**Study Title: Elagolix for Fertility Enhancement Clinical Trial (The EFFECT Trial)**

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## Background, Rationale and Context

Endometriosis is defined as the presence of endometrial glands and stroma outside the endometrial cavity. It affects at least 176 million women worldwide and over 10 million in the U.S.A., result in in an annual U.S. cost of over $22 billion. The impact on IVF has not been well studied, since most women with the disease are not counted, and those included in meta-analyses have already been surgically treated. Recent work from our laboratory has identified two endometrial proteins, BCL6 and SIRT1, that are associated with endometriosis and together cause progesterone resistance, the *sine quo non* of endometriosis (Evers-Hoeker et al 2017; Yoo et al, 2018). The over-expression of BCL6 has been shown to be associated with IVF failure (Almquist 2018; Figure 1) and had a high sensitivity and specificity for detection of endometriosis (Ever-Hoeker et al, 2017). Recurrent IVF implantation failure has also been shown to be treatable with suppression of menses using leuprolide acetate, letrozole, or both together (Surrey et al., 2017; Miller et al., 2012; Steiner et al., 2019). Furthermore, treatment of women with elevated BCL6 with surgery or medical suppression for 2 months prior to embryo transfer (Lupron) improves outcomes compared to controls (Likes et al, 2019).

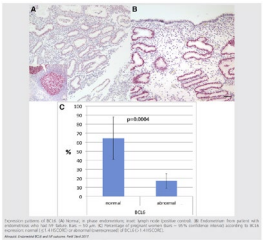

*Figure 1: BCL6 and IVF success. The darker staining for BCL6 provides an indication for the presence of endometriosis. When women entering an IVF cycle were tested, those positive for BCL6 (> 1.4 HSCORE) had a significantly decreased pregnancy rate compared to women with low BCL6.(Almquist et al., 2018).*

Elagolix (Orilissa) is a new generation FDA approved orally active GnRH antagonist that is rapidly reversible, for the treatment of endometriosis and pelvic pain (Taylor et al., NEJM 2018). There have been no randomized controlled trials examining the efficacy of elagolix for the treatment of endometriosis-associated infertility. Given the recent study in Fertility and Sterility demonstrating that IVF outcome prediction using BCL6 and subsequent preliminary data showing benefit using surgery and GnRH agonist therapy (Likes et al., 2019), and data on recurrent implantation failure responding to GnRH agonist and aromatase inhibitors (Steiner et al., 2019), there is adequate rationale to examine the use of this orally active, non-peptide compound (elagolix) for menstrual suppression prior to frozen embryo transfer in women who test positive for SIRT1 and BCL6 and therefore has suspected endometriosis. In addition, during the conduct of this RCT to study elagolix treatment to study pregnancy outcomes and miscarriage

(primary and secondary outcomes), we are collecting blood (estradiol and cytokine measurements) to study the effect of medical suppression on inflammatory biomarkers in blood as well.

**Objectives**

To perform a prospective, open label randomized controlled trial for women who failed IVF with euploid embryos who have suspected endometriosis based on elevated elevated SIRT1/BCL6, comparing 2 months of elagolix (200 mg BID) to oral contraceptives (orthocylen; OPCs).

Primary clinical objective is to determine methods to improve clinical pregnancy rates in these subjects who later undergo frozen embryo transfer with euploid (genetically tested) embryos.

**Justification**

The study will examine whether elagolix is superior to OCPs for fertility enhancement prior to frozen embryo transfer in women with unexplained prior implantation failure with euploid embryos. We will only use 200 mg twice a day dosing of elagolix, since the once daily approved dose of 150 mg does not reliably prevent ovulation and menstruation (Taylor et al., 2017). In women with menstruation, breakthrough bleeding or ovulation, a lower dose will efficiently eliminate the inflammatory changes that we are hoping to suppress. Further, we the goal of our study is to mimic depot leuprolide acetate which is completely suppressive of menstruation; thus the 200 mg BID dose, which is FDA approved for treatment of moderate to severe pelvic pain, will be used. It is possible that elagolix treatment will not be superior to OCPs. There has not been a head to head comparison of either elagolix or leuprolide acetate for endometriosis related symptoms of pain or infertility, so this type of comparison is needed.

**Methods and Measures**

Patients with at least one unexplained IVF failure after transfer of a normal euploid embryo who previously test positive for BCL6 and SIRT1 will be eligible to participate. Each will undergo initial endometrial biopsy, blood draw in the mid-secretory phase. Data will be evaluated separately to estimate the prevalence of positive BCL6 and/or SIRT1 in this group of women with failed euploid embryo transfer. Results of the biopsy will be shared with the patient and the clinicians performing the FET cycle, indicating whether they will be eligible for enrollment in the study. Inclusion criteria will include, therefore, over-expression of endometrial proteins SIRT1 and BCL6 and the availability of frozen euploid embryos for a future FET. Each subject who agrees to enroll will be randomized to one of two treatment arms (elagolix 200 mg BID or an oral birth control pill for two months).

After completion of 2 months of treatment, blood collection will be performed on all subjects. The treatments will not be blinded to the patient and the researchers. All subjects at the conclusion of treatment will undergo immediate entry into a

standardized frozen embryo cycle (FET) cycle using a single euploid embryo. Eight days after embryo transfer, serum hCG levels will be obtained on day 8 after the FET. Outcomes will be recorded including estradiol, progesterone and hCG measurements and documentation of pregnancy outcomes based on ultrasound. The outcome variables will include 1) cancellation; 2) not pregnant; 3) biochemical pregnancy; 4) ongoing pregnancy (sac with cardiac activity) and 5) live birth rate. Miscarriage rate will be calculated based pregnancy loss/number of pregnancies (not counting biochemical pregnancy).

FET cycles will begin without intervening menstruation. Subjects will begin estrace 2 mg for 4 days, going to 4 mg for 4 days and then back to 2 mg for 4 days. Progesterone in oil IM injections will be started when the lining is > 6 mm and has a trilaminar appearance. Transfer will occur 126 hours after the start of progesterone, which is continued until 10 weeks gestation if pregnant. If the hCG test is negative, POI will be discontinued.

If the FET is delayed for any reason, whatever treatment the subject is on (elagolix or OCPs) will be extended for up to 2 weeks to avoid interruption in treatment prior to embryo transfer.

**Design**

A prospective randomized open label controlled trial. Primary outcome will be live birth rate, with secondary outcomes including pregnancy rates, miscarriage rates, measures of inflammation, after treatment at the time of frozen embryo transfer.

Programmed FET Cycle

i. Patients will be advised to call within a week of stopping the last dose of treatment
ii. On day 3 after stopping, patients should begin taking estradiol 2mg PO BID
iii. First monitoring visit should be scheduled for cycle day 9-11 and should include E2, P4, LH, and pelvic ultrasound
    1. If endometrium is > 7mm and trilaminar, initiation of progesterone may be scheduled to facilitate FET on desired date
    2. If endometrium is < 7mm and/or not trilaminar, estradiol should be increased to 2mg PO TID and another monitoring visit should be scheduled in 4-5 days
iv. Timing of progesterone start
    1. If endometrium is > 7mm and trilaminar, initiation of progesterone may be scheduled to facilitate desired FET date
    2. In all cases, progesterone should be initiated with the goal of 126 hours of progesterone exposure prior to FET
    3. Progesterone to be utilized is progesterone in oil (PIO) 50mg IM daily

v. Consideration should be given to cycle cancellation in the following circumstances:
    1. Endometrium < 7mm after 2-3 weeks of exogenous estradiol
    2. Persistent non-trilaminar endometrium after 2-3 weeks of exogenous estradiol
    3. Presence of significant fluid in the endometrial cavity
    4. If follicular recruitment is noted, conversion to natural cycle FET may be considered

## Setting

The EFFECT trial will be conducted at three academic teaching institutions with accredited Reproductive Endocrinology and Infertility (REI) programs involved in In Vitro Fertilization cycles. Wake Forest will be the central IRB and provide data safety monitoring board (DSMB) capabilities for the study.

## Subjects selection criteria

Only patients with previous IVF failure who are suspected or known to have endometriosis on the basis of elevated endometrial BCL6 and SIRT1 will be included in this study. The selection criteria includes the use of these two biomarkers as which defines a specific study population of interest. This inclusion of biomarkers was part of an NIH proposal that was submitted to and funded by the NIH. SIRT1 is strongly induced by resveratrol which has been shown to reduce embryo implantation (Ochiai 2019a) and endometrial decidualization (Ochiai 2019b), supporting the biology as outlined in this proposal. We hypothesize that suppression of endometriosis will have the effect of reducing cytokines associated with inflammation which is part of the endometriosis pathogenesis. Each participant will be required to have at least 1 euploid embryo (preimplantation genetic tested and normal) with intentions to transfer that embryo in a frozen embryo transfer (FET) cycle.

- **Inclusion Criteria**

1. Age – 18 to 42
2. Parity – G0 or greater
3. AMH ≥ 0.5 and ≤ 10
4. Planning to undergo FET and PGT-A within 1 year of enrollment
5. Must have blastocyst(s) by day 6 that were biopsied for PGT-A
6. Has a previous endometrial biopsy showing elevated BCL6 and SIRT1 expression.
7. Endometrial thickness at time of transfer ≥ 6 and ≤ 14

- **Exclusion Criteria**

1. Uterine fibroids > 4 cm in size, if intramural
2. Polycystic ovary syndrome (PCOS) according with the Rotterdam criteria
3. Ovarian failure and subjects receiving donor oocytes/embryos
4. Failure to identify at least 1 euploid embryo for FET

5. Anti-cardiolipid and/or lupus anti-coagulant abnormalities by history
6. Diabetes mellitus (Type I or Type II)
7. Untreated hypothyroidism
8. Hyperprolactinemia
9. BMI ≤ 17 and ≥ 40
10. Uncorrected uterine anomaly
11. Women with osteoporosis
12. Women with moderate or severe hepatic impairment defined by Child-Pugh Classes B (moderate) and C (severe)
13. Women using strong CYP3A inhibitors (e.g. ketoconazole, clarithromycin)
14. Women at high risk of thromboembolic disorders including those that smoke, have cardiac valvular disease, atrial fibrillation, hypertension not controlled by medication, and a history of severe migraines.
15. Severe renal disease with GFR < 15 ml/min.

**Sample Size**

Based on previous studies (Likes et al., 2019) we calculated that 50 patients (25 per arm) are required to have a 90% chance of detecting, as significant at the 5% level, an increase in the primary outcome measure from 11% live birth in the OCP group to 50% in the elagolix group. We decided to include 50 subjects per treatment arm (elagolix 200 mg BID vs OCPs) for 2 months, because unlike the Likes study, we are not using a no treatment control arm, and OCPs may have some benefit over no treatment. Having a non-randomized, no treatment control group to follow will allow us some flexibility, however, in comparing outcomes over time.

Enrollment may vary between sites, but we hope to recruit approximately 1/2 of the subjects at our site with the remainder at each of the other 2 sites (UNC and Stanford). We will perform interim analyses after 25 subjects per arm for safety assessment. Based on the 12 months of treatment in the original study (Taylor et al., 2017), safety concerns are minimal. Nevertheless, we will halt the study using the following stopping rules:

1) If after 25 subjects per arm, the OCP ongoing pregnancy rate is higher than the treatment arm with elagolix
2) There is no difference between the no treatment controls and the elagolix treatment are at 25 patients per arm

We will perform an interim analysis after 25 subjects per arm to recalculate sample size requirements based on the outcomes at that point (Kuman and Chakraborty 2016).

The choice to use oral contraceptives as a comparator group was made based on surveys of potential subjects who voiced concerns about being assigned to a placebo (no treatment) arm. It was felt, and the NIH reviewers agreed, that having an active comparator group would be acceptable. OCPs, while a first line treatment for endometriosis, may not be as suppressive of endometriosis as traditional GnRH agonist therapy, although no head to head comparison studies are available. Finally, the

choice to **not compare 2 doses of elagolix** is based on published results from the Taylor study (Taylor et al., NEJM, 2017) showing a high proportion of subjects in the 150 mg per day dosing group that ovulated and/or menstruated. Bleeding, ovulation or menstruation is a clearly defined problem that would increase inflammation instead of decreasing it, which is the goal of this study.

**Interventions and Interactions**

*Visit 1: Screening and enrollment*. Following initial screening of potential subjects with an IVF failure, a positive SIRT1 and BCL6 on endometrial biopsy and available euploid embryos for transfer, patients will meet with the research nurse who will review all inclusion and exclusion criteria and obtain informed consent from eligible patients.

*Visit 2 First day of menses*. Patients will be instructed to call with the menses at which time an hCG measurement will be made to rule out pregnancy. Subjects will be prepared for their two treatment cycles. Patients will be randomized using computer generated assignments to receive either 200 mg of elagolix twice daily or daily OCPs (Ortho Cyclen). The medications will be formulated into 2 tablets per day to blind the subjects and researchers. The center will call and receive the treatment by means of an interactive voice-response system. The trials will be conducted in accordance with International Conference on Harmonisation guidelines and applicable regulations and ethical principles of the Declaration of Helsinki. All the women provided written informed consent for the study protocol. Women will be instructed to use two forms of non-hormonal contraception; monthly pregnancy tests were performed including an initial hCG test prior to beginning the medication. During the course of two months of treatment subjects will be encouraged to call in and speak to the research nurse or their clinician with any concerns or reports of side effects. Bone mineral or other safety parameters previously performed in the Elaris I-IV studies by Abbvie showed that we should not expect adverse events due to the brevity of the exposure.

*Visit 3:* All subjects will be seen at 1 month time interval and adverse reactions to the medial treatments reviewed and recorded. The second month of medications will be dispersed at that time. Adverse events will be coded with the use of the Medical Dictionary for Regulatory Activities, versions 19.0. We will use Fisher's exact test to compare the incidence of any adverse event in the elagolix group with the incidence in the OCP group. All reported P values will be considered two-sided, and confidence intervals for safety end points will be considered relevant at the 95% level.

Visit 4: After completion of 2 months of treatment with elagolix or OCPs, subjects will return for collection of blood (serum and plasma) which will be collected, processed and stored at -80° C for later evaluation. Subjects will be prepared for the upcoming FET cycle.

*Visit 5: Completion of the study*. Patients will return periodically for standardized treatments as part of their FET cycle for endometrial preparation, ultrasound and embryo transfer as dictated by the clinicians in the practice. Outcomes will be recorded and extracted from their SART database for pregnancy included extended follow-up for live birth rate.

Patients experiencing unanticipated problems with the protocol or who experience adverse events such that they do not tolerate the treatment they are assigned, will be allowed to discontinue treatment and be provided the opportunity to use the other treatment if they so choose. The outcomes of such cycles would be collected separately and included as observational data only and not included in the randomized data analysis. Alternatively, they can simply drop out of the study and resume medical therapy as appropriate or pursue frozen embryo transfer as previously planned.

**Outcome Variables**

The following outcome data will be collected on each subject:

1) BCL6/SIRT1 HSCOREs
2) hCG level (first)
3) Progesterone levels at first hCG measurement (ng/dl)
4) hCG level (second)
5) Pregnancy outcome (not pregnant, pregnant biochemical, pregnant miscarriage, pregnant ectopic, pregnant ongoing, pregnant delivered, pregnant $2^{nd}$ or $3^{rd}$ trimester loss)
6) Categorical outcome (pregnant/not pregnant; summary designation)
7) Comments related to overall outcomes
8) Number of sacs (0, 1, 2, 3)

**Independent Variables**

The following demographic data will be collected on each subject:

1) Age (based on date of birth)
2) Weight
3) BMI
4) Gravidy/Parity
5) Randomization group (elaglox, OCPs, non-randomized control)
6) Embryo grade
7) Number of remaining euploid embryos
8) PGT-A results (narrative)
9) Endometrial thickness
10) Peak estradiol levels (pg/dl)
11) Comments regarding embryo transfer
12) Embryo transfer physician (name)
13) Number of embryos transferred (confirm that 1 was transferred)

## Analytical Plan

*Statistical analysis*: Statistical analyses will be performed using the Student's t-test for data from the two groups. All data will be presented as means ± SEM. $p < 0.05$ was considered statistically significant. Fisher's exact test, relative risk, and 95% confidence interval (CI) will be used for comparisons of categorical data.

**Independent Variables.**

Measurement of hormones. Estrogen, progesterone and hCG assays will be run by the hospital's clinical laboratories using a competitive antibody technology. Venous blood will be drawn into serum separator tubes and taken immediately to the clinical lab for processing (available 24 hours per day).

Results will be analyzed initially using descriptive statistics. Comparison between groups will be done using chi square tests for proportions, and t-tests for continuous variables. Regression analysis will be performed to identify independent outcome predictors. Other inferential statistical analysis will be conducted as appropriate.

## Human Subjects Protection

### Subject Recruitment Methods

Subjects who failed IVF or FET with a previous endometrial biopsy showing elevated endometrial BCL6 and SIRT1 will be identified as part of ongoing care. We do not discriminate on the basis of race or economic status, although we do have age and BMI cut-off criteria defined in the protocol. Subjects will be approached in the privacy of the clinic and all conversations will be confidential.

- We plan to use a recruitment flyers in the clinic to help motivate and identify willing subjects. A copy of this poster is provided with the submission material for IRB review.
- Privacy will be protected and no records kept until subjects/patients agree to participate in the study?
- If an individual declines participation including randomization any and all documents will be destroyed.

### Informed Consent

Signed informed consent will be obtained from each subject by her physician or a clinic research nurse. Consent will be obtained in the CFEM clinical space where patients receive their care from the REI unit. Other centers will use clinical research staff to consent patients in private offices under similar conditions.

### Confidentiality and Privacy

Confidentiality will be protected by collecting only information needed to assess study outcomes, minimizing to the fullest extent possible the collection of any information that could directly identify subjects, and maintaining all study information in a secure manner. To help ensure subject privacy and confidentiality, only a unique study

identifier will appear on the data collection form. Any collected patient identifying information corresponding to the unique study identifier will be maintained on a linkage file, store separately from the data. The linkage file will be kept secure, with access limited to designated study personnel. Following data collection subject identifying information will be destroyed 3 years after completion of the study, consistent with data validation and study design, producing an anonymous analytical data set. Data access will be limited to study staff. Data and records will be kept locked and secured, with any computer data password protected. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study.

**Data and Safety Monitoring**

The principal investigator and PIs at UNC and Stanford will be responsible for the monitoring of the data and safety of study participants. The principal investigator will be assisted by other members of the study staff. A data safety monitoring board (DSMB) through WFBH will be established to oversee the entire project.

Per FDA request, we will assess suicidal ideation as part of our safety evaluation. At each visit subjects will be asked whether they have had any thoughts of self-harm as part of their encounter or if there has been any change in thoughts that include suicide. This screening will be two part, since this drug has been well studied and suicide risk is considered very low (Taylor et al., 2017). If a subject reports having suicidal ideation, we will use the PHQ-9 survery, commonly used in primary care. This instrument has a sensitivity of 78% and specificity of 70% (Runeson et al, 2017) and refer the individual for psychiatric evaluation. Subjects will be withdrawn from the study based on the outcomes of these two steps if they appear to be affected by either treatment toward self harm.

We will exclude subjects with moderate to severe hepatic or renal disease or osteoporosis. We have added to the consent information on avoidance of pregnancy.

Any pregnancy resulting in live birth that arises while taking active drug (elagolix or OCPs) will be identified and followed up to 1 year for any problems that might arise as a result of exposure as an early fetus.

Excessive or unexpected bleeding may be considered an adverse event since the purpose of this study is to achieve and maintain amenorrhea for subjects prior to their next frozen embryo transfer.

**Reporting of Unanticipated Problems, Adverse Events or Deviations**

Any unanticipated problems, serious and unexpected adverse events, deviations or protocol changes will be promptly reported by the principal investigator or designated member of the research team to the IRB and sponsor or appropriate government agency if appropriate.

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