Title: A pilot trial of 13-*cis* retinoic acid (isotretinoin) for the treatment of men with azoospermia

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Clinical Phase: II

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List of abbreviations

BMI	Body mass index
BP	Blood pressure
CRF	Case record form
CBC	Complete blood count
CI	Confidence interval
CK	Creatine kinase
FDA	Food and Drug Administration
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
ICSI	Intracytoplasmic sperm injection
IND	Investigational New Drug Application
IRB	Institutional Review Board
LC/MS	Liquid chromatography/mass spectroscopy
LH	Luteinizing hormone
MedDRA	Medical Dictionary for Regulatory Activities
NICHD	National Institute of Child Health and Human Development
OAT	Oligoasthenoteratozoospermia
PSA	Prostate Specific Antigen
SAE	Serious adverse event
T	Testosterone
TESE	Testicular sperm extraction

Synopsis

Study Title	A pilot trial of 13-*cis* retinoic acid for the treatment of men with azoospermia
Study Phase	II
Sponsor	National Institute of Child Health and Human Development
Study Drug	13-*cis* retinoic acid
Objective	**Primary Objective**: To determine the effect of 13-*cis* retinoic acid administration on the appearance of sperm in the ejaculate among men presenting with azoospermia **Approach:** Twenty men with infertility, aged 21-60, due to azoospermia (no apparent sperm in the ejaculate on two separate occasions) will be enrolled in a single-arm pilot trial of daily oral therapy of 20 mg twice daily of 13-*cis* retinoic acid for 32 weeks. The impact of treatment on the appearance of sperm in the ejaculate will be determined by monthly semen analyses. **Secondary Objectives**: • To determine the side effects associated with treatment with 13-*cis* retinoic acid
PI	Dr. John K. Amory MD, MPH
Rationale	Men with infertility from azoospermia and normal gonadotropins have few options for treatment. Recent research has demonstrated that lower intratesticular concentrations of 13-cis retinoic acid are associated with abnormal sperm production. Older studies of 13-cis retinoic acid administration to normal men demonstrated increases in sperm concentrations. Our recent pilot study in men with oligoasthenozoospermia demonstrated increased sperm production in roughly ½ of men with treatment, including several who started with sperm counts very close to zero. However, the effect of 13-cis retinoic acid on sperm concentrations in men with azoospermia has never been studied. If 13-cis retinoic acid allowed men with azoospermia to produce ejaculated sperm, it could help men with azoospermia father pregnancies using ICSI without the need for testicular biopsy or testicular sperm extraction (TESE).

Study Design	We will conduct an unblinded, pilot study to determine the impact of therapy with 13-*cis* retinoic acid on sperm indices in azoospermic men. Twenty azoospermic men, ages 21-60 will be enrolled in a 32-week study of 20 mg twice daily of 13-*cis* retinoic acid. The subjects will be closely followed for side effects related to treatment. The impact of treatment on the appearance of sperm in the ejaculate will be determined by monthly seminal fluid analyses. All aspects of this study will be performed in compliance with Good Clinical Practice (GCP) regulations, ICH guidelines, the Declaration of Helsinki, and under an FDA Investigational New Drug (IND) application.
Number of Subjects	Twenty men.
Duration of Trial	Enrollment should be completed in about 18 months, resulting in total study duration of about 36 months including screening, treatment phase, end of study visit procedures, close out and data analysis.
Duration of Treatment	The active treatment phase will be 32 weeks.
Dosage and Regimen	13-*cis* retinoic acid at 20 mg twice daily with meals for 32 weeks.
Inclusion Criteria	Subjects will be infertile men with azoospermia on at least two semen analyses separated by at least one week and no pregnancy with partner with normal cycles and normal hysterosalpingogram despite >1 year of unprotected intercourse.
Exclusion Criteria	Exclusion criteria include: hypogonadotropic hypogonadism (that might respond to gonadotropin injections), the use of anabolic steroids, illicit drugs, or the consumption of more than 4 alcoholic beverages daily, severe mental health problems, or current therapy with retinoic acid (e.g. Accutane) or vitamin A.
Efficacy Parameters	The appearance of sperm in the ejaculate during treatment
Safety Parameters	• CBC, clinical chemistry panel (glucose, liver and renal function tests including urea, creatinine, albumin, calcium alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin), CK, full lipid panel

	including: Total cholesterol, HDL, LDL and fasting triglycerides at baseline, week 8, 16, 24 and 32 of treatment, and study exit. Additional measures will be conducted if any abnormal values are found until the end of study visit. • Adverse event and concomitant medications throughout the study. Other safety parameters include vital signs; and changes in pre- and post-treatment physical examination results and score on the PHQ9 depression questionnaire.
Evaluations	The following determinations will be made in all subjects: • Semen analysis will be collected twice during screening and every four weeks during treatment and twice during recovery • Serum samples will be collected for the measurement of: vitamin A, all-trans and 13-cis retinoic acid, testosterone, FSH and LH. Serum for these measures will be collected before the beginning of the treatment, and every two months during treatment and sy study exit.
Other Assessments	Demographic characteristics, including age and race, will be collected.
Statistical Analysis	The primary endpoint is the appearance of sperm in the ejaculate of treated men between baseline and 32 weeks. This analysis will be conducted using a Wilcoxon signed-rank test.

1.0 Introduction and Background

Infertility affects 10-15% of all couples, with 1.5 million couples seeking medical assistance for infertility yearly (1). Infertility attributable to the male partner accounts for 30-40% of all cases of infertility. The most common form of male infertility involves some type of impairment in spermatogenesis (2). Unfortunately, over 80% of men with infertility from impaired spermatogenesis do not have a medically treatable cause, such as a gonadotropin deficiency (3). In men with idiopathic infertility, surgical sperm extraction from the testes coupled with *in vitro* fertilization or intra-cytoplasmic sperm injection offers some hope of fertility; however, these procedures are invasive, expensive, unsuccessful in 30-40% of cases, and don't address the underlying cause of infertility (4). Therefore, new approaches to the treatment of male infertility are sorely needed.

The essential role of vitamin A (retinol) in spermatogenesis has been long appreciated. Vitamin A deficient male mice are sterile due to impaired spermatogenesis and supplementation of deficient mice with vitamin A restores fertility (5, 6). Vitamin A is required for spermatogenesis as it is converted to retinoic acid in the seminiferous tubules via the activity of testes-specific retinol and retinal dehydrogenases. Retinoic acid is known to mediate most of the effects of vitamin A in tissues. In the testes, two retinoic acid receptors, α and γ, are present in both Sertoli cells and developing germ cells (7-10), and targeted deletion of these receptors in mice results in male infertility (11-13). From this work, it is clear that retinoic acid plays several essential roles in spermatogenesis, including necessary functions in spermatogonial differentiation, spermatid adhesion to Sertoli cells and spermiation (14). Given the crucial role of retinoic acid in spermatogenesis, it seems quite possible that some men with "idiopathic" infertility have intratesticular concentrations of retinoic acid below those necessary to initiate and/or maintain optimal spermatogenesis. In theory, poor dietary intake of vitamin A could lead to infertility; however, a nutritional cause of infertility seems improbable in the U.S.

More likely, low intratesticular concentrations of retinoic acid could occur in infertile men either due to impaired retinoic acid biosynthesis from vitamin A or increased metabolism of retinoic acid to inactive metabolites within the testes.

Despite the known importance of retinoic acid for spermatogenesis in animals, there has been relatively little work examining the role of retinoic acid on spermatogenesis in humans. Intriguingly, there is a suggestion that the administration of retinoic acid to men may improve sperm production. During the development of 13-cis retinoic acid (Accutane) for acne, three human studies examining the effect of oral administration of 13-cis-retinoic acid on sperm production in men were performed to determine if 13-cis retinoic acid was harmful to male fertility. Interestingly, men in these studies had increased sperm concentrations during treatment (see below), suggesting that 13-cis retinoic acid administration enhanced spermatogenesis in these normal men with acne (15-17). We recently completed a pilot study in 20 men with infertility from oligoasthenoteratozoospermia. In this study, roughly ½ of the men experienced significant improvements in their sperm production, suggesting this treatment is useful for men with oligoasthenoteratozoospermia. In this proposal, we will perform a clinical trial of retinoic acid administration to infertility patients with azoospermia to determine whether retinoic acid therapy can initiate sperm production in men with azoospermia. If the administration of retinoic acid were shown to improve spermatogenesis in some infertile men with azoospermia, it would have tremendous significance for our current approach to the treatment of men with infertility who could avoid the need for testicular biopsy and/or testicular sperm extraction. Because of its widespread use in humans for over 25 years, 13-cis retinoic acid is widely available in a generic formulation and could be quickly incorporated into male infertility treatment algorithms. Ideally, retinoic acid therapy would allow a man to make sufficient sperm for ICSI, dramatically increasing his chances of fathering a pregnancy.

Objectives

2.1 Primary Objective:

To determine the effect of 13-*cis* retinoic acid administration on the appearance of sperm in the ejaculation in men with azoospermia.

2.2 Secondary Objectives:

- To determine the side effects and tolerability of treatment with 13-*cis* retinoic acid in men with azoospermia

3. Rationale, Benefits and Risks

Male infertility is common and difficult to treat unless men have an easily identified gonadotropin deficiency. Many men have idiopathic infertility. We have shown that men with abnormal spermatogenesis have lower than normal levels of intratesticular retinoic acid, suggesting that intratesticular retinoic acid deficiency is associated with infertility, and have performed a pilot study in men with idiopathic oligoasthenozoospermia demonstrating that half of these men increase their sperm production on treatment. In this study, we aim to determine if retinoic acid therapy can convert men with azoospermia into men with oligozoospermia. The medication, 13-*cis* retinoic acid, is widely available and prescribed for the treatment of acne, suggesting that serious adverse events are unlikely, especially at the low doses to be tested.

4. Compliance

All aspects of this study will be performed according to Good Clinical Practice GCP regulations, the Declaration of Helsinki and ICH guidelines, under an active FDA Investigational New Drug (IND) (#120703).

5. Study Design

We will conduct an open-label pilot trial to determine the impact of therapy with 13-*cis* retinoic acid on sperm indices in infertile men. Twenty infertile men with azoospermia will be enrolled in a 32-week study of 20 mg twice daily of 13-*cis* retinoic acid. The subjects will be closely followed for side effects related to treatment. The impact of treatment on indices of spermatogenesis will be determined by monthly seminal fluid analyses.

6. Duration of the Study

Subjects: Subjects will undergo a screening phase of up to 60 days. The first 20 subjects meeting the enrollment criteria will be enrolled. The treatment phase will last for 32 weeks and subjects will have two post-treatment visits, about 12 and 24 weeks after the end of treatment.

Enrollment should be completed in approximately 18 months, resulting in total study duration of about 36 months including screening, treatment phase, end of study visit procedures, close out and data analysis.

7. Number of Subjects

20 men will participate in the study. We may need to screen up to 50 men to find 20 men eligible to participate.

8. Dosage and Administration

13-cis retinoic acid at 20 mg twice daily (purchased commercially) with meals

9. Selection of Subjects

9.1.1 Inclusion Criteria

1. Subjects will be infertile men (no pregnancy with partner with normal cycles and normal hysterosalpingogram despite >1 year of unprotected intercourse).
2. Azoospermia (no sperm apparent in centrifuged semen) as assessed by semen analysis on two occasions separated by one week.
3. In the opinion of the investigator, is able to comply with the protocol, understand and sign an informed consent and HIPAA form.

9.2 Exclusion Criteria

Men who meet any of the following criteria are NOT eligible for enrollment in the trial:

1. Men participating in another clinical trial
2. Men not living in the catchment area of the clinic
3. Clinically significant abnormal findings at screening
4. Hypogonadotropic hypogonadism (that might respond to gonadotropin injections),
5. The use of anabolic steroids, illicit drugs, or the consumption of more than 4 alcoholic beverages daily
6. Severe mental health problems requiring medications
7. Current therapy with retinoic acid (e.g. Accutane) or vitamin A
8. Score of greater than 15 on the PHQ9 (mood) questionnaire
9. Abnormal serum chemistry values according to local laboratory normal values which indicate liver or kidney dysfunction. Other abnormal lab values may also be exclusionary, at the discretion of the investigator
10. Men with a personal history of serious psychiatric disorders
11. Men currently receiving tetracycline containing medications
12. Men currently receiving phenytoin
13. Men with a history of inflammatory bowel disease
14. Men with a history of bone disease
15. Men who have used isotretinoin within eight weeks of the start of dosing
16. Men with elevated serum triglycerides

10. Concomitant Treatment

Concomitant medications that are exclusionary include:

- Use of sex hormones for treatment
- Use of androgens or other compounds for body building
- Use of retin-A or Accutane for the treatment of acne

Concomitant medications are discouraged except as prescribed for the treatment of co-exising medical conditions. All concomitant treatments will be recorded on the subject's case record form (CRF), including the generic name of the drug, start and stop dates, and reason for use.

11. Study Materials

11.1 Study Medication

Each subject will receive the study drug Isotretinoin 20 mg twice daily for 32 weeks. The first dose will be administered at the clinic under the observation of the study staff. The day and time of the start of treatment will be recorded on the CRF in the appropriate space provided. The subjects will be given enough supply of medication until their next visit.

A Study drug log (diary) will be provided to the subjects for recording the times of taking study medication. These drug logs will be reviewed at each visit. The investigator will retain the copies of these logs as source documents.

The prescriber Dr. Amory, is registered in iPLEDGE. All subjects will be registered in iPLEDGE and undergo the extensive counselling recommended by this program.

11.2 Storage

Study drug will be stored at a temperature not to exceed 25^{o}C in a locked storage area at the study site. The study drug will be accessible only to study personnel designated to handle study drug.

11.3 Disposal

Empty used pill bottles will be returned to the clinic and retained at the site in a locked storage area. All unused study drug will be returned to the University of Washington investigational pharmacy for destruction once final drug accountability has been performed.

12. Study Procedures

12.1 General

The study will consist of 3 periods:

1. Pre-treatment period (including screening) for up to 60 days.
2. Treatment period for 32 weeks
3. Recovery period of 24 weeks

The treatment period will begin with Day 1 of treatment, twice daily for 32 weeks. The recovery period will last for 24 weeks.

12.2 Laboratory Samples

Blood samples for laboratory analyses will be collected at the first screening visit, and at the treatment visits on weeks 8, 16, 24, 32 and 56 with the subject in a fasting state (no food or drink other than 4 to 6 ounces of water or non-sweetened clear liquids for a minimum of 8 hours). A snack will be provided after the blood is drawn. If the subject is in a non-fasting state, the investigator will reschedule the visit within two days. The investigator will review all laboratory reports and file a copy with each subject's chart and CRFs. The CBC and blood chemistry samples will be analysed at the local hospital laboratory at the University of Washington.

The serum samples for measurements of retinoic acid will be frozen for analysis at the end of study. In terms of processing, the blood samples will be collected into vacutainers (containing serum separating tube gel and clot activator) wrapped aluminum foil to prevent light exposure that can degrade retinoic acid,, and gently mixed. The samples will be allowed to clot for 30 minutes at room temperature before centrifugation at 1000-2000g for 15 minutes. Following centrifugation, the serum samples will be frozen in (light protection?) tubes at –80 C.

Semen Analysis: Semen will be collected after at least 48 hours of abstinence from ejaculation at the two screening visits, treatment visits 2-9, and all follow up visits. The semen will be assessed for the presence of sperm, and, if present, for count and motility. In addition, a known volume of semen will be diluted and washed for making smears for morphology assessments. These

procedures allow for accurate total sperm count and motility assessments following the WHO protocol (18).

12.3 Visits in Pre-treatment period

All visits will be scheduled between 7:00am and 3:00pm.

12.3.1 Screening Visit 1

The following screening procedures will be performed at the first screening visit:

- Each participant must sign an IRB-approved informed consent form before any of the study-related procedures can be performed. The original signed informed consent form will be kept on file by the investigator with the subject's record and a copy will be given to the subject.
- Subjects will also complete an Isotretinoin-specific consent form as provided by the iPLEDGE program
- A general medical history will be taken and will include information on current and previous diseases and treatment.
- Vital signs (pulse, blood pressure) will be taken and recorded.
- Complete physical exam, including measurement of testicular volume, will be performed.
- Fasting blood samples will be obtained for the following measurements: Complete blood count (CBC), clinical chemistry including glucose, liver and renal function tests (urea, creatinine, albumin, alanine aminotransferase, aspartate aminotransferase, gamma glutamine transferase, alkaline phosphatase), creatine kinase (CK), bilirubin, albumin, full lipid panel including total cholesterol, HDL, and LDL, triglycerides, hematology (hematocrit, hemoglobin), and hormones (LH, FSH, T)
- Semen analysis will be done to determine if sperm are present
- Height and weight will be collected
- Participants will be questioned regarding past? And concomitant medications and adverse events.
- Once lab results are obtained and considered consistent with idiopathic infertility from azoospermia by the investigator as per the inclusion/ exclusion criteria for enrollment, the

participant is informed by telephone of his lab results. If conformance to the inclusion/exclusion is confirmed, he is scheduled to return to the clinic for the 2nd sperm collection one week later.

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12.3.2. Screening Visit 2

Vital signs (pulse, blood pressure) will be taken and recorded.

- Participants will be questioned regarding concomitant medications and adverse events.

  If this 2nd sperm collection also meets enrollment criteria, the subject is scheduled to begin on treatment.

- If any results are abnormal, according to local laboratory standards and in the clinical judgment of the investigator, the subject will be informed and excluded from the study and referred to his primary physician.
- Subjects will be informed that they cannot donate blood during treatment and for 12 weeks after treatment is completed.

12.4 Visits in treatment period

Nine visits will occur in the treatment period: week 0, week 4, week 8, week 12, week 16, week 20, week 24, week 28 and week 32. All visits are ± 6 (does this include one week later?) eg following Tuesday?days.

12.4.1 Visit 1 (week 0)

This is the day 1 of treatment. The following will be done at this visit:

- Vital signs (pulse, blood pressure) and weight will be taken and recorded.
- Subjects will be questioned regarding concomitant medications and adverse events. If the subject is taking a medication that is listed as disallowed the subject should not be enrolled in the study.
- The subject will complete the PHQ9 questionnaire (see appendix 5)
- Subjects will be given enough supply of study medication for 30 days. This doesn't allow enough medication, in case subject has to come in at week 4 + 6 days.

- A study drug log will be dispensed to record the date and time of administration of the study drug. Subjects will be instructed to take the study drug twice a day, except on the day of the visit. Subjects will be scheduled to return to the clinic after 28 +/- 6 days.

12.4.2 Visits 2-9 (Treatment Visits)(+/- 6 days)

These visits are for weeks 4, 8, 12, 16, 20, 24, 28 and 32. The following procedures will be done at these visits:

- Vital signs (pulse, blood pressure) and weight will be taken and recorded.
- The subject will complete the PHQ9 mood questionnaire
- Complete physical exam including and testicular examination and volume measurement will be performed.
- Subjects will be questioned regarding concomitant medications and adverse events.
- Any unused study drug counted for drug accountability and returned to subject? At treatment visit 32 or early exit vist, all unused study drug will be collected.
- Study drug log will be reviewed to check the date and time of drug administration. These logs will be collected at each treatment visit.
- A blood sample will be drawn at week 8, 16, 24 and 32. The time of day of the blood draws will be recorded in the source document.
- A semen sample will be obtained for the presence of sperm, and, if present, the measurement of sperm count, concentration, motility and morphology
- Fasting blood samples will be obtained for the following measurements: CBC, clinical chemistry including glucose, liver and renal function tests (urea, creatinine, albumin, calcium, alanine aminotransferase, aspartate aminotransferase, gamma glutamine transferase, alkaline phosphatase, bilirubin, and albumin), creatine kinase, full lipid panel including total cholesterol, HDL and LDL, triglycerides, hematology (hematocrit, hemoglobin), and testosterone, FSH and LH at weeks 8, 16, 24, and 32.
- In addition, blood samples will be collected at each visit for two serum samples to be frozen for the future analysis of serum retinoid levels.
- Subjects will be scheduled to return to the clinic for follow-up visits.
- Subjects will be given the next month's supply of study drug at visits 2-8 (weeks 4, 8, 12, 16, 20, 24 and 28).

12.4.3 Visit 10 and 11 (Follow-up Visits) +/- 6 days

These visits are for weeks 44 and 56. The following will be done at these visits:

- Vital signs (pulse, blood pressure and respiratory rate) will be taken and recorded.
- The subject will complete the mood (PHQ-9) questionnaire.
- Subjects will be questioned regarding concomitant medications and adverse events.
- Fasting blood samples (week 56 only) will be obtained for the following measurements: CBC, clinical chemistry including glucose, liver and renal function tests (urea, creatinine, albumin, calcium, alanine aminotransferase, aspartate aminotransferase, gamma glutamine transferase, alkaline phosphatase, bilirubin, and albumin), creatine kinase, full lipid panel including total cholesterol, HDL and LDL, triglycerides, hematology (hematocrit, hemoglobin), and testosterone, FSH and LH. In addition, two serum samples will be frozen for the future analysis of serum retinoid levels.
- A semen sample will be obtained to ascertain the presence of sperm, and, if present, the measurement of sperm count, concentration, motility and morphology

13. Study Withdrawals

Subjects withdrawn from the study can be replaced only if the withdrawal occurs before the start of the treatment. Subjects who have had study drug will not be replaced, regardless of the reason for discontinuation. All efforts will be made to contact any subject who decides to discontinue study participation before the end of study safety procedures (Visit 11). The necessary clinical and laboratory examinations planned at the last visit should be performed at the end of the study whenever it occurs. The reasons for discontinuation will be documented.

Post-Admission Withdrawal Criteria

Subjects may be discontinued prematurely for any of the following reasons:

- Emergence of a severe condition(s) such that, in the judgment of the investigator, continuation in the trial would negatively impact the health of the subject.
- Personal reasons (*e.g.* withdrawn consent).
- Non-compliance with study drug

- Subject lost to follow-up
- A severe skin reaction judged by the investigator to potentially be related to the study drug
- A persistent elevation of serum triglycerides
- Onset of neutropenia
- New onset of hearing disorder
- New onset of visual changes
- Onset of abdominal pain, rectal bleeding or severe diarrhea
- Persistent, significant increase of liver enzymes or bilirubin
- New onset of significant serum glucose elevation
- New onset of headache associated with nausea and vomiting
- Persistent elevation of serum calcium
- Development of kidney stones
- Increase in the PHQ9 score to >15 (suggestive of moderately severe depression)

14. Statistical Considerations

Data analysis and Interpretation of Results: The primary endpoint is the presence of sperm in the ejaculate at week 32 of treatment compared with its absence at baseline. The presence of sperm after 32 weeks of treatment will be compared with baseline using a Wilcoxon sign-rank test. Linear regression will be performed to determine if significant relationships between baseline (or delta) serum concentrations of 13-cis retinoic acid and changes in sperm quality are present using STATA Version 10.0 (College Park, TX, USA). For all comparisons, an alpha of 0.05 will be considered significant.

14.1 Sample size consideration

Inclusion of 20 men will allow for an 80% power to detect a response rate of greater than 15% in terms of sperm production and allowfor a drop-out rate of 10% at an alpha of 0.05 (The primary outcome).

14.2 Efficacy

The primary efficacy outcome is the presence of sperm in the ejaculate of formerly azoospermic men.

14.3 Safety

Laboratory data, physical examination results, vital signs and chemistry levels will be assessed and compared for each subject from screening to end of study. All adverse events will be recorded and coded using the body system coding of MedDRA.

The incidence of adverse events will be examined by using descriptive statistics. Clinical chemistries and allied data will be examined for change over time.

15. Adverse Events

Adverse Events (AE) will be carefully monitored and an AE form is included in the CRF. Serious adverse events (SAE) as defined below will be reported on the SAE form included as appendix #3.

All adverse experiences must be recorded in the study event record of the subject's CRF, and will include the following information (when applicable):

- Specific condition or event
- Indication of whether the condition was pre-existing or not and if yes, whether it has worsened in severity (including an increase in frequency)
- Date of occurrence
- Date of resolution
- Relationship to study drug as evaluated by the investigator (causality assessment). The investigator must enter their opinion of causality on the AE forms.
- Action taken (study drug continued or not) and outcome
- Seriousness according to the approved regulatory classification {i.e. any event that is fatal, life-threatening, disabling, incapacitating, results in or prolongs hospitalization, or is a medically significant event, e.g. an intervention to prevent one of the above outcomes, or any other serious criteria (cancer, congenital anomaly, overdose, other significant) is considered serious}.

When any serious adverse drug experience, regardless of causality, is encountered during this clinical trial at an investigator's site, the investigator must notify the Study Monitor by facsimile using the form provided as an appendix. This report must be submitted within 2 calendar days from the time the investigator's staff is notified of the event.

All serious adverse events that are unexpected will be reported to the FDA by the principal investigator or his designated surrogate. All serious adverse events will be followed until fully characterized. The investigator will collect and forward to the FDA via fax, all available supporting documentation (with subject name redacted) for serious events, including at a minimum hospital discharge summaries and death certificates (where applicable). Additional supporting documentation that should be collected whenever possible, to verify the medical diagnosis, includes autopsy reports (where applicable), surgical procedure summaries, histology reports, and imaging reports.

16. Ethical Considerations

16.1 Informed Consent

Principal investigators will provide the NICHD with a copy of the Informed Consent approved by their local Institutional Review Board (IRB). The Informed Consent will be translated and certified into the language of the respondent as needed.

Under this consent, the subject shall understand that he is authorizing access to medical records as required for monitors, auditors, IRBs and regulatory authorities.

16.2 Conflicts of Interest

The investigators will not profit from results, either positive or negative, with regard to the product being evaluated.

16.3 Subject Recruitment

The subjects will be recruited from UW and Community Infertility and Urology clinics who see >200 such men annually. The study coordinator will screen potential subjects over the phone by inquiring about their age and medical history. Potential subjects who qualify by phone screen will be scheduled for an appointment to consult with the investigator about further eligibility requirements for the study.

17. Confidentiality

The information on individual subjects arising from this study is to be considered confidential and transmitted to the sponsor only in a form that will not permit identification of the individual. Regulatory and sponsoring agencies may request access to the study records and related medical records of each participating subject, and if requested, the subject's identity will remain confidential to the extent permitted by the applicable laws and regulations. All records will be kept in a secure storage area with limited access.

18. Investigative Record Management

All investigative site records will be kept in a secure storage area with limited access. NICHD should be notified before destruction of any site records.

19. Data Transmission

Not applicable

20. Publication Policy

Data on the use of the study drug and results of all clinical and laboratory studies are considered private and confidential. NICHD will encourage publication of the results of the study.

21. Investigator Documentation

Prior to beginning the study, the investigator will be asked to demonstrate compliance with ICH E6, 8.2 and 21 CFR 312 by providing the following essential documents, including but not limited to:

1. An Institutional Review Board (IRB) -approved Informed Consent (as described in section 18.1) in the local language.
2. Local IRB approval.
3. Form FDA 1572, fully executed, and all updates on a new fully executed Form FDA 1572.
4. Current curriculum vitae (CV) for each principal investigator and each sub investigator listed on Form FDA 1572.

22. References

1. Center for Disease Control. Infertility Facts, acessed at http://www.cdc.gov/nchs/fastats/fertile.htm. On June 3rd, 2013.
2. Jequier AM, Holmes SC. Primary testicular disease presenting as azoospermia or oligozoospermia in an infertility clinic. Br J Urol 1993 71:731-735.
3. deKretser DM. Male infertility. Lancet 1997 349:787-790.
4. Schlegel PN. Nonobstructive azoospermia: a revolutionary surgical approach and results. Semin Reprod Med 2009 27:165-170.
5. Napoli J. Retinoic acid: Its biosynthesis and metabolism. In: "Progress in Nucleic Acid Research and Molecular Biology" (Ed. Moldave K.), Academic Press, San Diego, 2000 63:139-188.
6. Doyle TJ, Braun KW, McLean DJ, Wright RW, Griswold MD, Kim KH. Potential functions of retinoic acid receptor A in Sertoli cells and germ cells during spermatogenesis. Ann N Y Acad Sci 2007 1120:114-130.
7. Koubova J, Menke D, Zhou Q, Capel B, Griswold MD, Page DC. Retinoic acid regulates sex-specific timing of meiotic initiation in mice. Proc Natl Acad Sci USA 2006 103:2472-2479.
8. Anderson EL, Baltus AE, Roepers-Gajadein HL, Hassold TJ, de Rooij DG, van Pelt AMM, Page DC. *Stra*8 and its inducer, retinoic acid, regulate meoitic initation in both spermatogenesis and oogenesis in mice. Proc Natl Acad Sci, USA 2008 105:14976-14980.
9. Chung SS, Wang X, Wolgemuth DJ. Expression of retinoic acid receptor alpha in the germline is essential for proper cellular association and spermiogenesis during spermatogenesis.. Development 2009 136:2091-2100.
10. Dufour JM, Kim KH. Cellular and subcellular localization of six retinoid receptors in rat testis during postnatal development: identification of potential heterimeric receptors. Biol Reprod 1999 61:1300-1308.
11. Lohnes D, Kastner P, Dierich A, Mark M, LeMeur M, Chambon P. Function of retinoic acid receptor gamma in the mouse. Cell 1993 73:643-658.
12. Lufkin T, Lohnes D, Mark M, Dierich A, Gorry P, Gaub MP, LeMeur M, Chambon P. High postnatal lethality and testis degeneration in retinoic acid receptor alpha mutant mice. Proc Natl Acad Sci USA 1993 90:7225-7229.

13. Kastner P, Mark M, Leid M, Gansmuller A, Chin W, Grondona JM, Décimo D, Krezel W, Dierich A, Chambon P. Abnormal spermatogenesis in RAR beta mutant mice. Genes Dev 1996 10:80-92.
14. Ghyselinck NB, Vernet N, Dennefeld C, Giese N, Nau H, Chambon P, Viville S, Mark M. Retinoids and spermatogenesis: lessons from mutant mice lacking the plasma retinol binding protein. Dev Dyn 2006 235:1608-1622.
15. Hoting VE, Schutte B, Schirren C. Isotretinoin and acne conglobata: andrological evaluations (German). Fortschur Med 1992 23:427-430.
16. Vogt HJ, Ewers R. 13-*cis*-retinoic acid and spermatogenesis (German). Der Hautarzt 1985 36:281-286.
17. Torok L, Kadar L, Kasa M. Spermatological investigations in patients treated with etretinate and isotretinoin. Andrologia 1987 19:629-633.
18. Nya-Ngatchou JJ, Arnold SLM, Walsh TJ, Muller CH, Page ST, Isoherranen N, Amory JK. Intratesticular 13-cis Retinoic Acid is Reduced in Men with Abnormal Semen Analyses. Andrology 2013 March; 1: 325-331.
19. WHO. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge, United Kingdom: Cambridge University Press; 1999.

23. List of appendices

1. Schedule of Events
2. PHQ-9 Questionnaire
3. Serious Adverse Event Report Form

Appendix 1: Schedule of Events

		Screening		Treatment		Follow-up	
	Visit #	S1	S2	T1	T2-T9	F1	F2/exit
	Study Day	-60 to -1	-53 to 1	1	Wks: 4, 8,12,16, 20, 24, 28, 32	Wk 44	Wk 56
Administrative	Informed consent/HIPAA	X					
	Vital Signs	X	X	X	X	X	X
Medical	Medical History	X					
	Physical Exam	X		X	X	X	X
	AE & Con Med	X	X	X	X	X	X
	Semen Analysis	X	X	X	X	X	X
	PHQ9 Questionnaire	X		X	X	X	X
Blood Sampling	CBC,Chemistry/Lipids, CK	X		X	X**	X	X
	FSH, LH, T & Retinoids	X		X	X**	X	X
Drug Administration	Dispense Study Meds			X	X*		
	Dispense study med log			X	X		
	Collect and review med log				X		
	Collect unused study meds				X		
Reimbursement ($)		30	20	40	40	30	30

- Visit T9, week 32: No drug dispense **Blood draws only week 8, 16, 24 and 32

- **Abbreviations:**
- HIPAA: Health insurance portability and accountability act
- PHQ9: Patient health questionnaire, #9
- FSH: follicle-stimulating hormone
- LH: luteinizing hormone
- T: testosterone
- AE: adverse event
- Con Med: concomitant medications
- S1/S2: screening visit 1/2
- T1-T7: treatment visits 1-7
- F1/F2: follow-up visit 1/2
- Wk: week

Appendix 2: PHQ9 Questionnaire

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

NAME:_____ DATE:_____

Over the last *2 weeks*, how often have you been bothered by any of the following problems?
(use "✓" to indicate your answer)

	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself—or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed. Or the opposite — being so figety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead, or of hurting yourself	0	1	2	3
		add columns	+	+

(Healthcare professional: For interpretation of TOTAL, please refer to accompanying scoring card). TOTAL:

10. If you checked off *any problems*, how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?	Not difficult at all	_____
	Somewhat difficult	_____
	Very difficult	_____
	Extremely difficult	_____

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Appendix 3: Serious Adverse Event Report Form

SERIOUS ADVERSE EVENT REPORT	CBR SERIAL NO:

I. EVENT INFORMATION

1. PATIENT INITIALS	1.a COUNTRY	2. DATE OF BIRTH Day Month Year	2.a. AGE years	3. SEX	3.a. HEIGHT cm	3.b. WEIGHT kg

7.- 13. DESCRIBE EVENT. ___EXPECTED UNEXPECTED

DIAGNOSIS:

.....

Description:

4.-6. EVENT ONSET
Day Month Year

_____

_____

8.-12. SERIOUSNESS CRITERIA
CHECK ALL APPROPRIATE TO EVENT

___DEATH (DATE)
Day Month Year

___HOSPITALIZATION

___DISABILITY OR INCAPACITY

___LIFE-THREATENING

___OTHER SERIOUS CRITERIA
(Cancer, congenital anomaly, overdose, significant)

___NOT APPLICABLE

II. SUSPECT DRUG INFORMATION

14. SUSPECTED DRUG(S) (include generic name(s))

15. DAILY DOSE (include schedule)	16. ROUTE OF ADMINISTRATION

20. DID EVENT ABATE AFTER STOPPING DRUG?
___YES
___NO
___NOT APPLICABLE

17. INDICATION(S) FOR USE		21. DID EVENT RE-APPEAR AFTER REINTRODUCTION? __YES __NO __NOT APPLICABLE
18. THERAPY DATES (from/to) Day Month Year Day Month Year	19. THERAPY DURATION UNTIL REACTION ONSET	

III. CONCOMITANT DRUGS AND HISTORY

22. CONCOMITANT DRUGS AND DATES OF ADMINISTRATION (exclude those used to treat reaction)

23. OTHER RELEVANT HISTORY (e.g. diseases, allergies, pregnancy etc.)

IV.IND HOLDER INFORMATION

V. INVESTIGATOR'S ASSESSMENT

24.a. NAME AND ADDRESS OF IND HOLDER Population Council Center for Biomedical Research 1230 York Avenue New York, NY 10021 USA Fax:+1(212)327 86 73		23.d. OUTCOME __ Recovered __ Recovering __ Not Recovered __ Sequelae __ Fatal __ Unknown	23.e. CAUSALITY __ Not Related __ Unlikely __ Possible __ Probable __ Highly Probable __ Insufficient Data
BATCH NO. (when relevant)	24.b. PATIENT IDENTIFIER NO		
24.c. DATE RECEIVED Day Month Year	24.d. TRIAL NUMBER	26. NAME AND ADDRESS OF REPORTING PHYSICIAN	
24.e. DATE OF THIS REPORT Day Month Year	25.a. REPORT TYPE __INITIAL __FOLLOW-UP		

Add another page if not sufficient space. If this case is from literature, please attach an original article