(3):297–314.
10. George O, Vallée M, Le Moal M, et al. Neurosteroids and cholinergic systems: implications for sleep and cognitive processes and potential role of age-related changes. *Psychopharmacology (Berl)*. 2006;186(3):402–413.
11. Vallée M, Mayo W, Le Moal M. Role of pregnenolone, dehydroepiandrosterone and their sulfate esters on learning and memory in cognitive aging. *Brain Res Brain Res Rev*. 2001;37(1-3):301–312.
12. Pérez-Neri I, Montes S, Ojeda-López C, et al. Modulation of neurotransmitter systems by dehydroepiandrosterone and dehydroepiandrosterone sulfate: mechanism of action and relevance to psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008;32(5):1118–1130.
13. Flood JF, Morley JE, Roberts E. Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. *Proc Natl Acad Sci U S A*. 1992;89(5):1567–1571.
14. Bowlby MR. Pregnenolone sulfate potentiation of N-methyl-D-aspartate receptor channels in hippocampal neurons. *Mol Pharmacol*. 1993;43(5):813–819.
15. Mathis C, Vogel E, Cagniard B, et al. The neurosteroid pregnenolone sulfate blocks deficits induced by a competitive NMDA antagonist in active avoidance and lever-press learning tasks in mice. *Neuropharmacology*. 1996;35(8):1057–1064.
16. Marx CE, Shampine LJ, Duncan GE, et al. Clozapine markedly elevates pregnenolone in rat hippocampus, cerebral cortex, and serum: candidate mechanism for superior efficacy? *Pharmacol Biochem Behav*. 2006;84(4):598–608.
17. Gursoy E, Cardounel A, Kalimi M. Pregnenolone protects mouse

hippocampal (HT-22) cells against glutamate and amyloid beta protein toxicity. *Neurochem Res*. 2001;26(1):15–21.
18. Johnson MD, Bebb RA, Sirrs SM. Uses of DHEA in aging and other disease states. *Ageing Res Rev*. 2002;1(1):29–41.
19. Wen S, Dong K, Onolfo JP, et al. Treatment with dehydroepiandrosterone sulfate increases NMDA receptors in hippocampus and cortex. *Eur J Pharmacol*. 2001;430(2-3):373–374.
20. Naert G, Maurice T, Tapia-Arancibia L, et al. Neuroactive steroids modulate HPA axis activity and cerebral brain-derived neurotrophic factor (BDNF) protein levels in adult male rats. *Psychoneuroendocrinology*. 2007;32(8-10):1062–1078.
21. Veiga S, Garcia-Segura LM, Azcoitia I. Neuroprotection by the steroids pregnenolone and dehydroepiandrosterone is mediated by the enzyme aromatase. *J Neurobiol*. 2003;56(4):398–406.
22. Eser D, Romeo E, Baghai TC, et al. Neuroactive steroids as modulators of depression and anxiety. *Neuroscience*. 2006;138(3):1041–1048.
23. Sundström Poromaa I, Smith S, Gulinello M. GABA receptors, progesterone and premenstrual dysphoric disorder. *Arch Women Ment Health*. 2003;6(1):23–41.
24. Roberts E, Fitten LJ. Serum steroid levels in two old men with Alzheimer's disease (AD) before and after oral administration of dehydroepiandrosterone (DHEA). Pregnenolone synthesis may be rate-limiting in aging. In: Kalimi M, Regelson W, eds. *The Biological Role of Dehydroepiandrosterone (DHEA)*. Berlin, Germany: de Gruyter; 1990:43–63.
25. Ritsner M, Maayan R, Gibel A, et al. Differences in blood pregnenolone and dehydroepiandrosterone levels between schizophrenia patients and healthy subjects. *Eur Neuropsychopharmacol*. 2007;17(5):358–365.
26. McGAVACK TH, Chevalley J, Weissberg J. The use of delta 5-pregnenolone in various clinical disorders. *J Clin Endocrinol Metab*. 1951;11(6):559–577.
27. Pincus G, Hoagland H. Effects of administered pregnenolone on fatiguing psychomotor performance. *J Aviat Med*. 1944;15:98–115.
28. Pincus G, Hoagland H. Effects on industrial production of the administration of pregnenolone to factory workers, II. *Psychosom Med*. 1945;7:347–352.
29. Meieran SE, Reus VI, Webster R, et al. Chronic pregnenolone effects in normal humans: attenuation of benzodiazepine-induced sedation. *Psychoneuroendocrinology*. 2004;29(4):486–500.
30. Nachshoni T, Ebert T, Abramovitch Y, et al. Improvement of extrapyramidal symptoms following dehydroepiandrosterone (DHEA) administration in antipsychotic treated schizophrenia patients: a randomized, double-blind placebo controlled trial. *Schizophr Res*. 2005;79(2-3):251–256.
31. Ritsner MS, Gibel A, Ratner Y, et al. Improvement of sustained attention and visual and movement skills, but not clinical symptoms, after dehydroepiandrosterone augmentation in schizophrenia: a randomized, double-blind, placebo-controlled, crossover trial. *J Clin Psychopharmacol*. 2006;26(5):495–499.
32. Strous RD, Maayan R, Lapidus R, et al. Dehydroepiandrosterone augmentation in the management of negative, depressive, and anxiety symptoms in schizophrenia. *Arch Gen Psychiatry*. 2003;60(2):133–141.
33. Strous RD, Stryjer R, Maayan R, et al. Analysis of clinical symptomatology, extrapyramidal symptoms and neurocognitive dysfunction following dehydroepiandrosterone (DHEA) administration in olanzapine treated schizophrenia patients: a randomized, double-blind placebo controlled trial. *Psychoneuroendocrinology*. 2007;32(2):96–105.
34. Bloch M, Schmidt PJ, Danaceau MA, et al. Dehydroepiandrosterone treatment of midlife dysthymia. *Biol Psychiatry*. 1999;45(12):1533–1541.
35. Schmidt PJ, Daly RC, Bloch M, et al. Dehydroepiandrosterone monotherapy in midlife-onset major and minor depression. *Arch Gen Psychiatry*. 2005;62(2):154–162.
36. Wolkowitz OM, Reus VI, Keebler A, et al. Double-blind treatment of major depression with dehydroepiandrosterone. *Am J Psychiatry*. 1999;156(4):646–649.
37. Guy W. *ECDEU Assessment Manual for Psychopharmacology*, Rev ed. Washington, DC: Department of Health, Education and Welfare; 1976.
38. Kay SR, Fiszbein A, Opler LA. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. *Schizophr Bull*. 1987;13(2):261–276.
39. Chouinard G, Margolese HC. Manual for the Extrapyramidal Symptom Rating Scale (ESRS). *Schizophr Res*. 2005;76(2-3):247–265.
40. Chouinard G, Ross-Chouinard A, Annable L, et al. Extrapyramidal Symptom Rating Scale [abstract]. *Can J Neurol Sci*. 1980;7:233.
41. Barnes TR. A rating scale for drug-induced akathisia. *Br J Psychiatry*. 1989;154(5):672–676.
42. Robbins TW, James M, Owen AM, et al. Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. *Dementia*. 1994;5(5):266–281.
43. Sahakian BJ, Owen AM. Computerized assessment in neuropsychiatry using CANTAB: discussion paper. *J R Soc Med*. 1992;85(7):399–402.
44. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*, 2nd Edition. Hillsdale, NJ: Lawrence Erlbaum; 1988.
45. Hintze JL. *NCSS 2007. Statistical System for Windows*. Kaysville, Utah: User's Guide. Number Cruncher Statistical Systems; 2006.
46. Pantelis C, Stuart GW, Nelson HE, et al. Spatial working memory deficits in schizophrenia: relationship with tardive dyskinesia and negative symptoms. *Am J Psychiatry*. 2001;158(8):1276–1285.
47. Flood JF, Morley JE, Roberts E. Pregnenolone sulfate enhances post-training memory processes when injected in very low doses into limbic system structures: the amygdala is by far the most sensitive. *Proc Natl Acad Sci U S A*. 1995;92(23):10806–10810.
48. Robel P, Young J, Corpéchot C, et al. Biosynthesis and assay of neurosteroids in rats and mice: functional correlates. *J Steroid Biochem Mol Biol*. 1995;53(1-6):355–360.
49. Melchior CL, Ritzmann RF. Neurosteroids block the memory-impairing effects of ethanol in mice. *Pharmacol Biochem Behav*. 1996;53(1):51–56.
50. Muneoka KT, Shirayama Y, Minabe Y, et al. Effects of a neurosteroid, pregnenolone, during the neonatal period on adenosine A1 receptor, dopamine metabolites in the fronto-parietal cortex and behavioral response in the open field. *Brain Res*. 2002;956(2):332–338.
51. Maciejak P, Członkowska AI, Bidziński A, et al. Pregnenolone sulfate potentiates the effects of NMDA on hippocampal alanine and dopamine. *Pharmacol Biochem Behav*. 2004;78(4):781–786.
52. Bitran D, Shiekh M, McLeod M. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABAA receptors. *J Neuroendocrinol*. 1995;7(3):171–177.
53. Fahey JM, Lindquist DG, Pritchard GA, et al. Pregnenolone sulfate potentiation of NMDA-mediated increases in intracellular calcium in cultured chick cortical neurons. *Brain Res*. 1995;669(2):183–188.
54. Horak M, Vlcek K, Chodounska H, et al. Subtype-dependence of N-methyl-D-aspartate receptor modulation by pregnenolone sulfate. *Neuroscience*. 2006;137(1):93–102.
55. Monnet FP, Mahé V, Robel P, et al. Neurosteroids, via sigma receptors, modulate the [^{3}H]norepinephrine release evoked by N-methyl-D-aspartate in the rat hippocampus. *Proc Natl Acad Sci U S A*. 1995;92(9):3774–3778.
56. Shirakawa H, Katsuki H, Kume T, et al. Pregnenolone sulphate attenuates AMPA cytotoxicity on rat cortical neurons. *Eur J Neurosci*. 2005;21(9):2329–2335.
57. Begg C, Cho M, Eastwood S, et al. Improving the quality of reporting of randomized controlled trials. The CONSORT statement. *JAMA*. 1996;276(8):637–639.
58. Chow SC, Liu JP. *Design and analysis of clinical trials: concepts and methodologies*. New York: Wiley; 1998.
59. Leucht S, Engel RR, Bäuml J, et al. Is the superior efficacy of new generation antipsychotics an artifact of LOCF? *Schizophr Bull*. 2007;33(1):183–191.
60. Strous RD. Dehydroepiandrosterone (DHEA) augmentation in the management of schizophrenia symptomatology. *Essent Psychopharmacol*. 2005;6(3):141–147.

 © Copyright 2010 Physicians Postgraduate Press, Inc.