Study Title: Impact of Escitalopram on Sperm DNA Fragmentation

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TITLE: SSRI Impact On Sperm Quality: A Randomized Placebo Controlled Trial

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Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from WCM.

List of Abbreviations

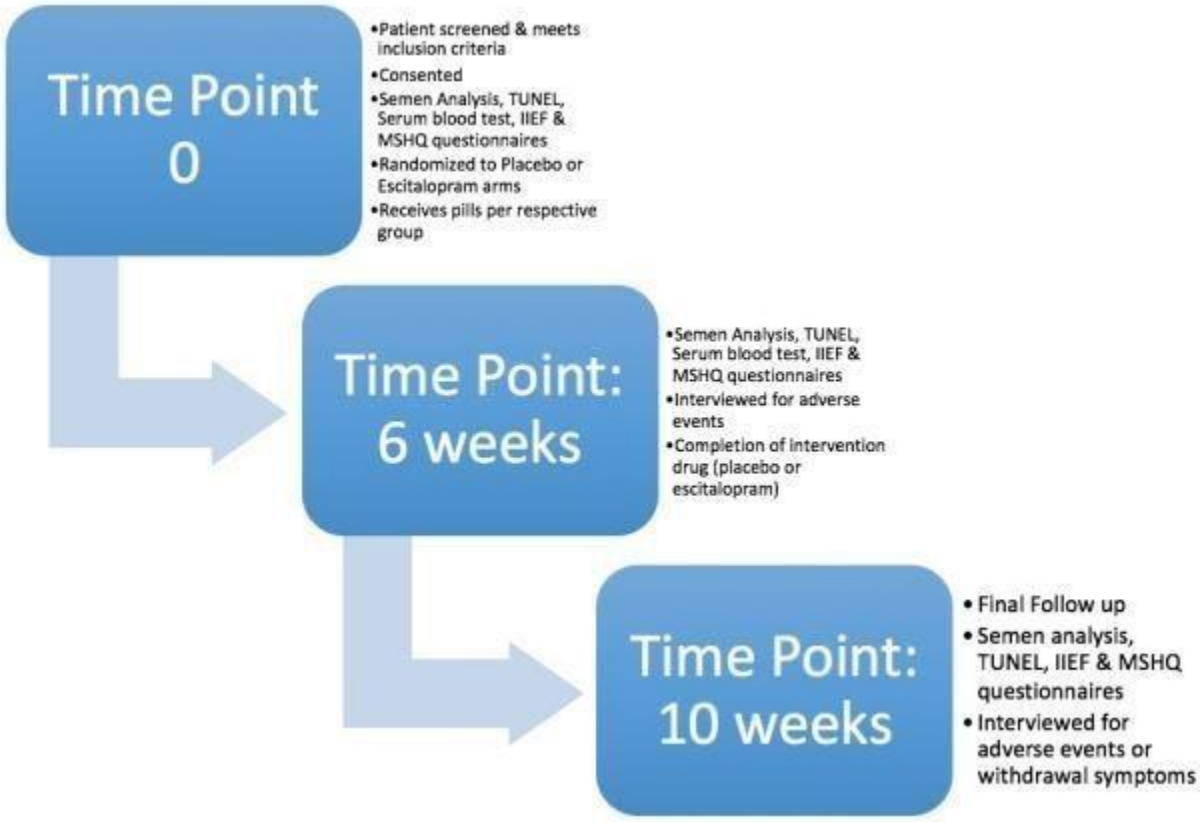
AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
CTSC	Clinical Translational Science Center
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HRBFA	Human Research Billing Analysis Form
HUD	Humanitarian Use Device
ICF	Informed Consent Form
IIEF	International Index of Erectile Function
IDE	Investigational Device Exemption
IND	Investigational New Drug
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
MSHQ	Male Sexual Health Questionnaire
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
UAP	Unanticipated Problem
WCM	Weill Cornell Medicine

Protocol Summary

Full Title:	SSRI Impact On Sperm Quality: A Randomized Placebo Controlled Trial
Short Title:	*SSRI Impact on Sperm*
Clinical Phase:	*II*
Principal Investigator:	*Jonathan Gal, MD*
Sample Size:	*N*=45 per arm. 2 arms, placebo and study drug. Thus, N=90 in total.
Accrual Ceiling:	Samples size required is 45 per arm (90 total), however, anticipating a 20% attrition or non-eligibility rate, 108 will be screened and consented.
Study Population:	Healthy men aged 18-65 years of age, with normal semen analyses (semen analyses with at least 5 million sperm) and no psychiatric history of depression, bipolar, mania or suicidal ideation. These men must be willing to engage in at least weekly sexual activity, with a partner or alone for the duration of the 10- week study
Accrual Period:	We anticipate accrual will take 1 year with 9 patients per month.
Study Design:	Double-blind placebo-controlled randomized trial of daily escitalopram for 6 weeks in healthy men. Hormone profiles, semen analysis and sperm DNA fragmentation, and sexual function will be measured at baseline, after 6 weeks of therapy, and 4 weeks after discontinuation of therapy (10 weeks into study).
Study Duration:	Total length for each subject will be 10 weeks. This includes Baseline, 6-week assessment and 10 week assessment. The full study will be 78 months long, from June 2017 through December 31, 2028.
Study Agent/ Intervention Description:	Escitalopram 10mg by mouth daily for 6 weeks or a matched placebo control by mouth for 6 weeks.
Primary Objective:	The primary objective of this analysis is to determine the negative effect of Escitalopram on sperm DNA fragmentation. Specifically, to determine the proportion of patients who convert from normal to abnormal DNA fragmentation levels as

	measured by TUNEL (abnormal defined as >7%) following 6 weeks of escitalopram therapy.
Secondary Objectives:	Secondary objectives will include assessing absolute change in DNA fragmentation via TUNEL assay from baseline to 6 weeks and 10 weeks. Other objectives include: changes in semen analysis parameters (motility, progressive motility, viability, and concentration) by bright field microscopy from baseline to 6 and 10 weeks.
Exploratory Objectives:	Exploratory objectives include assessing for change in serum testosterone, LH, FSH, estrogen, prolactin, as well as sexual and erectile function using the International Index for Erectile Function (IIEF), Male Sexual Health Questionnaire (MSHQ) questionnaires from baseline to 6 and 10-week time points.
Endpoints:	The primary endpoint will be the proportion of subjects treated with Escitalopram that develop an abnormal TUNEL (>7%) following 6 weeks of therapy compared to placebo subjects.

SCHEMA


Time Point 0
•Patient screened & meets inclusion criteria
•Consented
•Semen Analysis, TUNEL, Serum blood test, IIEF & MSHQ questionnaires
•Randomized to Placebo or Escitalopram arms
•Receives pills per respective group
Time Point: 6 weeks
•Semen Analysis, TUNEL, Serum blood test, IIEF & MSHQ questionnaires
•Interviewed for adverse events
•Completion of intervention drug (placebo or escitalopram)
Time Point: 10 weeks
• Final Follow up
• Semen analysis, TUNEL, IIEF & MSHQ questionnaires
• Interviewed for adverse events or withdrawal symptoms

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1. Study Objectives

To determine if 6 weeks of Escitalopram therapy increases DNA fragmentation and decreases sperm quality compared to placebo.

1.1 Primary Objectives

To assess the proportion of patients who convert from normal to abnormal DNA fragmentation levels as measured by TUNEL (abnormal defined as >7%) following 6 weeks of Escitalopram therapy.

1.2 Secondary Objectives

To assess the proportion of patients who convert from abnormal to normal DNA fragmentation levels as measured by TUNEL (abnormal defined as >7%) 4 weeks after discontinuing 6 weeks of Escitalopram therapy (10 week visit vs 6 week visit). Other objectives include to assess the absolute change in DNA fragmentation via TUNEL assay from baseline to 6 weeks and 10 weeks. Other objectives include: changes in semen analysis parameters (motility, progressive motility, viability, and concentration) by bright field microscopy from baseline to 6 and 10 weeks.

1.3 Exploratory Objectives

To assess for change in serum testosterone, LH, FSH, estrogen, and prolactin from study baseline to 6 week duration. Further, to assess sexual and erectile function using the IIEF, MSHQ questionnaires from baseline to 6 weeks of treatment and after 4 weeks of discontinuation of therapy (10-weeks total study time).

2. Background

2.1 Disease

In an early series, 2 patients initially on antidepressants with infertility were found to have improved semen analysis (SA) parameters 1-2 months following cessation of therapy. In the first patient taking citalopram, he had marked oligospermia and 1% motility; 1 month after cessation of citalopram, his SA parameters were normal. He was then started on bupropion, which induced a decrease in concentration to 21million/ml, 10% motility, and he had a high DNA fragmentation of 76%. One month following bupropion cessation, his SA improved with 75% motility, concentration of 41million/ml which was stable at 2-months post cessation of citalopram. The second patient was taking sertraline and found to have a concentration of 20,000 with no motile sperm. 3 months after cessation of sertraline, all parameters were normal with 40 million total motile sperm. With bupropion added after this, then venlafaxine for refractory symptoms, SA revealed no motile sperm. One month following cessation of these medications SA results again normalized[1]. Similarly, a report of a 30 year old man on

citalopram discontinued the SSRI with improvement of concentration, motility, progressive motility and normal morphology at the first time point 4 months following cessation of SSRI[2].

In a cohort-control study, 74 fertile but depressed men on SSRI's were compared to 44 healthy fertile volunteers. Semen analyses were performed revealing sperm concentration of 61.2+/-11.4 million vs 186+/- 31.4 million in SSRI and controls respectively, 48.2%+/-4.6% vs 66.2%+/-4.4%, normal morphology in 19% vs 52.3%, DNA fragmentation rate by SCSA of 43.2% +/-11.4% vs 21.4% +/-10.6% respectively. A variety of SSRI's were used by the patient population including citalopram, escitalopram, fluoxetine, paroxetine, sertraline and no differential correlations were made by SSRI type. A correlation was also present between SSRI duration and effect on SA parameters[3].

In a prospective series of 35 patients, healthy men provided a SA prior, during and 1 month after a 5-week duration of paroxetine. DNA fragmentation using TUNEL was measured at baseline and after 4 weeks of paroxetine; TUNEL was higher on paroxetine (30.3%) compared to baseline (13.8%), with an associated OR of 9.33 (95% CI, 2.3-37.9). SA parameters such as volume, concentration, motility, and morphology did not change with therapy. Testosterone decreased form 844ng/dL to 605ng/dL[4]. Another series of 25 patients with premature ejaculation (PE) received a 12 week intervention of escitalopram. After 3 months of therapy, sperm concentration (68+/-27.1 vs 26.4 +/-16.1 million), motility (58.2+/5.2 vs 23.4+/-7.5%), and normal morphology (19.2+/-4.8 vs 7.3+/-3.3%) decreased compared to normal values[5].

In a single blinded clinical trial of 60 men with premature ejaculation, men were randomized into sertraline or behavioral therapy. Men in the sertraline group demonstrated decreased sperm concentration (118.61+/-54.47 vs 147.47+/-75.73 million/ml) compared to baseline, as well as reduced normal morphology (24.80+/-3.28 vs 44.19+/-3.94 %) and had higher DNA fragmentation (31.10+/-3.22 vs 15.73+/-3.50%) using the SCD method[6].

SSRI & Sperm Effects in the Lab

SSRI's have been postulated to potentially impair spermatogenesis through central actions as well as exerting effects on spermatozoa. SSRI's have been demonstrated in a rat model to induce secretion of prolactin in female rats prior to ovulating due to inhibition of dopamine[7]. A human study demonstrated that fluoxetine potentiates a 5-HT induced increase in plasma cortisol and prolactin secretion among those with depression or OCD[8]. Hyperprolactinemia is thought to decrease GnRH secretion via negative feedback, leading to decreased LH & FSH secretion from the anterior pituitary, which are integral in promoting and maintaining spermatogenesis[9].

SSRI's have also been demonstrated to bind sulfhydryl groups, which exist on spermatozoa[10]. This may have implications in sperm capacitation because sulfhydryl carrying proteins have been shown to be important during the process of sperm capacitation[11]. An in vitro experiment exposed semen to varying concentrations of SSRI's, each fluoxetine, sertraline, fluvoxamine, paroxetine and citalopram demonstrated spermicidal activity[12]. Mice given citalopram doses

also demonstrated increased DNA fragmentation among sperm[13]. Mice given fluoxetine in escalating doses demonstrated decreased sperm count and motility as well as increased sister-chromatid exchanges[14]. Rats given fluoxetine for 60 days demonstrated decreased sperm counts, motility, testicular weights, decreased testosterone and FSH levels[15].

2.2 Investigational Agent or Device

Escitalopram is commonly used as an antidepressant and anxiolytic. Dosage is between 10 and 20mg daily[16]. The effects of escitalopram on sperm and fertility are largely unknown, but limited data available is mentioned above. Risks and side effects related to Escitalopram include: nausea (17%), sweating (8%), diarrhea (7%), insomnia (7%), somnolence (5%), erectile dysfunction (5%), dry mouth (5%), rhinitis (5%), upper respiratory tract infections. The rate of withdrawal of Escitalopram is 17.3%[18]. Common symptoms of withdrawal of similar SSRI type drugs include: flu-like symptoms, fatigue, weakness, tiredness, headache, tachycardia, dyspnea, gait instability, ataxia, dizziness, light-headedness, vertigo, paresthesia, and electric shock sensations. Withdrawal symptoms may last days to weeks[19]. Antidepressants of the SSRI class have been shown to increase the risk of suicidal attempts among depressed patients[20]. Escitalopram is absolutely contraindicated in combination with the use of monoamine oxidase inhibitors (MAOIs)[16].

Lexapro (Escitalopram) is an FDA approved drug that is being used off-label for research purposes to investigate effects on sperm quality in healthy men with no depression. As no changes in labelling are being requested (ie. no change in dose, route of administration, or population) researchers are requesting an IND exemption.

2.3 Rationale

SSRI medications, specifically escitalopram is a very commonly prescribed medication among men of reproductive age. Significant evidence exists that they may be harmful for paternal fertility potential in both animal and human studies. However, high quality data is lacking, particularly among commonly used SSRI's such as escitalopram. As such, it is important to properly evaluate the potential effect of escitalopram in a randomized placebo controlled fashion. Results will be important in guiding urologists, psychiatrists and family practitioners regarding discussion surrounding SSRI use in their patients interested in fertility.

2.4 Risk/Benefit Assessment

Risks to the subjects largely include side effects of Escitalopram as well as the potential for withdrawal symptoms. Side effects include: nausea (17%), sweating (8%), diarrhea (7%), insomnia (7%), somnolence (5%), erectile dysfunction (5%), dry mouth (5%), rhinitis (5%), upper respiratory tract infections. Withdrawal symptoms include: flu-like symptoms, fatigue, weakness, tiredness, headache, tachycardia, dyspnea, gait instability, ataxia, dizziness, lightheadedness, vertigo, paresthesia and electric shock sensations. Withdrawal symptoms may last days to weeks[19]. Antidepressants of the SSRI class, like Escitalopram, have been shown to increase the risk of suicidal attempts among depressed patients[20]. Other risks to subject are

minimal and include discomfort and potential bruising associated with venipuncture for serum hormone testing.

The potential benefit to the subject includes the financial stipend per each study visit and the benefit of a fertility evaluation at no cost to the patient. More significantly, the benefit in knowledge will contribute the highest quality data in shaping care in future patients and clinical care pathways and guidelines. SSRI's and specifically Escitalopram are very commonly used among men of reproductive ages. Thus, these results may help physicians and care providers appropriately counsel couples who may be taking antidepressants such as Escitalopram regarding the potential risks. These findings will be published in an internationally accessible journal to help shape care globally.

3. Subject Selection

3.1 Study Population

Healthy men aged 18-65 years of age, with normal semen analyses, or semen analyses with at least 5 million sperm, with no psychiatric history of depression, bipolar, mania, or suicidal ideation. These men must be willing to engage in at least weekly sexual activity, with a partner or alone for the duration of the 10-week study.

3.2 Inclusion Criteria

1. Healthy men aged 18-65 years of age
2. Normal semen analyses, or semen analyses with at least 5 million sperm
3. Willing to engage in at least weekly sexual activity, with a partner or alone for the duration of the 10-week study

3.3 Exclusion Criteria

1. Azoospermia or severe oligospermia
2. Presently attempting to conceive pregnancy
3. Sexual dysfunction preventing ability to provide semen analysis throughout study or engage in weekly sexual activity
4. Current psychiatric disorder including: bipolar, mania, depression, generalized anxiety, social phobia, panic attacks, obsessive compulsive disorder, and schizophrenia.
5. Family history of bipolar disorder, or suicide (including 2nd degree relatives)
6. Present use of psychotropic agents (prescription or herbal) or anticonvulsants
7. Use of sleeping pills.
8. Alcohol consumption greater that 2oz/day
9. Use of illicit drugs
9. Inability to read, follow instructions or complete questionnaires in English.
10. Use of hormonal medications in past 3 months (androgens, androgen blockade, anabolic steroids, estrogens, herbal)
11. History of chemotherapy or pelvic radiation in past 12 months
12. Use of Monoamine Oxidase inhibitors (MAOI) or tricyclic antidepressants (TCA) within 14 days
13. Liver disease

4. Registration Procedures

4.1 Patient Registration

Patients will be centrally registered with the Office of Billing Compliance. To register a patient, submit the following documents via the JIRA Registration Process:

- Legible copy of the HRBAF
- First and last page of signed informed consent form

Registration must be completed within 24 hours of the signing of informed consent.

5. Study Procedures

Healthy subjects will be recruited from internet sources and study flyers. Inclusion and exclusion criteria will be screened if agreed. Eligible subjects will be brought into the clinic for a baseline assessment. In short, patients will be given 6 weeks of Escitalopram at baseline and will be evaluated at the end of the treatment. They will again be evaluated after 4 weeks of discontinuation (week 10).

At baseline, subjects will be consented, interviewed for past medical history, and randomly assigned to Escitalopram or placebo treatment groups in a 1:1 ratio using a computer-generated randomization scheme developed by the study statistician. Subjects will be given a 6-week supply of their study drug which they have been assigned to. They will then have serum testing for testosterone, estrogen and prolactin. Semen specimen will also be collected to perform a semen analysis, and TUNEL assay. Subjects will also complete IIEF and MSHQ questionnaires. Subjects will complete the Ask Suicide-Screening Questionnaire – Suicide Risk Screening Tool to establish a baseline for suicide ideation.

At 1-week, subjects will be called to review adverse events and complete the Ask Suicide-Screening Questionnaire – Suicide Risk Screening Tool.

At 6-weeks, subjects will have just completed their 6-week duration of study drug (Escitalopram or placebo) given at the baseline visit. Subjects will be interviewed for adverse events or signs/symptoms of withdrawal, suicide ideation, and any left-over pills will be counted. Subjects will undergo serum testing for LH, FSH, testosterone, estrogen and prolactin. Semen specimen will also be collected to perform a semen analysis, and TUNEL assay. Subjects will also complete IIEF and MSHQ questionnaires. Subjects will be asked questions from the standardized Ask Suicide-Screening Questionnaire – Suicide Risk Screening Tool to screen for suicide ideation.

At 10-weeks, subjects will be interviewed for adverse events or signs/symptoms of withdrawal and suicide ideation. Subjects will undergo serum testing for testosterone, estrogen and prolactin. Semen specimen will also be collected to perform a semen analysis, and TUNEL assay. Subjects will once again complete IIEF and MSHQ questionnaires. Subjects will be asked questions from the

standardized Ask Suicide-Screening Questionnaire – Suicide Risk Screening Tool to screen for suicide ideation.

5.1 Schedule of Evaluations

Table 1. Schedule of trial events

	Baseline	Wk 1	Wk 6	Wk 10
Drug Administration	X			
Informed consent	X			
Demographics	X			
Medical history	X			
Serum (testosterone, Estradiol, Prolactin, LH, FSH)	X		X	
Semen Analysis	X		X	X
TUNEL Assay	X		X	X
Compliance (pill counting)			X	
Adverse Events Interview		X	X	X
IIEF & MSHQ Questionnaires	X		X	X
Ask Suicide-Screening Questionnaire – Suicide Risk Screening Tool	X	X	X	X

5.1.1 Screening Visit

Screening will take place over the telephone.

- Medical, Psychiatric, Fertility history
- Medication history
- Inclusion and Exclusion Criteria will be reviewed
- Study overview provided.

5.1.2 Treatment Phase

This section will list all of the necessary study procedures by visit day.

5.1.2.1 Visit 1 (baseline)

- Review of inclusion & exclusion Criteria

- Informed Consent
- Serum Testosterone, Estrogen, Prolactin, LH, FSH
- Semen analysis, Sperm TUNEL assay
- MSHQ & IIEF questionnaires
- Medical History assessing for exclusion criteria & contraindications to Escitalopram

5.1.2.2 Telephone call after 1 week of medication

- Assess for medication side effects and suicide ideation

5.1.2.3 Visit 2: 6 weeks (± 7 days)

- Interview for adverse events, suicide ideation, and pill count
- Serum Testosterone, Estrogen, Prolactin, LH, FSH
- Semen analysis, Sperm TUNEL assay
- MSHQ & IIEF questionnaires

5.1.2.4 Visit 3: 10 weeks (± 7 days)

- Interview for adverse events, suicide ideation, and signs or symptoms of withdrawal
- Semen analysis, Sperm TUNEL assay
- MSHQ & IIEF questionnaires

5.2 Treatment Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications for Investigational agent are described in Section 6.

Patients will receive 1 bottle of study pills. These pills will either be Escitalopram 10mg pill, or a matched placebo. A complete supply of: 2 pills daily for 4 weeks and 1 pill daily for the final 2 weeks (week 5 & 6) will be provided to each subject. Escitalopram may be taken with or without food.

5.3 General Concomitant Medication and Supportive Care Guidelines

Escitalopram is absolutely contraindicated in patients taking MAOi's. Other agents patients will be warned to avoid include those that may increase SSRI toxicity: alcohol, St. John's wort, pseudoephedrine, zolpidem, sibutramine, lithium and diuretic type medications such as furosemide or hydrochlorothiazide. These may lead to elevated serotonin levels and risk of serotonin syndrome. Drugs at risk of altered metabolism or drug levels include the following: warfarin, digoxin, propafenone, flecainide, propranolol, metoprolol, alprazolam, diazepam, midazolam, triazolam, carbamazepine, cisapride, clozapine, cyclosporine, haloperidol, thioridazine, phenytoin, pimozide,

tramadol, theophylline, arsenic, cimetidine, halofantrine, nilotinib, omeprazole, phenothiazines, aripiprazole, clozapine, and risperidone.

5.4 Duration of Therapy and Criteria for Removal from Study

In the absence of treatment delays due to adverse event(s), treatment may continue for 6 weeks or until one of the following criteria applies:

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable event(s)
- Patient decides to end treatment. Patients will be encouraged here to return for follow up visits if able and willing.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.5 Duration of Follow Up

Patients will be followed for 10 weeks from start of treatment.

6. Dosing Delays/Dose Modifications

Escitalopram 10mg PO daily will be given for 4 weeks. Subjects will then be asked to take Escitalopram 5mg daily to wean the medication for the final 2 weeks of the intervention period. The same procedure will occur for the placebo group. No additional changes will be made to study dose.

7. Pharmaceutical Information

Table 2. Pharmaceutical Information

Group	AGENT(S)	DOSE	ROUTE	Frequency	Duration	Start date
Intervention	Escitalopram	10 mg	PO	Daily	4 weeks	Baseline visit
	Escitalopram	5mg	PO	Daily	2 weeks	5th week of trial
Placebo	Placebo	10mg	PO	Daily	4 weeks	Baseline visit
	Placebo	5mg	PO	Daily	2 weeks	5th week of trial

7.1 Investigational Agent

Escitalopram will be supplied in capsule form. Dose will be 5 mg capsules. No special handling or storage instructions are required.

Escitalopram is absolutely contraindicated in patients taking MAOi's. Other agents with which caution should be taken include those that may increase SSRI toxicity: alcohol, St. John's wort,

pseudoephedrine, zolpidem, sibutramine, lithium and diuretic type medications such as furosemide or hydrochlorothiazide. These may lead to elevated serotonin levels and risk of serotonin syndrome. Drugs at risk of altered metabolism or drug levels include the following: warfarin, digoxin, propafenone, flecainide, propranolol, metoprolol, alprazolam, diazepam, midazolam, triazolam, carbamazepine, cisapride, clozapine, cyclosporine, haloperidol, thioridazine, phenytoin, pimozide, tramadol, theophylline, arsenic, cimetidine, halofantrine, nilotinib, omeprazole, phenothiazines, aripiprazole, clozapine, and risperidone.

7.2 Availability

Escitalopram is commercially available and is supplied to the investigators by Vividus pharmacy.

7.3 Agent Ordering

Escitalopram and matching placebo pills will be ordered from Kenneth Zielinski at Sentrix pharmacy:

Sentrix Pharmacy
3285 W McNab Rd.
Pompano Beach, FL 33069
(855)472-1894

Orders will require at least 10 days to process and ship. Shipments will be prepared and shipped prior to enrollment of the first patient.

7.4 Agent Accountability

Escitalopram Inventory Records – The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of all agents received from Sentrix on a Drug Accountability Record Form (DARF).

8. Correlative/Special Studies

No correlative or special studies will be conducted.

9. Measurement of Effect

Response to therapy will be measured by the sperm DNA fragmentation TUNEL assay. A negative response will be demonstrated by increasing the proportion of men in the Escitalopram group with a TUNEL score (>7%). Other negative responses that will be measured are the percent change in TUNEL score.

9.1 Response Criteria

Number of men converting from a normal TUNEL result (<7%) to an abnormal TUNEL result (> 7%).

9.2 Duration of Response

Duration of overall response: Primary response will be measured at 6 weeks from start of treatment. Duration of response will be assessed at 10 weeks (4 weeks beyond cessation of treatment) to determine if the effects of Escitalopram are still present 4 weeks after discontinuation.

9.4 Other Response Parameters

Additional response parameters include absolute and percent changes in TUNEL DNA fragmentation percentage, as well as, changes in semen analyses parameters such as motility, viability, concentration and total motile count. Further parameters include: serum LH, FSH, testosterone, estrogen and prolactin levels at 6 weeks, as well as questionnaire responses to IIEF and MSHQ at 6 and 10-week study time points.

10. Data Reporting / Regulatory Considerations

10.1 Data Collection

The data collection plan for this study is to utilize REDCap to capture all treatment, toxicity, efficacy, and adverse event data for all enrolled patients.

10.1.1 REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill-Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web- based data-entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted (SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

10.2 Regulatory Considerations

All protocol amendments and consent form modifications will be made by the Principal Investigator.

11. Statistical Considerations

11.1 Study Design/Endpoints

The primary endpoint of this analysis is the proportion of patients who convert from normal to abnormal DNA fragmentation levels as measured by TUNEL (abnormal defined as >7%) following 6 weeks of escitalopram therapy. Patients with normal TUNEL (lower than 7%) will be randomized to escitalopram or placebo. Patients will return to the clinic 6-weeks after randomization (+/- 3 days) and will have their DNA fragmentation re-tested. A Fisher's exact test will be used to compare the proportion of patients whose TUNEL increased after 6 weeks of escitalopram treatment sufficiently to become abnormal (all were normal at baseline), in the treatment and placebo arms. With 45 patients per arm, we will have 88% power to detect a 24% absolute increase in the proportion of patients with a TUNEL becomes abnormal after 6 weeks of escitalopram therapy (5% in placebo and 31% in treatment patients).

Secondary analyses will include comparing the proportion in each arm at week 10 (4 weeks after discontinuation) as well as baseline to 10 weeks. We will also similarly compare baseline to 6week values and 10 week values of semen analysis, testosterone, LH, FSH, estrogen, prolactin, IIEF, MSHQ. Categorical and continuous values will be summarized with proportions and medians, and analyzed with Chi- squared or Fisher's exact and Kruskal-Wallis tests, respectively. Exploratory linear regression analyses of change in TUNEL over 6 weeks will be used to adjust for clinical and demographic variables found to be imbalanced within each arm. All tests will be two-sided with significance considered at the 0.05 level. All analyses will be performed using R 3.2.3.

11.2 Sample Size/Accrual Rate

Sample size has been calculated to be 45 patients per arm, 90 total. However, accounting for an attrition rate of 20%, our total number of patients screened is anticipated to be 108. Patients with normal TUNEL (lower than 7%) will be randomized to escitalopram or placebo using varying block sizes (2,4,6). Patients will return to the clinic 6-weeks after randomization (+/- 3 days) and will have their DNA fragmentation re-tested. A Fisher's exact test will be used to compare the proportion of patients whose TUNEL increased after 6 weeks of escitalopram treatment sufficiently to become abnormal (all were normal at baseline), in the treatment and placebo arms. With 45 patients per arm, we will have 88% power to detect a 24% absolute increase in the proportion of patients with a TUNEL becomes abnormal after 6 weeks of escitalopram therapy (5% in placebo and 31% in treatment patients).
We anticipate accruing about 9 patients per month, leading to a 1-year accrual for 108 patients.

11.3 Stratification Factors

Patients will not be stratified.

11.4 Analysis of Endpoints

11.4.1 Analysis of Primary Endpoints

The primary endpoint in this study is the proportion of patients who have abnormal (>7%) TUNEL DNA fragmentation levels after 6 weeks of treatment (all men have normal levels at baseline). Fisher's exact test will be used to estimate 95% confidence intervals for the difference and perform a two-sided test with an alpha level of 5%. Response rates for each arm will also be estimated at this level.

11.4.2 Analysis of Secondary Endpoints

There are two secondary endpoints in this study. First, we will compare the proportion of patients who have abnormal DNA fragmentation levels at 10 weeks (4 weeks following cessation of treatment). Secondly, we will compare the absolute increase in DNA fragmentation percentage across treatment groups at both 6 and 10 weeks. This will be done using a Kruskal Wallis test for location shift as non- normal data are expected.

Exploratory analyses will include comparison across arms of semen parameters including motility, viability, concentration, and total motile count. Comparisons will be done at 6 and 10 weeks (4 weeks post cessation of treatment). Fisher's exact and Kruskal Wallis tests will be used for categorical and continuous variable, respectively. Furthermore, comparisons across arms of serum LH, FSH, testosterone, estrogen and prolactin will be compared at baseline, and 6 weeks. Responses to both the IIEF and MSHQ questionnaires will be compared at baseline to 6 and 10 weeks.

11.5 Interim Analysis

No interim analysis is planned for this study.

11.6 Reporting and Exclusions

11.6.1 Evaluation of toxicity

All patients will be evaluable for toxicity from the time of their first treatment with Escitalopram. Patients will be asked to report any adverse events experienced at each follow up visit. The research team will call the patient after 1 week of therapy to ensure they are tolerating the drug. Counts and rates of all adverse events will be reported in the final analysis and SAEs reported immediately as required.

11.6.2 Evaluation of response

The primary analysis will include all patients randomized for whom 6-week data are available. Additional per-protocol analyses may be done, excluding patients who have missed 7 or more days of treatment, determined either by self-report or pill count.

12. Adverse Event Reporting Requirements

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safe use of the drug or device under investigation. Safety will be monitored by evaluation of adverse events reported by patients or observed by investigators or research staff, as well as by other investigations such as clinical laboratory tests, x-rays, electrocardiographs, etc.

12.1 Adverse Event Definition

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

12.1.1 Investigational Agent or Device Risks

Adverse events for Escitalopram that have been previously described include the following: Risks and side effects related to Escitalopram include: nausea (17%), sweating (8%), diarrhea (7%), insomnia (7%), somnolence (5%), erectile dysfunction (5%), dry mouth (5%), rhinitis (5%), upper respiratory tract infections. Patients may also have symptoms of withdrawal: flu-like symptoms, fatigue, weakness, tiredness, headache, tachycardia, dyspnea, gait instability, ataxia, dizziness, light-headedness, vertigo, paresthesias, electric shock sensations, myalgias, neuralgias, tinnitus, altered taste, pruritis, visual changes, blurred vision, tremor, myoclonus, ataxia, muscle rigidity, jerkiness, muscle aches, facial numbness, sweating, flushing, chills insomnia, vivid dreams, nightmares, hypersomnia, lethargy, nausea, vomiting, diarrhea, anorexia, abdominal pain, anxiety, agitation, tension, panic, depression, intensification of suicidal ideation, irritability, impulsiveness, aggression, anger, bouts of crying, mood swings, de- realization and depersonalization, visual and auditory hallucinations, confusion, decreased concentration, amnesia, genital hypersensitivity, premature ejaculation. Withdrawal symptoms may last days to weeks[19]. Antidepressants of the SSRI class have been shown to increase the risk of suicidal attempts among depressed patients[20]

12.1.2 Adverse Event Characteristics and Related Attributions

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Attribution of the AE:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely – The AE is doubtfully related to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

12.1.3 Recording of Adverse Events

All adverse events will be recorded on a patient specific AE log. The AE log will be maintained by the research staff and kept in the patient's research chart.

12.1.4 Reporting of AE to WCM IRB

All AEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link:
http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf

12.2 Definition of SAE

SAE's include death, life threatening adverse experiences, hospitalization or prolongation of hospitalization, disability or incapacitation, overdose, congenital anomalies and any other serious events that may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed in this definition.

12.2.1 Reporting of SAE to IRB

All SAEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link: http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf

12.2.2 Reporting of SAE to FDA [For protocols where WCMC is the Sponsor-Investigator]

If an SAE occurs on this study, the event will be filed on a MedWatch form with the FDA. The investigator must notify the FDA of any SAE's as soon as possible but no later than 7 calendar days after the initial receipt of the information

Select appropriate reporting branch below

CDER INDs:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Human Drug information Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

CDER-only Biologic INDs:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biologic Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

12.4 AE/SAE Follow Up

All SAEs and AEs reported during this study will be followed until resolution or until the investigator confirms that the AE/SAE has stabilized and no more follow-up is required. This requirement indicates that follow-up may be required for some events after the patient discontinues participation from the study.

13. Data and Safety Monitoring Plan (DSMP)

In this section, please include a written plan of the measures that will be taken to ensure the safety of clinical research subjects and protect the validity and integrity of research data. The following questions should be addressed as a part of the DSMP and must be incorporated into your WCM eIRB application:

1. We will not use the 'Data and Safety Monitoring Plan' external to the study personnel as the study agent is an FDA approved drug being used off-label and has reported adverse effects such as nausea, fatigue, increased sweating, somnolence (tiredness), diarrhea, dizziness, anorexia (loss of appetite), impotence (unable to get an erection) constipation, weight increase, yawning, decreased libido (desire for sex), unable to ejaculate, suicidal thoughts, and serotonin syndrome. The study agent is also commercially available.
2. Subjects will be interviewed for adverse events and side effects at 6 and 10 week follow ups. They will also be provided with the contact number of study personnel to contact if they are experiencing any unwanted side effects.
3. Serious adverse events that are life threatening or require significant medical or surgical intervention will be considered and reviewed for termination of the study.
4. All serious adverse events as previously identified will be reported within the timeframe mentioned in section 12. Minor adverse events will be reported every 6 months and study end.
5. Monitoring for adverse events will be performed semi-annually.
6. The monitoring entity's comments/review will be disseminated to the IRB at the time of continuing review, at interim analysis and study end.

For additional information and guidance on developing a DSMP, please contact the Quality Assurance Unit (JCTOQAU@med.cornell.edu).

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