
Prophylactic antibiotics prior to embryo transfer: a randomized
controlled trial

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A Introduction

A1 Study Abstract

Over 200,000 *in vitro* fertilization (IVF) cycles were initiated in the United States in 2015,¹ allowing thousands of infertile couples to start families. Although most of the steps in IVF protocols are based on strong evidence, one, prophylactic antibiotic administration, is commonplace but not proven to produce benefit. This practice began in 1978 as a means to prevent bacterial contamination of the tip of the catheter used to transfer embryos into the uterus.² Although subsequent studies showed that bacterial contamination of the transfer catheters had a negative impact on IVF cycle outcomes,^{3,4} a more recent, robust randomized controlled trial powered to detect catheter tip contamination rates noted a decrease in contamination with routine administration of amoxicillin-clavulanate but no difference in clinical pregnancy rate.⁵ Current standard of care in our practice for a fresh IVF cycle is to administer one dose of 1 gram oral azithromycin (to both male and female partners) on day one of the IVF cycle start. Given the limited evidence for benefit and the true risk for subjecting patients to unnecessary treatments, we must determine whether prophylactic antibiotics actually improve the IVF outcome that matters most to couples: pregnancy.

A2 Primary Hypothesis

Our *hypothesis* is that withholding antibiotic prophylaxis will be non-inferior to routine administration.

A3 Purpose of the Study Protocol

The *objective* of this study protocol is to determine the impact on clinical pregnancy rate of withholding routine prophylactic antibiotic therapy during IVF.

As a smaller pilot study within our RCT we aim to assess alterations and influence of the vaginal microbiome of women going through IVF cycles with a mid-vaginal swab at three time points in the first 30 women enrolled (15 in each arm): at the time of baseline testing, prior to egg retrieval and at the time of embryo transfer. Additionally we will assess the embryo catheter tip at the time of embryo transfer to evaluate the consistency of the vaginal microbiota with endometrial microbiota. Lastly we plan to obtain a swab at the time of embryo transfer to assess the human virome, which has never been described or evaluated in the IVF population.^{6,7} We will correlate our findings with the clinical outcomes we plan to track as a part of our primary study. We will consent and enroll the first 30 women (15 in each arm) who enroll in the PAPET trial for the microbiome and virome pilot study as well.

B Background

B1 Prior Literature and Studies

Limited evidence supporting a common IVF practice: Although patients are routinely prescribed prophylactic antibiotics during an IVF cycle to improve embryo transfer success rates, little evidence supports this practice. A 2012 Cochrane review analyzed randomized controlled trials from the literature but only identified one study that was suitable for inclusion.⁸ In that trial, 350 women were randomized to receive either prophylactic amoxicillin and clavulanic acid on the day before and the day of embryo transfer, or no antibiotic treatment.⁵ The primary and secondary outcomes were catheter contamination rate and clinical pregnancy rate, respectively. Although the catheter contamination rate was significantly lower in the antibiotic group than in the control group (49.4% versus 62.3%, $P<0.03$), there was no difference in clinical pregnancy rate (36% versus 35.5% respectively, $P=0.83$). Impact on live birth rate was not assessed. A more recent retrospective analysis of 876 embryo transfers assessed the impact of discontinuation of routine corticosteroid and antibiotic administration during IVF cycles and found no significant difference in clinical pregnancy rate (56.1% in medicated group versus 61.5% in un-medicated group, $P=0.10$) or other secondary outcomes including clinical miscarriage and live birth rate.⁹ Given this limited evidence about the clinical usefulness of prophylactic antibiotics, a summary statement on embryo transfer from the American Society for Reproductive Medicine states that, “a recommendation for routine prophylactic antibiotics cannot be made”.¹⁰ Thus, we need a well-designed randomized controlled study to rigorously address this question and inform recommendations for the thousands of IVF cycles completed each year.

Potential of prophylactic antibiotics to cause harm: The impact of broad-spectrum antibiotic exposure in the preconception period is not well described but could have the unintended outcome of altering the follicular and endometrial microbiome, which may play a significant role in reproductive competence and IVF outcomes.^{11,12} For example, a prospective study of 35 infertile patients undergoing IVF demonstrated that a *Lactobacillus*-dominated endometrial environment was associated with higher rates of implantation (60.7% vs. 23.1%, $P=0.02$), pregnancy (70.6% vs. 33.3%, $P=0.03$), ongoing pregnancy (58.8% vs. 13.3%, $P=0.02$) and live birth (58.8% vs. 6.7%, $P=0.002$).¹³ Vaginal microbiota diversity has also been implicated as a causative factor in preterm delivery which is known to occur with higher incidence in the IVF population.^{1,14} The microbial community structure of the vagina and endometrium could be negatively affected by potentially unnecessary broad-spectrum antibiotic exposure. We intend to provide proof of concept with our smaller exploratory study evaluating the vaginal and endometrial microbiome and changed across an IVF cycle and as a result of prophylactic antibiotic exposure. Additionally, the *critical* importance of avoiding the misuse and overuse of antibiotics is continually discussed across all fields of medicine because of the increasing incidence of widespread antibiotic resistance.¹⁵⁻¹⁷ Finally, most antibiotics cause negative side effects including nausea, emesis, and yeast vaginitis and may add unneeded stress in the already-complicated IVF treatment process.

B2 Rationale for this Study

Non-inferiority RCT:

Current standard of care in our practice for a fresh IVF cycle is to administer one dose of 1 gram oral azithromycin (to both male and female partners) on day one of the IVF cycle start. This practice is of unproven clinical benefit and is based on theoretical risk and dated, poor-quality data.^{2-5,8,9} Although prophylactic antibiotic treatment may

decrease contamination of the embryo transfer catheter tip, no studies have been powered to determine impact on clinical pregnancy rate, the most direct correlate with successful IVF outcome.² This will be the first randomized controlled trial powered to detect a difference in the primary endpoint of clinical pregnancy rate.

Pilot Study:

Profiling the vaginal microbiome and human virome in a smaller cohort of patients is an exploratory portion of this study. Existing studies are limited and conflicting regarding influence of the microbiome on IVF cycle outcomes.¹¹⁻¹³ This would be the first study to evaluate alterations in the vaginal microbiome across the IVF cycle in response to medications and supraphysiologic hormone levels, and the first to evaluate the human virome in this patient population.^{6,7} We would examine differences within and between groups and assess for the potential impact and association clinical outcomes of those patients exposed to prophylactic azithromycin, and those who are not. Optimizing the vaginal microbiome could serve as a potential target for therapy to help increase IVF success rates in the future with the goal of a full term, live birth.

C Study Objectives

C1 Primary Aim

Specific Aim 1: Determine the effect of withholding prophylactic antibiotic administration on clinical pregnancy rate (defined as a gestational sac, with fetal pole and cardiac activity, on ultrasound).

We *hypothesize* that withholding prophylactic antibiotics before embryo transfer will be non-inferior (absolute difference $\leq 15\%$) to routine prophylaxis in terms of clinical pregnancy rates.

C2 Secondary Aim

Specific Aim 2: Evaluate the impact of prophylactic antibiotic administration on miscarriage rate (pregnancy loss per clinical pregnancy) before 20 weeks.

Our *working hypothesis* is that miscarriage rates will be equivalent between the two groups.

Specific Aim 3: Determine the effect of no prophylactic antibiotic administration on embryo development.

We *hypothesize* that embryo development, as evaluated by fertilization rate (number of two-pronuclei embryos per mature oocyte), blastocyst conversion rate (number of day 5 embryos from two-pronuclei embryos), and blastocyst utilization rate (number of day 5 embryos transferred or frozen per two-pronuclei embryos) will be equivalent between the two groups.

Specific Aim 4: Determine the impact of prophylactic antibiotics or withholding prophylaxis on the vaginal and endometrial microbiome.

We *hypothesize* that azithromycin will impact the vaginal microbiome. Additionally, we expect to see changes across the IVF cycle that may be associated with cycle outcome endpoints.

Specific Aim 5: Evaluate the implications of the human virome on IVF outcomes.

We hypothesize that subclinical eukaryotic viruses will be associated with negative IVF cycle outcomes.

C3 Rationale for the Selection of Outcome Measures

We expect to define the clinical impact of withholding antibiotic prophylaxis before embryo transfer as measured by markers of a successful IVF cycle. As we hypothesize that withholding prophylaxis will be non-inferior to routine antibiotic administration, these results are expected have a positive clinical impact by decreasing unnecessary broad-spectrum antibiotic exposure and potentially creating a paradigm shift in IVF protocols.

D Study Design

D1 Overview or Design Summary

This will be a prospective randomized controlled non-inferiority trial in which 178 couples will receive the standard of care prophylactic antibiotics (control) and 178 will not receive prophylactic antibiotics (experimental). The primary outcome is clinical pregnancy rate. The expected primary outcome is that withholding prophylactic antibiotics will be non-inferior to antibiotic prophylaxis in terms of clinical pregnancy rate. Secondary outcomes include spontaneous miscarriage rate and embryo development as measured by fertilization rate, blastocyst conversion rate, blastocyst utilization rate, and live birth rate.

D2 Subject Selection and Withdrawal

2.a Inclusion Criteria

The female partner must be aged 18–43 years and going through a fresh IVF cycle.

2.a Exclusion Criteria

Couples will be excluded from enrollment if they have any contraindication to antibiotic treatment (ie: allergy), are not intending to undergo embryo transfer (fertility preservation patients and oocyte donors), require use of extended antibiotic coverage at time of egg retrieval, are already on antibiotics for any reason (e.g., upper respiratory infection), have a recent history of pelvic infection, or are planning on limited insemination (inseminating a limited number of the eggs retrieved).

2.b Ethical Considerations

Participation will be completely voluntary. Patients care will not be impacted in any way by their decision to participate or lack thereof. Incidence of pelvic infection following embryo transfer approaches zero in this low risk population therefore patients are not

being placed at any additional undo risk. They may be spared from experiencing potential adverse side effects of the oral antibiotic.

2.c Subject Recruitment Plans and Consent Process

Couples going through fresh IVF cycles will be identified and their charts reviewed to assess eligibility for inclusion. Eligible couples will be contacted via phone by a trained study coordinator and offered enrollment. Those who express interest will have a time arranged for both partners to provide written consent. Couples will only be included in the study during one IVF cycle. Subjects going through their first IVF cycle will also have an introduction to the study during IVF orientation.

2.d Randomization Method and Blinding

Couples will be randomized after providing written consent. Written consent will be obtained separately for both the male and female partner. In the case of same sex couples, only the female undergoing embryo transfer will be consented. The first 50 women enrolled in the noninferiority trial will also be consented for the microbiome and virome profiling exploratory study. After consent, randomization in a 1:1 ratio to prophylactic antibiotics or no antibiotics will be performed in a block-randomized fashion per cycle number via computer random number generator. Couples randomized to the antibiotic group will receive the current standard regimen in our clinic: 1 gram azithromycin oral once for both partners the day controlled ovarian stimulation is initiated with injectable gonadotropins (IVF cycle start). In cases of same-sex couples, only the female undergoing the embryo transfer receives prophylaxis. Couples randomized to the no-antibiotic treatment group will not be prescribed oral antibiotic prophylaxis. Couples who agree to participate will have their charts marked indicating participation in the study. The couples' respective nurses will receive a message that will remain part of the electronic medical record regarding patient treatment group allocation so they can prescribe antibiotics appropriately. Embryologists who assess the embryo outcome measures will be blinded to patient treatment group allocation. The patients and their providers and nurses will not be blinded to treatment group allocation.

2.e Risks and Benefits

Potential problems with our study may be insufficient patient recruitment. The process of IVF is already stressful, time and user intensive and we will have to consent both partners. Patients may be hesitant to deviate from our previous standard of care. On the other hand, patients may want to avoid additional medication and may not take the antibiotic regardless to which arm they are randomized. We would mitigate this risk by a thorough informed consent and expressing our goal is to improve our current IVF protocols and that the best approach is not known while emphasizing the importance of compliance. Reportable severe events include development of pelvic infection following embryo transfer, a significant increase in spontaneous miscarriage rates or a note in increased contamination rates in the lab. Risks of pelvic infection in this low risk population following embryo transfer is remarkably rare as stated above and we do not anticipate this occurring. We anticipate miscarriage rates to be equivalent or improved in the non-prophylaxis group. The lab uses genatmicin routinely in embryo culture

media to decrease contamination rates and will continue to be closely monitored for any increase in contamination rates. Reportable non-severe adverse events include yeast vaginitis, nausea and vomiting or other adverse reactions from antibiotic prophylaxis.

Obtaining a mid-vaginal swab poses little to no risk to the patient. The patient may experience some slight discomfort from one additional speculum exam at the time of baseline testing. The embryo transfer catheter tip will be sequenced for comparison to the vaginal swab following embryo transfer and will require no additional invasive testing or exams.

2.f Early Withdrawal of Subjects

While loss to follow up is extremely rare in this circumstance, some patients who are enrolled and randomized may not make it to egg retrieval or may not get an embryo transfer due to either a poor response to medications or poor embryo quality. We will account for these patients in our 5% drop out estimation and they will not be included in analysis.

2.g When and How to Withdraw Subjects

Patients can withdraw from the study at any time if they choose to do so. Their care will not be impacted by their participation or lack thereof.

2.h Data Collection and Follow-up for Withdrawn Subjects

Subjects who withdraw will not be included in the final data analysis.

D3 Study Drug –

3.a Description

Azithromycin is routinely given to both partners (in male/female couples) or to the embryo recipient in same sex couples or gestational carriers when they start their IVF cycle medications and is our current standard of care.

3.b Treatment Regimen

1 gram oral azithromycin, once the day of IVF cycle start (when they start injectable gonadotropin medications) which is our current standard of care.

3.c Method for Assigning Subjects to Treatment Groups

Computer random number generator.

3.d Preparation and Administration of Study Drug

Azithromycine will be prepared and dispensed by the patients preferred pharmacy as is standard practice if they are randomized to the antibiotic prophylaxis group.

3.e Subject Compliance Monitoring

Patients will give verbal confirmation of compliance.

3.f Blinding of Study Drug

Patients will not be blinded to which group they're in. A placebo control will not be used.

3.g Receiving, Storage, Dispensing and Return

Azithromycin will be prepared and dispensed by the patients preferred pharmacy as is standard practice if they are randomized to the antibiotic prophylaxis group.

E Study Procedures

E1 Screening for Eligibility

Couples will be screened for eligibility by their primary physician, the primary investigator, Dr. Eskew or the Research Project Coordinator. If they meet the inclusion and exclusion criteria they will be offered enrollment.

E2 Schedule of Measurements

Outcomes measures are routinely tracked by our clinical to report to the Society of Assisted Reproductive Technology (SART). Patients who participate in the study will not be required to make any additional visits beyond consent.

E3 Visit 1

Outcome measures for specific aim 3 will be tracked from the patients initial oocyte retrieval and embryo transfer visit. The lab routinely tracks this data and no additional query or visits will be required in term of embryo development data.

E4 Visit 2 etc.

Specific Aim 1 will be addressed and tracked when patients come in for their viability ultrasound at 6-7 weeks gestation which is routine practice in our clinic. Clinical pregnancy is defined as a gestational sac, with fetal pole and cardiac activity, on ultrasound. No additional visits will be required. Patients will continue to be tracked once they are released from our practice to their primary OBGYN to track miscarriage rate (spontaneous loss prior to 20 weeks) and subsequent live birth outcomes.

Schedule of Visits for Microbiome/Virome Study:

Outcome measures will be assessed the same as th above aforementioned schedule of measurements for the noninferiority trial. Collection of specimens for the exploratory study will occur as described below:

Visit 1-

Women enrolled in the smaller exploratory study will have a midvaginal swab obtained when they get their baseline testing prior to ultrasound. This visit is already required in the IVF cycle and will not require an additional visit to collect the specimen.

Visit 2-

The second specimen will be obtained with a midvaginal swab prior to egg retrieval. This visit is already required in the IVF cycle and will not require an additional visit to collect the specimen.

Visit 3-

The third and final specimen will be obtained via midvaginal swab prior to embryo transfer. This visit is already required in the IVF cycle and will not require an additional visit to collect the specimen.

All specimen swabs collected for microbiome and virome profiling will be flash frozen at -80 celsius until all specimens have been collected and can be batch analyzed at the same time.

E5 Safety and Adverse Events

5.a Safety and Compliance Monitoring

As administration of 1 gram oral Azithromycin is standard of care in our practice no additional risk will be incurred from prescribing the antibiotic prophylaxis. Patients will be asked regarding compliance with medication administration and if any adverse side effects were incurred including yeast vaginitis, nausea and vomiting or other adverse reactions from antibiotic prophylaxis.

5.b Medical Monitoring

i Institutional Data and Safety Monitoring Board

In addition to principle investigator review of adverse events, a data and safety monitoring board (DSMB) will be established. This will include Ann Pitcavage, Joan Riley, PhD, Kristina Cipolla and Emily Jungheim, MD, MSCI. The designated group will meet every 6 months to review occurrence of adverse events in addition to assessing recruitment progress and will review for futility of the trial in the context of lack of adequate recruitment.

5.c Definitions of Adverse Events

Severe adverse events: pelvic infection (endometritis, cervicitis or pelvic inflammatory disease) as diagnosed by cervical culture and physical exam, spontaneous miscarriage (pregnancy loss prior to 20 weeks gestation) or increased contamination rates in the lab which is routinely monitored for by our embryologists.

Non-severe adverse events include adverse effects of antibiotic administration including yeast vaginitis, abdominal pain, diarrhea, nausea and vomiting.

5.d Classification of Events

Reportable severe events include development of pelvic infection following embryo transfer, a significant increase in spontaneous miscarriage rates or a note in increased contamination rates in the lab. Risks of pelvic infection in this low risk population following embryo transfer approaches zero and we do not anticipate this occurring. We anticipate miscarriage rates to be equivalent or improved in the non-prophylaxis group but will be closely tracked for any increase in incidence. The lab uses gentamicin routinely in embryo culture media to decrease contamination rates and will continue to be closely monitored as is standard for quality control measures in our lab for any increase in contamination rates.

Reportable non-severe adverse events include yeast vaginitis, nausea and vomiting or other adverse reactions from antibiotic prophylaxis which the patients will report to their nurses.

5.e Data Collection Procedures for Adverse Events

In addition to principle investigator review of adverse events, a data and safety monitoring board (DSMB) will be established. This will include Ann Pitcavage, Joan Riley, PhD, Kristina Cipolla and Emily Jungheim, MD, MSCI. The designated group will meet every 6 months. Adverse events will be noted in the patients chart and in the redcap database for complete capture and tracking. Data will be analyzed if a noted increase in severe adverse events is noted or otherwise every 6 months when the DSMB meets.

5.f Reporting Procedures

Patients will be instructed to report any adverse symptoms to their respective nurse or doctor. These events will be immediately reported to the PI or research project coordinator.

5.g Adverse Event Reporting Period

Until study completion.

F Statistical Plan

F1 Sample Size Determination and Power

Given the conflicting evidence toward benefit of routine antibiotic prophylaxis and IVF outcomes, a non-inferiority approach was selected to test our hypothesis that no prophylaxis is not inferior to routine antibiotic administration. A total sample size of 356 patients (178 per arm) was determined based on an average baseline clinical pregnancy rate of 41% within our practice in this population with a non-inferiority margin of 15%, 80% power, and using a one sided $\alpha=0.025$ while estimating a 5% drop out

rate. Our practice completed approximately 500 fresh IVF cycles in 2016. Our exclusion criteria are broad and we do not anticipate patient recruitment to be difficult. We believe 356 patients is feasible and estimate a 14 month timeline to complete recruitment if we enroll 7 patients at minimum per week.

F2 Interim Monitoring and Early Stopping

Interim analysis will be performed using the Haybittle-Peto approach. This interim analysis approach will occur in 3 steps under the guiding principle that if an interim analysis shows a probability of equal to a very small alpha or greater than a very large critical value ($p < 0.001$) that a difference as extreme or more between the treatments is found, given that the null hypothesis is true, then the trial should be stopped early: Step 1) At 50% enrollment (180 women total), analysis will be performed to assess the primary endpoint, clinical pregnancy rate between the two groups, with a significance level of $p < 0.001$ required to stop; Step 2) At 75% enrollment ($n=270$), analysis will again be performed to assess the primary endpoint, clinical pregnancy rate between the two groups with a $p < 0.001$ required to stop and Step 3) Final analysis at 100% enrollment with $p < 0.05$ level of significance. This approach will be ideal for interim analysis as well as the final analysis using a $p < 0.05$ level of significance as normal, which is our chosen level of significance. The interim analysis will be performed by a statistician in our division who is on the IRB, and not by the primary investigator. The interim analysis results will be provided to the PI, the research team, and the Data Safety Monitoring Board for continuing review.

F3 Analysis Plan

See Table 1 for timetable of patient enrollment and analysis. Our practice is required to track patient demographic data and cycle outcome measures to report to the Society for Assisted Reproductive Technology. Data are recorded and tracked through Research Electronic Data Capture (REDCap). Data from enrolled patients will be exported and tracked in a separate secure REDCap dataset from our existing dataset. All of our outcome measures are routinely tracked, and no additional data query will be required to address potential confounders as listed in Table 2.

F4 Statistical Methods

Intention-to-treat and per protocol analyses will be performed. Chi-squared and Fisher's exact tests will be used to compare categorical variables as appropriate (see Figure 2). Kolmogorov-Smirnov test will be initially used to assess the distribution pattern of continuous variables. Normally distributed variables will be analyzed via Student's t-test. Non-normally distributed continuous variables will be analyzed with the Mann-Whitney U test.

The primary analysis is based on difference in clinical pregnancy rate between the two groups. The sample size estimation for the study is based on this comparison with a superiority approach as described below with an estimated effect size of 15%. Chi-square analysis will be utilized to compare the difference in clinical pregnancy between groups. Secondary analysis will include spontaneous miscarriage (clinical pregnancy loss prior to 20 weeks) and will also be determined using chi-square. Difference in embryo development as determined by number of blastocysts from 2 pronuclei embryos will be determined by independent samples t-test. Each microbiota sample will have the taxon indicated as relative abundance. To assess the diversity across an IVF cycle,

– after log transformation, linear mixed regression will be applied. Wilcoxon rank sum will be used to measure for difference in richness and diversity with clinical outcomes of clinical pregnancy rate, miscarriage rate and embryo outcomes.

Baseline demographic and cycle level factors as noted above will be collected and compared between the two groups to assess for potential cofounders using appropriate statistical tests for continuous and categorical variables as discussed above and noted in Figure 2.

Multivariable logistic regression will then be employed to control for known confounders that impact clinical pregnancy and IVF outcomes including: maternal age, BMI, infertility diagnosis and IVF cycle number. As we plan to use an intent-to-treat approach, patients will be analyzed with the group they are randomized to even if they fail to make it to embryo transfer. Missing data is infrequent with patients going through an IVF cycle however, if we are unable to obtain miscarriage data on a patient that will be analyzed with the intention to treat approach as not having a miscarriage. We estimate the number of patients missing will be less than 1% of a sample size based on follow up data from the past 5 years at our institution.

A p-value <0.05 will be considered statistically significant. Analysis will be performed using IBM® SPSS® statistics version 23.

G Data Handling and Record Keeping

G1 Confidentiality and Security

Our practice is required to track patient demographic data and cycle outcome measures to report to the Society for Assisted Reproductive Technology (SART). Data is currently recorded and tracked through Research Electronic Data Capture (REDCap). REDCap is a secure web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.¹⁸ Enrolled patients will be exported and tracked in a separate REDCap data set from our existing dataset.

G2 Records Retention

Study records will be stored and tracked through REDCap as noted in G1. Signed consents will be kept in a locked cabinet in a locked office. Data will be retained until study enrollment and analysis is complete.

H Study Administration

H1 Organization and Participating Centers

Washington University Department of Reproductive Endocrinology and Infertility.

H2 Funding Source and Conflicts of Interest

The PI, Ashley Eskew, MD is a recipient of funding from NIH 5T32HD055172 as a part of her Masters of Science and Clinical Investigation clinical research track. There are no conflicts of interest to report.

H3 Subject Stipends or Payments

Not applicable.

H4 Study Timetable

See Table 1.

I Publication Plan

We plan on presenting our findings at national meetings including those for American Society for Reproductive Medicine and Society for Reproductive Investigations. We plan to publish our results in journals that have a high impact among reproductive endocrinologists within 6 months of completing our data collection.

J Attachments

J1 Tables

Figure 1: Timetable (months)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Patient Recruitment	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Analysis															X	X	X		
Manuscript Composition																	X	X	X

Table 2. Potential confounders

Patient history	Cycle level factors	Cycle outcome
Demographic data: Maternal age, race, ethnicity Medical history: BMI, tobacco use Obstetric history: gravidity, parity Infertility diagnosis Duration of infertility Number of prior IVF cycles Baseline labs: Follicle stimulating hormone or antimullerian hormone level, antral follicle count	Total amount of gonadotropin administered Stimulation protocol Number oocytes collected Number mature oocytes Insemination method (conventional vs. intracyto-plasmic sperm injection [ICSI] vs. partial ICSI) Number oocytes normally fertilized (two pronuclei) Day of embryo transfer Number of embryos transferred Number of embryos frozen	Clinical pregnancy rate Spontaneous miscarriage rate Live birth rate

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