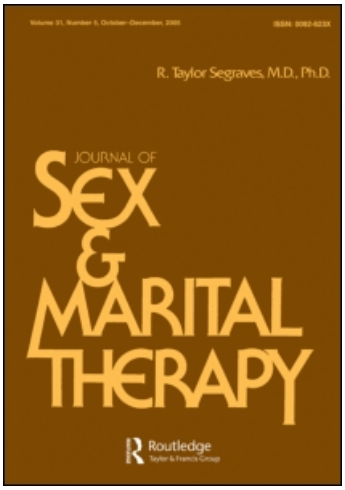

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Randomized, Placebo-Controlled, Double-Blind, Parallel Design Trial of the Efficacy and Safety of Zestra® in Women With Mixed Desire/Interest/Arousal/Orgasm Disorders

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Randomized, Placebo-Controlled, Double-Blind, Parallel Design Trial of the Efficacy and Safety of Zestra® in Women With Mixed Desire/Interest/Arousal/Orgasm Disorders

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Over 256 women, age 21 to 65, with acquired mixed female sexual disorders participated in a 16-week randomized, placebo-controlled, double-blind study of Zestra®, a topical botanical preparation. Routine outcome instruments measured efficacy and safety. Zestra® was well tolerated. The only significant safety finding was mild-to-moderate genital burning seen only in Zestra®-treated subjects (14.6%). Zestra® provided significant desire, arousal, and treatment satisfaction benefits for a broadly generalized group of women with sexual difficulties.

Investigation of female sexual dysfunction (FSD) is complex. Emotional, relational, situational, experiential, cultural, physiological, and pharmacological variables interact in each woman to produce a particular array of sexual outcomes. The FSDs were categorized in the *DSM-IV R* and again in 1998 into specific disorders: desire disorders, arousal disorder, orgasmic disorder, and sexual pain disorders (Basson et al., 2000). These categories were convenient in providing working definitions and an accepted lexicon

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for researchers and therapists. However, there is potential to incorrectly assume that these disorders are fully independent of each other. Both case studies and epidemiologic studies demonstrate that these disorders can overlap and may be interdependent. In some cases, it may be possible to identify the primary disorder that led to the others, but in many cases, this may be impossible. Thus, there is a population of women with mixed interest/desire/arousal/orgasm disorders with varying presentations of each component. A second International Definitions Committee presented revised definitions based on the discrepancies noted above as well as new observations. The current proposed definitions are independent of etiology and primacy (Basson et al., 2004).

There are no FDA-approved drugs available for the treatment of any form of FSD. Numerous nutraceutical and cosmetic products are marketed to enhance women's sexual pleasure. Of these, two have published well-designed, well-controlled trials in peer-reviewed journals: ArginMax® and Zestra® (Fourcroy, 2003). In 2001, a randomized, double-blinded, crossover proof-of-principle study (Ferguson et al., 2003) was conducted to evaluate the efficacy and safety of Zestra® compared to placebo oil in 10 women with, and 10 women without, Female Sexual Arousal Disorder (FSAD) in conditions of home use in conjunction with sexual activities. Subjects were screened by history, physical examination, sex therapist interviews, and questionnaires. Qualified subjects were randomized to treatment paths and given five doses of test article and diaries (Female Sexual Encounter Profile (FSEP), Ferguson, 2002) to use at home. At visit 2, they were assessed by questionnaires and given five doses of crossover test articles and diaries to use at home. At the final visit, they were assessed by questionnaires. Safety was assessed by adverse event reports. Primary efficacy was assessed by responses to a diary question regarding satisfaction with arousal. Secondary efficacy instruments included remaining diary questions, recall-based questionnaires, global assessment questions, and a consumer-testing questionnaire.

All 20 subjects completed the study. Three subjects (15%) reported single incidences of mild genital burning sensations lasting 5–30 minutes after use of Zestra®. Both normal and FSAD women showed statistically significant improvements (relative to placebo) in level of arousal, level of desire, satisfaction with arousal, genital sensation, ability to have orgasms, and sexual pleasure. Although FSAD women showed a greater magnitude of response, the presence of FSAD had no effect on response rates. Zestra® was just as effective in women using selective serotonin reuptake inhibitor (SSRI) anti-depressants as in women not using anti-depressants. Zestra® improved sexual function in normal and FSAD women under conditions of home usage.

The present study was intended to build on the results of the previous study incorporating changes in the women's sexual health field

and knowledge gained from the previous study. The results of the previous study indicated that Zestra® had clinically significant effects on desire and orgasm domains, as well as arousal in women for whom FSAD was their chief complaint. Numerous studies since the presentation of this model have shown large numbers of women who have a syndrome of mixed desire/arousal/orgasm disorders with widely varying components of each. Heiman et al. (2004) suggested that a viable strategy for clinical trials is to select a group of women who meet diagnostic criteria for one disorder and have subsyndromal levels of another sexual disorder. That strategy was employed in this trial.

METHODS

Objective

This study was conducted to evaluate the efficacy and safety of Zestra® compared to placebo oil in women diagnosed as having acquired mixed interest/desire/arousal/orgasm disorders with varying presentations of each component in conditions of home use in conjunction with sexual activities.

Design

This was a multiple site, double-blind, placebo-controlled, parallel design study with an open-label placebo run-in (to establish baselines) to investigate the efficacy and safety of topical application of Zestra® in women with acquired mixed desire/interest/arousal/orgasm disorders. This design was consistent with the Paris 2003 recommendations for FSD clinical trials (Heiman et al., 2004) and the FDA Guidance on Clinical Development of Products for the Treatment of Female Sexual Dysfunction (U.S. FDA, 2000), although the duration of active treatment in this study is 12 weeks rather than the 6 months suggested by the FDA for Phase 3 studies. Thirteen investigators were recruited to conduct this study. Each research site was under supervision of either a local or central institutional review board (IRB). Subjects were recruited from investigators' practices and also with IRB-approved advertising. There was one screening visit (visit 1), four intermediate visits (visits 2–5), and a final visit (visit 6).

A sufficient number of subjects were enrolled to ensure 200 subjects successfully completed visit 3. Dropouts and dismissed subjects were replaced. At visit 1, each subject who signed an informed consent form (her partner also had to provide written consent) was interviewed to obtain a sexual history and a medical history, underwent a physical examination, submitted blood and urine specimens for laboratory evaluation, completed the Single Question Assessment of Erectile Dysfunction (SQAED, Derby et al., 2000) regarding her partner, the Female Sexual Distress Scale (FSDS), and underwent a review of the inclusion/exclusion criteria. Female sexual

dysfunction diagnoses were established by interview. Qualifying subjects returned for Visit 2 where adverse events and concomitant medications were reviewed. They received 15 sachets of placebo test article (open-label) with instructions for storage and use, and 15 copies of the FSEP with training on their use following each sexual encounter. Each subject was told to use the study medication in conjunction with sexual activities at least two times per week over the next 4 weeks.

At visit 3, adverse events and concomitant medications were reviewed. Diaries and used and unused sachets of the placebo test article were collected. Subjects who had at least three sexual encounters using the test article were allowed to continue. They completed the following instruments to establish baseline values: Female Sexual Function Index (FSFI), Women's Inventory of Treatment Satisfaction (WITS), Zestra Consumer Testing Survey (ZCTS), two global assessment questions (GAQ), the Beck Depression Inventory (BDI), the Dyadic Adjustment Scale (DAS), and the FSDS. They received 15 sachets of double-blinded test articles (either placebo or Zestra®) with instructions for storage and use, and 15 copies of the FSEP with training on their use following each sexual encounter. Each subject was told to use the study medication in conjunction with sexual activities at least two times per week over the next 4 weeks. At visits 4 and 5, adverse events and concomitant medications were reviewed. Diaries and used and unused sachets of the double-blinded test article were collected. They again received 15 sachets of the double-blinded test article (either placebo or Zestra®) they were randomized to, with instructions for storage and use, and 15 copies of the FSEP with training on their use following each sexual encounter. Each subject was told to use the study medication in conjunction with sexual activities at least two times per week over the next 4 weeks. At visit 6, adverse events and concomitant medications were reviewed. Diaries and used and unused sachets of the double-blinded test article were collected. They completed the following instruments to compare with baseline values established at visit 3: FSFI, WITS, ZCTS, GAQ, BDI, DAS, and the FSDS. A physical examination was performed, blood and urine specimens were collected for laboratory evaluation, and the subjects were dismissed from the study. Safety was assessed by soliciting adverse events and reviewing physical examinations and laboratory evaluations.

Inclusion Criteria

The inclusion/exclusion criteria were consistent with those recommended for FSD studies by Rosen, Barsky, and Ferguson, 2006. To be included in this study, female subjects, 21 to 65 years of age, had to have been previously "functional" or experienced sexual desire/arousal/orgasm for at least 3 years. They had to be post-menopausal (no menses for 1 year and appropriate elevation of FSH), or using hormonal contraception for at least 3 months

prior to study entry, or have had a tubal ligation at least 3 months prior to study entry or confining all sexual intercourses to a vasectomized partner. Subjects had to provide written, informed consent, have a stable heterosexual relationship with a male partner for at least 1 year, and their partners had to attend the screening visit and sign a separate informed consent form. Subjects had to assess their partners as "Not impotent" or "Minimally impotent" on the SQAED. Subjects and their partners had to be willing to engage in sexual activities with intent to attain orgasm at least two times per week. Those who completed at least three valid attempts during the run-in period were allowed to continue into the double-blind phase of the study. The women had to meet the diagnostic criteria (based on a interview) for one or more of the following acquired disorders: Sexual Interest/Desire Disorder (DD), Subjective Sexual Arousal Disorder (SAD), Genital Sexual Arousal Disorder (GAD), or Orgasmic Disorder (OD). They had to score $\geq$15 in the FSDS. Subjects had to be willing and able to understand and comply with all study requirements.

Exclusion Criteria

Subjects with any of the following conditions or meeting any of the following criteria were excluded from the study: evidence of unresolved sexual trauma or abuse; primary anorgasmia, vaginismus, sexual pain disorder, sexual aversion disorder, or persistent genital arousal disorder; female sexual dysfunction caused by untreated endocrine disease, e.g., hypopituitarism, hypothyroidism, diabetes mellitus; pregnant or nursing; sensitivity to any of the ingredients in Zestra®; chronic or complicated urinary tract or vaginal infections within previous 12 months; pelvic inflammatory disease within previous 12 months; currently active sexually transmitted disease; chronic dyspareunia not attributable to vaginal dryness within previous 12 months; currently active moderate-to-severe vaginitis; cervical dysplasia within previous 12 months; significant cervicitis as manifested by mucopurulent discharge from the cervix; significant gynecologic conditions such as uterine fibroids, vulvar vestibulitis, or vaginismus that may (in the investigator's opinion) interfere with the subject's ability to comply with study procedures; psychoses and bipolar disorder; use of coumadin, neuroleptics, or lithium within previous 3 months; unwillingness to forego any medications, herbal treatments, or dietary supplements intended to enhance sexual function during the course of the study; history of myocardial infarction within the previous 6 months; history or evidence of significant renal or hepatic disease within previous 6 months; significant central nervous system diseases within the last 6 months i.e., stroke, spinal cord injury, multiple sclerosis, etc; any condition which in the investigator's opinion would interfere with the subject's ability to provide informed consent, to comply with study instructions, or which might confound the interpretation

of the study results; and any condition that would endanger the participant if she participated in this trial.

Outcome Measures

Primary efficacy assessments included the FSFI (Rosen et al., 2000) and the subjects' assessments of their sexual encounters as recorded in a diary: the FSEP (Ferguson, 2002). Secondary evaluations of efficacy included a treatment satisfaction questionnaire (WITS), a consumer testing survey (ZCTS), two GAQs, the BDI, the DAS, a distress scale (FSDS) (Derogatis, 2000), sexual encounter frequency, and dropout rates.

The selection of outcomes measures in this study is based on the most current information available (Rosen, Barsky, & Ferguson, 2006). The FSEP has been used in many FSD studies and specifically meets the FDA recommendations for a primary outcome measure (U.S. FDA, 2000). The initial validation study of the FSEP was presented at the 11th World Congress of the International Society for Sexual and Impotence Research in Buenos Aires by Gittelman and Peterson (2004). The FSFI, the FSDS© , and GAQs are routinely used in FSD trials, and the first two have been subjects of ongoing validation for a number of years. Global assessment questions have historically been used to calculate response rates. The ZCTS is a consumer testing survey that addresses issues ultimately impinging on product marketability (Ferguson et al., 2003). The WITS is a female-oriented treatment satisfaction instrument developed by Stanley E. Althof, Eric W. Corty, and Miki Wieder (2004). The authors' authorized use of this instrument in this study as part of the ongoing validation for this questionnaire. Since depression can contribute to FSD, and FSD can contribute to depression, seriously confounding diagnostic and treatment schema (Heiman et al., 2004), the commonly used BDI (Beck, Steer, Ball, & Ranieri, 1996) was administered at the end of the baseline run-in period and then at the end of the double-blinded treatment period. Relational factors have the same confounding issues as depression; thus the participants' attitudes about their relationship to their partners were assessed at baseline and at the end of treatment using the DAS (Spanier, 1976). Sexual encounter frequency may prove a useful outcome measure since a beneficial effect of treatment may be reinforcing, while lack of efficacy by the placebo may be discouraging. A similar hypothesis is the basis of dropout analysis.

Laboratory Evaluations

At the screening visit (visit 1), the following were performed: urine pregnancy test (positive result was grounds for dismissal), complete blood count with differential, urinalysis, thyroid stimulating hormone (TSH), luteinizing

hormone (LH), follicle stimulating hormone (FSH), dehydroepiandrosterone sulfate (DHEA-S), sex hormone binding globulin (SHBG), total testosterone, free testosterone, % free testosterone, estradiol, estrone, estriol, glucose, creatinine, and blood urea nitrogen (BUN). Urine pregnancy tests were repeated at all visits. At the exit visit (visit 6), the following were performed: complete blood count with differential, urinalysis, glucose, creatinine, and BUN. Hormones were assayed at Quest Diagnostics Nichols Institute, Chantilly, VA, using state-of-the-art assays. Estrone was determined by radioimmunoassay. Estradiol was assayed by chemiimmunoluminescence. Dehydroepiandrosterone sulfate was measured by immunoassay. Sex hormone binding globulin was determined by immunoassay. Total and free testosterone were measured by equilibrium dialysis and liquid chromatography/mass spectroscopy/mass spectroscopy.

Test Articles

Zestra® is a patented topically-applied formulation (U.S. Patent 6,737,084; EP Patent EP1328281) composed of borage seed oil, evening primrose oil, angelica root extract, and coleus forskohlii extract as its active ingredients invented by Martin Crosby, B.S. Pharmacy, Charleston, S.C. Ascorbyl palmitate, U.S.P (fat-soluble Vitamin C) and dl-alpha tocopherol U.S.P. (Vitamin E) are included as antioxidants to stabilize the edible oils (borage seed oil and evening primrose oil) against oxidation. This use is consistent with the manner in which these antioxidants are used in edible cooking oils and other food products that are regulated by FDA. Likewise, the flavor oil in Zestra® which is used to mask the characteristic herbal scent of Angelica, has FDA generally recognized as safe (GRAS) status and is in lower concentration than when used in candies, lipsticks, other body oils, or other massage oils. This product was designed, developed, and intended to be marketed under U.S. Cosmetics Regulations and has been available to the public as a consumer product since mid-2003. The components in this formulation are available in the United States either as dietary supplements, in foods or cosmetics, or are considered to be on the GRAS List by FDA, and on the Everything Added to Food in the United States (EAFUS list).

The placebo was soybean oil, coloring (riboflavin), and fragrance which matched Zestra® based on odor, color, lubricity, viscosity, and absorbency. The placebo did not contain ascorbyl palmitate or dl alpha tocopherol. All test articles were packaged in individual sachets containing 0.8 milliliter by volume, suitable for a single usage. Sachets for Zestra® and the placebo were prepared in separate production and packaging runs. All sachets were coded according to a randomization scheme held by the manufacturer. Sets of placebo and double-blinded test articles were prepared for each subject according to the randomization scheme. The study medication supplies were manufactured, labeled, and distributed according to the requirements of U.S.

law by Martin Crosby, Zestra Laboratories, Inc., Charleston, SC. All study medications were stored, inventoried, reconciled, and destroyed according to applicable state and federal regulations.

Statistical Plan

Sample Size (Power Calculation)

The calculation of sample size was based on the binary response data of the primary efficacy variable (FSEP question regarding satisfaction with arousal) from the previous Zestra® study. A sample size of 33 per group will allow, with 95% power, the ability to detect a significant difference in proportions (with FSEP arousal satisfaction rates of 55% on Zestra and 15% on placebo) using the chi-square test with continuity correction at a one-sided .05 level of significance. Actual group sizes ($N = 100$/group) were selected on the need to better assess adverse events, and the need to generate more convincing and generalizable efficacy data in a larger population sample than used in the previous study.

Randomization

The randomization scheme was prepared by sequential blocks of four treatment assignments using all combinations. The treatment assignment ratio was 1:1. The randomization code was held by the medical director for the study and was not revealed to the participating clinical sites. The medical director was allowed to unblind specific treatment assignments in the event that a medically disturbing adverse event trend appeared.

Subject Definitions

All subjects who used at least one dose of double-blinded study medication (dispensed at visit 3) were included in the intent to treat (ITT) cohort. Since all of the efficacy variables were comparisons of changes from baseline and many of the instruments were only administered at visit 3 and visit 6, a per protocol (PP) cohort was defined as those subjects who completed the entire schedule.

Safety Variables

The primary safety variables were adverse events and findings from the physical and laboratory exams. The incidences of study-emergent adverse experiences were tabulated by treatment group, event, and body system. The overall incidence rates were compared between treatments using Fisher's Exact test. Data from the entire ITT cohort were evaluated.

Efficacy Variables

Primary efficacy assessments were the change from baseline (visit 3) in the FSFI domains and the FSEP questions 3 through 10 which address level of desire, satisfaction with level of desire, lubrication, level or arousal, satisfaction with level of arousal, achievement of orgasm, and satisfaction and success of the encounter, respectively. The FSEP questions 1 and 2 are informational and not efficacy-related. Secondary evaluations of efficacy were a treatment satisfaction questionnaire (WITS), a consumer testing survey (ZCTS), two GAQs, the BDI, the DAS, the FSDS, sexual encounter frequency, and dropout rates.

Baseline Measurements

Subject demographic data, baseline medications and concurrent illnesses, and baseline values for all outcomes measures were recorded and summarized by treatment group assignment. Student's *t*-tests (continuous variables) and Fisher's Exact tests (discrete variables) were used to determine the adequacy of the randomization in assigning subjects equally to the treatments.

Analyses

Since the FSEP responses were collected over a number of encounters, each subject's responses within a protocol period were normalized by dividing the sum of the responses to each question of the diaries by the number of valid attempts. A valid attempt was defined as an encounter in which the subject used the test article. The FSEP data from the ITT cohort were analyzed according to plan. After the entire study was analyzed, it was suggested that the FSEP data also be analyzed for the PP cohort to bring these data into congruency with the other efficacy variables. The FSFI individual item responses at baseline were subtracted from the response at end of treatment for each subject (change from baseline variable). Individual domain scores were calculated. The WITS and DAS were handled similarly. The baseline comparisons between treatment groups (placebo versus Zestra) were made using a Student's *t*-test with continuous variables and Fisher's Exact tests were used for the discrete variables. The change from baseline in FSFI mean domain scores were analyzed using analysis of covariance (ANCOVA) and the change from baseline in total number of positive responses to FSEP questions were analyzed using linear mixed effects analysis of covariance models. Dropout incidence was analyzed by logistic regression. After review of the results, the FSFI arousal domain was selected as the most sensitive indicator of response. ANCOVA was then used to seek significant covariates from the following baseline variables: age, race,

menopausal status, hysterectomy, hormonal replacement therapy, hormonal contraception, baseline free testosterone, baseline estradiol, concomitant medications, and co-morbidities. Results from the FSFI and the WITS were also converted to binary data (subjects who improved or did not improve) and analyzed using linear logistic regression. All inferential statistical tests were two-sided at a significance level of .05.

RESULTS

Subject Participation

Two hundred ninety-six women were screened at Visit 1. Two hundred fifty-six women qualified and entered into the open-label placebo run-in period at visit 2. Two hundred seventeen women began use of the double-blinded test article at visit 3 (this was the intent-to-treat cohort); 112 were randomized to placebo; 105 were randomized to Zestra®. Among the women receiving double-blinded placebo, 7 dropped out by visit 4; 7 more dropped out by visit 5; and 5 more dropped out by visit 6. Among the women receiving double-blinded Zestra®, 9 dropped out by visit 4; 9 more dropped out by visit 5; and 2 more dropped out by visit 6. One hundred seventy-eight women completed the study; this was the per protocol cohort). Ninety-three placebo subjects completed the study, while 85 Zestra® subjects completed the study.

Demographics

Two hundred fifty-six women, age 21 to 65 years, enrolled in this study. The mean age at entry into the study was 48. Distribution by race was Caucasian–208; African-American–23; Hispanic–23; Other–2. The average age of menarche in the participants was 12.6 years, while the age of first intercourse was 18. In the month preceding screening for the study, the mean number of sexual encounters was 3.8. Twenty-one women had a history of resolved sexual trauma or abuse. Sixty percent were post-menopausal, and 31% were on hormonal therapy. Twenty-six percent were taking anti-depressants. The majority of women had more than one of the FSDs. Ninety percent had at least-DD, while 66% had at least SAD. Desire disorder and SAD were present in 60.5%. All disorders were present in 22.7% of the participants.

Safety

Baseline and exit physical examinations and laboratory evaluations exhibited no clinically significant changes. Adverse events were solicited by interview at each subject visit. One adverse event emerged as treatment related. Only "genital burning" had a significantly ($p < .001$) higher incidence (14.6%) in

the Zestra®-treated group than in the placebo-treated group (0.0%). Of the 14 women who reported genital burning, 7 described it as "mild," and 7 described it as "moderate" in severity. Eight women said it occurred only in the first four or fewer uses with no reports thereafter. Five women (5.2%) discontinued the study, citing the "genital burning" as the reason. All reports of this symptom were considered by the investigators to be related to the treatment. Eighty-two (85.4%) of the 96 women treated with Zestra in the current study did not report this symptom. No unexpected adverse events that could be considered related to Zestra® were reported in this study. There were no serious adverse events associated with Zestra® treatment.

Baseline Efficacy Variables

Table 1 compares the baseline values of the efficacy variables between the treatments at the end of the open-label placebo run-in period (visit 3) and post double-blind treatment (visit 6).

Efficacy

A total of 178 women completed the entire study: placebo 93; Zestra® 85. This constitutes the per protocol cohort. Table 2 compares the changes from baseline in the efficacy variables between Zestra® and placebo. The results for individual variables are presented below.

FSFI

Zestra® produced a greater mean improvement than placebo in the desire domain ($p = .0448$) and the arousal domain ($p = .0064$, highly significant). Placebo produced greater mean improvements than Zestra® in the lubrication and pain domains, but they were not significant. Zestra® showed a greater mean improvement in the orgasm domain, but it was not significant. In the satisfaction domain, Zestra® showed an improvement in mean score while the placebo score was slightly worse. This difference was near significance ($p = .0588$). Lastly, total FSFI score showed a nonsignificant trend ($p = .0918$) for Zestra® over placebo.

FSEP

Diaries (FSEP) were collected at visit 3 (placebo-controlled baseline) and at visits 4, 5, and 6 (double-blinded treatment). Since these were the only efficacy data that were collected throughout the study, the statistical analyses were conducted by plan on the intent-to-treat population. That is, all data from all subjects were analyzed regardless of whether they completed the

TABLE 1. Efficacy Variables at Baseline (Visit 3) and Post-Treatment (Visit 6)

Variable	Baseline Placebo Mean ± SE), $N = 93$	Baseline Zestra (Mean ± SE), $N = 85$	Baseline p	Post-Treatment Placebo (Mean ± SE), $N = 93$	Post-Treatment Zestra (Mean ± SE), $N = 85$
FSFI: Desire	2.71 ± 0.114	2.64 ± 0.113	.6659	2.87 ± 0.114	3.11 ± 0.111
FSFI: Arousal	3.11 ± 0.121	2.98 ± 0.124	.4647	3.23 ± 0.126	3.54 ± 0.136
FSFI: Lubrication	4.44 ± 0.163	4.65 ± 0.141	.3444	4.79 ± 0.154	4.96 ± 0.126
FSFI: Orgasm	3.08 ± 0.168	2.84 ± 0.159	.2999	3.33 ± 0.179	3.36 ± 0.181
FSFI: Satisfaction	3.88 ± 0.118	3.75 ± 0.138	.4458	3.88 ± 0.142	4.12 ± 0.134
FSFI: Pain	5.11 ± 0.136	5.39 ± 0.111	.1136	5.19 ± 0.133	5.24 ± 0.131
FSFI: Total	22.33 ± 0.557	22.24 ± 0.547	.9109	23.29 ± 0.591	24.34 ± 0.592
FSEP Question 3: Level of desire	1.29 ± 0.067	1.30 ± 0.071	.9118	1.38 ± 0.069	1.55 ± 0.075
FSEP Question 4: Satisfaction with level of desire	0.44 ± 0.034	0.43 ± 0.037	.8285	0.54 ± 0.039	0.60 ± 0.041
FSEP Question 5: Adequate lubrication	0.79 ± 0.034	0.84 ± 0.029	.2553	0.84 ± 0.034	0.86 ± 0.028
FSEP Question 6: Level of arousal	1.33 ± 0.067	1.34 ± 0.071	.8986	1.42 ± 0.073	1.58 ± 0.076
FSEP Question 7: Satisfaction with arousal	0.42 ± 0.033	0.41 ± 0.038	.7486	0.53 ± 0.039	0.56 ± 0.043
FSEP Question 8: Achieved orgasm	0.40 ± 0.033	0.38 ± 0.037	.7328	0.46 ± 0.036	0.43 ± 0.041
FSEP Question 10: Successful and satisfactory encounter	0.50 ± 0.035	0.49 ± 0.037	.9557	0.54 ± 0.037	0.58 ± 0.042
Successful and satisfactory encounters on meds (absolute)	4.59 ± 0.387	4.38 ± 0.394	.6981	4.86 ± 0.386	4.96 ± 0.433
WITS: Treatment satisfaction	0.43 ± 0.140	0.45 ± 0.155	.9136	0.27 ± 0168	0.76 ± 0.174
WITS: Sex life satisfaction	−0.10 ± 0.157	−0.20 ± 0.172	.6951	0.06 ± 0.181	0.40 ± 0.193
WITS: Self-perception	0.28 ± 0.132	0.24 ± 0.163	.8530	0.44 ± 0.156	0.87 ± 0.151

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WITS: Partner perception	1.45 ± 0.107	1.42 ± 0.106	.8196	1.10 ± 0.133	1.37 ± 0.134
WITS: Relationship satisfaction	1.56 ± 0.134	1.37 ± 0.161	.3709	1.47 ± 0142	1.55 ± 0.146
WITS: Total score	16.88 ± 4.191	14.6 ± 4.508	.7109	16.35 ± 5.173	28.59 ± 5.414
Beck Depression Inventory	6.11 ± 0.506	6.95 ± 0.700	.3291	5.53 ± 0.568	6.58 ± 0.740
DAS: Satisfaction	39.32 ± 0.554	38.52 ± 0.627	.3355	39.17 ± 0.590	38.32 ± 0.725
DAS: Consensus	50.32 ± 0.767	50.60 ± 0797	.8024	50.45 ± 0.771	50.48 ± 07654
DAS: Cohesion	16.68 ± 0.374	16.52 ± 0.403	.7712	16.24 ± 0.366	15.91 ± 0.416
DAS: Affection	12.28 ± 0.527	11.84 ± 0.615	.5823	12.63 ± 0.527	12.98 ± 0.584
DAS: Total score	118.60 ± 1.722	117.47 ± 1.984	.6658	118.49 ± 1.921	117.68 ± 2.094
ZCTS Question 1: Genital sensation change	0.13 ± 0.044	0.22 ± 0.048	.1489	0.22 ± 0.050	0.64 ± 0.060
ZCTS Question 2: Was change pleasurable	0.16 ± 0.043	0.21 ± 0.046	.4344	0.19 ± 0.047	0.41 ± 0.072
ZCTS Question 6: Sexual pleasure	0.19 ± 0.044	0.29 ± 0.055	.1519	0.31 ± 0.057	0.39 ± 0.077
ZCTS Question 7: Lubrication change	0.49 ± 0.058	0.49 ± 0.062	.9953	0.55 ± 0.060	0.594 ± 0.063
ZCTS Question 8: Ease of orgasm	0.10 ± 0.041	0.12 ± 0.042	.7227	0.15 ± 0.048	0.29 ± 0.060
ZCTS Question 9: Enhancement of sexual experience	0.18 ± 0.048	0.22 ± 0.045	.5414	0.26 ± 0.050	0.33 ± 0.072
ZCTS Question 10: Price per dose, mean (visit 6 only), P from paired t against baseline	2.08 ± 0.3674	2.49 ± 0.427	.4727	2.50 ± 0.463	3.53 ± 0.440
Female Sexual Distress Scale	25.41 ± 1.023	24.82 ± 1.142	.7024	21.98 ± 1.264	19.68 ± 1.227
Sexual encounter frequency	8.99 ± 0.295	8.45 ± 0.340	.2277	8.57 ± 0.276	8.14 ± 0.319

TABLE 2. Efficacy: Changes from baseline

	Placebo	Zestra	*p* value
FSFI Domains: Least squares mean changes			
Desire	0.161	0.466	.0448*
Arousal	0.123	0.561	.0064**
Lubrication	0.348	0.311	.7533
Orgasm	0.249	0.527	.2561
Satisfaction	−0.004	0.376	.0588
Pain	0.086	−0.146	.2381
Total	0.963	2.095	.0918
FSEP: least squares means (ITT analysis)			
Question 3: Level of desire	1.5357	1.6692	.0307*
Question 4: Satisfaction with level of desire	0.3999	0.5886	.3614
Question 5: Adequate lubrication	2.0855	1.7350	.2389
Question 6: Level of arousal	1.5371	1.6962	.0251*
Question 7: Satisfaction with arousal	0.4364	0.5465	.5952
Question 8: Achieved orgasm	0.0316	0.1734	.4590
Question 10: Successful and satisfactory encounter	0.6373	0.8141	.3580
Successful and satisfactory encounters on meds (absolute)	1.3807	1.6422	.5519
WITS: Least squares mean changes			
Treatment satisfaction	0.0848	0.5727	.0207*
Sex life satisfaction	0.5160	0.9430	.0246*
Self perception	0.4226	0.8842	.0102*
Relationship satisfaction	0.0865	0.2721	.2677
Partner perception	−0.2386	0.0677	.0319*
Total score	0.2336	0.6364	.0086**

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GAQs: Least squares mean changes			
Question 1: Sexual Satisfaction improved	−0.6800	−0.4374	.3797
Question 2: Number of successful encounters increased	−0.1896	−0.2060	.9475
BDI: Least squares mean changes	−0.0983	−0.0737	.4288
DAS: Least squares mean changes			
Satisfaction	0.0211	0.0047	.7779
Consensus	0.0205	0.0057	.8037
Cohesion	−0.0410	−0.0858	.5629
Affection	0.2451	0.3933	.3203
Total score	0.0377	0.0397	.9700
Zestra Consumer Testing Survey: Percentage of subjects improved (Questions 1, 2, 6, 7, 8, and 9 only)			
Question 1: Genital sensation change	18.3	45.9	.000094**
Question 2: Was change pleasurable	13.8	33.3	.0033**
Question 6: Sexual pleasure	23.7	29.4	.4001
Question 7: Lubrication change	20.4	22.4	.8551
Question 8: Ease of orgasm	12.9	24.7	.0536
Question 9: Enhancement of sexual experience	18.3	25.9	.2768
Question 10: Price per dose, mean (visit 6 only), P from paired t against baseline	$2.50(0.3673)	$3.53(0.0237)*	.3064
FSDS: Least squares mean changes	−0.3831	−0.5186	.2164
Sexual Encounter Frequency: Least squares mean changes	−0.4042	−0.7790	.2251
Dropout percentage by end of study	17.0	19.0	.7260

*statistically significant ($p < .05$).

**highly statistically significant ($p < .01$).

study. Table 2 shows the least square means (not changes) for the pertinent diary questions and the p value for comparison of Zestra® to placebo. Significant improvements for Zestra® over placebo were seen in questions 3 ($p = .0307$) and 6 ($p = .0251$) which query level of desire and level of arousal, respectively. No other questions demonstrated statistically significant differences. When the FSEP data were analyzed for the per protocol cohort, the results were consistent with those for the ITT analysis, although Question 6 (level of arousal) just failed to meet significance ($p = .0653$), likely due to a loss in power because of the reduction of the number of observations. Since ITT data are considered purer, only they are presented in Table 2.

WITS

The WITS is a 36-question subject assessment questionnaire that was administered to subjects at visit 3 (end of open label placebo run-in) and at visit 6 (end of double-blind treatment with Zestra® or placebo). The WITS has five domains: treatment satisfaction, sex life satisfaction, self perception, relationship satisfaction, and partner perception. Answers are Likert scales. A total score for the questionnaire can also be evaluated.

The results show that Zestra® provided a significant benefit over placebo in 4 out of 5 domains: treatment satisfaction, sex life satisfaction, self perception, and partner perception. Additionally, Zestra® showed a highly significant benefit over placebo for the total score ($p = .0086$).

ZCTS

The ZCTS has a number of marketing-related questions that are suitable for statistical analysis: questions 1, 2, 6, 7, 8, 9, and 10; the other questions have *ad lib* prose answers. Question 1 addresses whether genital sensation increased, stayed the same, or decreased. Zestra® showed a highly significant ($p = .000094$) benefit (increase) over placebo. Question 2 asks if the change in sensation is pleasurable, neutral, or unpleasurable. Again, Zestra® showed a highly significant ($p = .0033$) benefit over placebo. Although the percent of subjects with positive results were higher for Zestra® than for placebo in questions 6, 7, 8, and 9, none of these differences was statistically significant. Question 10 asks what the subject would be willing to pay for a single dose of the study test article. At visit 6, the subjects who had received double-blind placebo indicated they would pay $2.50. This was not significantly more than they had answered at baseline (after 1 month of open-label placebo). On the other hand, those subjects who received double-blind Zestra® indicated they would be willing to pay $3.53 for a single dose. This was significantly ($p = .0237$) higher than what they had answered at baseline (after 1 month

of open-label placebo). Comparison of changes from baseline between the treatment groups was not statistically significant.

GAQ

The GAQs asked the subjects 1) if sexual satisfaction improved and 2) if the number of successful sexual encounters increased. Answers were yes or no. There were no significant differences between placebo and Zestra®.

BDI

The BDI is a widely used validated instrument of 21 questions with graded answers. A lower score indicates less depression. There was no significant difference between placebo and Zestra®.

DAS

The DAS is a widely used validated instrument of 32 questions with graded answers used to assess four domains of relationships. Higher scores indicate improvement. There were no significant differences between placebo and Zestra® for any of the domains or the total score.

FSDS

The FSDS is a widely used validated instrument of 12 questions with graded answers. A higher score indicates more distress. Both placebo and Zestra-treated subjects showed a decrease in distress by the end of the study, but there was no significant difference in the changes.

Sexual Encounter Frequency

No significant differences. Both groups decreased their frequency slightly from baseline but maintained an average of approximately eight encounters per month.

Drop Out Analysis

By the end of the study (visit 6), 17.0% of the subjects (19) receiving double-blind placebo had dropped out of the study. On the other hand, 19.0% of the subjects (20) who received double-blind Zestra® dropped out. This difference was not statistically significant ($p = .7260$).

Efficacy Response Covariates

The following factors were tested for significance as covariates of efficacy using standard ANCOVA methods: age, race, menopausal status,

hysterectomy, hormonal replacement therapy, hormonal contraception, baseline-free testosterone, baseline estradiol, concomitant medications, and comorbidities. Race was a highly significant covariate. African-American women responded more strongly than Caucasian women ($p = .0095$). The difference was also judged to be clinically significant. Presence of osteoporosis was a significant covariate. Women with osteoporosis were less responsive than women without osteoporosis ($p = .0244$). The difference was also judged to be clinically significant.

All of the following factors were tested and found not to be significant covariates of efficacy: age, menopausal status, hysterectomy, hormonal replacement therapy, hormonal contraception, baseline-free testosterone, baseline estradiol, anxiety/depression, anti-depressants, narcotics, SSRI and selective serotonin and norepinephrine reuptake inhibitors (SSNRI), non-steroidal anti-inflammatory drugs (NSAIDs), statins, restless leg syndrome, bladder disease, bowel disease, allergy/sinusitis, arthritis, gastro-esophageal reflux disease (GERD), hypertension, diabetes, asthma, hypothyroid disease (treated), lipidemia, and migraine. The following factors were not tested: body mass index (BMI), oopherectomy, tubal ligation, and bupropion.

DISCUSSION

The arousal and desire benefits seen in the first clinical trial in highly selected subjects was now confirmed in a population that mirrors the general population of women with sexual difficulties. The significant benefits of Zestra® in desire and arousal were seen in both the FSFI domains and the FSEP questions regarding levels of desire and arousal. Interestingly, neither the FSFI nor the FSEP results indicated a significant sexual satisfaction benefit, while the WITS data showed significant benefits in 4 of the 5 treatment satisfaction domains. These apparently contradictory results may relate to the differences in the construction and intent of the three different instruments. Both the FSFI and the FSEP were developed with arousal disorder as the prominent target condition. The satisfaction domain of the FSFI and the satisfaction question of the FSEP address sexual satisfaction, while the WITS instrument was specifically developed to measure satisfaction with treatment. The WITS instrument is relatively new and subject to further validation studies. Thus, the benefits demonstrated by the WITS results may be considered tentative.

There were several efficacy instruments that failed to show any differences between the placebo and Zestra® treatments: GAQ, BDI, DAS, FSD, sexual encounter frequency, and drop-out analysis. The GAQ questions had yes and no answers that may have been too great a barrier for a change in response when compared to questions with graded responses such as those in the FSFI. Similar barriers may have contributed to the lack of significant

responses in the FSEP questions that had yes and no responses. Althof et al. (2005) have pointed out the inherently greater sensitivity associated with subject assessment questionnaires that employ Likert scales when compared to diaries that have binary responses. This study supports their views. The inclusion of the BDI and the DAS in the current study was based on hypotheses that improving sexual response could relieve depression and also improve a couple's relationship. The results in this study do not support those hypotheses. It may be possible that a 12-week trial is not long enough to produce changes in relationship satisfaction. The FSDS results showed improvements for both the placebo and the Zestra® groups, but there was no significant difference.

Participants had been told to attempt at least two sexual encounters per week during the 1 month run-in period to qualify for the study. Those who completed at least three valid attempts were allowed to continue into the double-blind phase of the study. Sexual encounter frequency decreased slightly in both groups over the 12 weeks of double-blind treatment, but there was no significant difference; both groups maintained an average frequency of 7 to 8 encounters per month during double-blind treatment. This frequency suggests that the population recruited was not the most severely affected group of women with sexual difficulties, a group for whom this frequency would have been an insurmountable burden. A dropout analysis showed no significant difference between placebo and Zestra® treatments.

Efficacy covariate analyses revealed several interesting findings. African-American women were significantly more responsive to Zestra® than were Caucasian women. Women with self-reported osteoporosis were significantly less responsive to treatment than were those who did not report osteoporosis. Since these women represented a minority of the subjects in this study, the clinical significance of these findings is only tentative. Usage of anti-depressants of all types and the SSRI-SSNRI classes made no difference in responsiveness. This confirms the findings from the previous Zestra® study.

The only statistically significant adverse event was genital burning which was seen only in Zestra®-treated subjects. The incidence of this symptom (14.6%) confirms the incidence (15%) seen in the previous clinical trial of Zestra®. No new adverse events emerged. There were no serious adverse events associated with Zestra® treatment. There were no other significant safety findings.

CONCLUSION

This multicenter, randomized, double-blind, placebo-controlled trial of Zestra in women with mixed desire/interest/arousal/orgasm disorders showed that Zestra is well tolerated with a 14.6% incidence of mild-to-moderate genital burning in only the Zestra® treatment group. Zestra® provides significant

desire, arousal, and treatment satisfaction benefits for a broadly generalized group of women with sexual difficulties.

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DECLARATION OF INTEREST

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