Title: Effect of ejaculatory abstinence on semen parameters in male factor infertility patients: a randomized controlled study

NCT #: NCT04206800

Document date: 12/11/2019

PART B
STUDY DESCRIPTION

Title of Protocol	Effect of ejaculatory abstinence on semen parameters in male factor infertility patients: a randomized controlled study
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B1. PURPOSE OF PROTOCOL

The purpose of this study is to compare semen parameters with in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) when male factor infertility patients abstain from ejaculation more than 48 hours (routine care) or less than 24 hours. We hypothesize that total motile sperm will be improved with EA less than 24 hours. Information from this trial could allow us to optimize chances for a successful pregnancy in patients who need IVF/ICSI.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

The semen analysis is currently one of the main tests in diagnosing male factor infertility but does not measure fertilization potential (1,2). There are numerous parameters in a semen analysis that are affected by different factors with one being ejaculatory abstinence (EA) prior to artificial reproductive technology (ART). Current guidelines from the World Health Organization (WHO) recommend between 2 to 7 days of EA while the European Society of Human Reproduction and Embryology advises 3 to 4 days of EA prior to a semen sample (3,4). This EA period has been utilized for diagnostic purposes and was inherited in IVF/ICSI treatment procedures with minimal investigation.

Two prior studies showed optimal semen parameters when following the current WHO guidelines on EA (5,6). Conversely, other studies suggest shorter EA periods may provide benefit on certain semen parameters (7–13). Furthermore, a shorter EA may result in a reduced exposure to reactive oxygen species which leads to improved sperm quality (14,15). ROS may be a contributory factor to a male's infertility (16).

Two prior systematic reviews evaluated EA periods and correlations to semen quality in fertile and infertile males (17,18). Ayad et al reported despite the varied quality of studies, there may be enough evidence to favor a shorter EA period and guidelines should be revisited (17). Hanson et al reported that certain semen parameters improved with longer or shorter EA periods and no clear recommendations could be given (18). Both of these systematic reviews demonstrated conflicting results when evaluating total motile sperm.

To our knowledge, this is the first randomized control trial to evaluate semen parameters with varying EA periods in male factor infertility patients when IVF/ICSI is utilized for first time IVF patients. In addition, we will survey patients' ejaculatory mode and frequency during the IVF cycle. This will provide useful and practical information for physicians and patients when determining how to optimize chances for a successful pregnancy.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Overview
This will be a randomized controlled trial to compare semen parameters following EA for either more than 48 hours (routine care) or less than 24 hours among male factor infertility patients who receive IVF/ICSI.

Methods
Study participation will not influence any element of IVF treatment and all patients will be receiving the standard of care at Boston IVF. For participants who meet eligibility criteria and provide informed verbal consent, baseline demographic data will be obtained from their medical record including for example; age, race, education level, height, weight, baseline semen analysis, testosterone level, follicle stimulating hormone (FSH) level. Demographic data on the female partner will include parameters such as: age, race, education level, height, weight, gravidity, parity, anti-mullerian hormone (AMH) level, and day 3 FSH.

Eligible participants will have the study introduced by the medical care team. Eligible participants will be given information on study details (see study details for participants) as well as an ejaculatory calendar/survey (see survey and calendar) that can be used if the participant enrolls in the study (see study timeline). The medical care team will inform eligible participants that they will receive a recruitment email from the research team at Bivfresearch@bostonivf.com followed by a phone call from the research team to see if eligible participants are willing to participate (see recruitment email). Eligible participants who meet inclusion and exclusion criteria whom do not get information regarding the study from the medical care team will also receive a recruitment email. If so, the research member will call the eligible participant to obtain verbal consent to enroll in the study (see verbal consent). If the participant does not answer, a voicemail will be recorded followed by a second phone call the following day. The recruitment email and verbal consent will occur between the pre-IVF period and before stimulation day 4 of the IVF cycle. If a participant consents to enrollment, we will make a note in the treatment plan for their cycle. Enrolled participants will receive a phone call from a member of the research team during stimulation day 6 to 8 to randomize the participant to EA > 48 hours (routine care) or EA < 24 hours (see randomization call). If the participant does not answer, a voicemail will be recorded followed by a second phone call the following day.

The semen sample provided on day of oocyte retrieval will be analyzed with a basic and advanced semen panel. Any remaining samples will be discarded per routine practice. Prior to providing the semen sample, participants turn in the completed survey/ejaculation history calendar to a member of the research team. Blank copies will be available for participants the day of egg retrieval if needed.

There is no clinical risk to your IVF cycle as the procedures followed are standard clinical practice. Study participation will not influence any element of the IVF treatment except for EA counseling. Currently there is no set protocol on how EA counseling is performed. Counseling on EA varies among the medical care teams at Boston IVF as some patients may or may not receive explicit EA instructions. Patients may be counseled by the medical care team with recommendations to abstain for > 48 hours at the time of their consult. All patients in this study are using ICSI to fertilize eggs and the number of sperm needed for treatment will not be compromised by ejaculatory abstinence < 24 hours. Participants who crossover to the alternate treatment arm will continue their participation in the study so that we can collect cycle outcome data. IVF stimulation protocols will be according to standard practice. ICSI and embryology practice will follow standard practice. Selection of embryos will be based on the current embryology laboratory selection protocols or by preimplantation genetic testing for aneuploidy (PGT-A). The number of embryos transferred will be based on the usual American Society of Reproductive Medicine guidelines. Any unused embryos will be disposed of or cryopreserved per

standard clinical protocols.

Outcomes
Primary: Total motile sperm (motility x concentration x volume)

Secondary:
Clinical data from the IVF cycle randomized to intervention or control condition, will include but not be limited to the following: fertilization rate; blastocyst rate; number of morphologically normal blastocysts; number of euploid and aneuploid embryos (if preimplantation genetic testing-aneuploidy [PGT-A] performed); number of embryos transferred; implantation rate; biochemical pregnancy rate (defined as positive beta-hCG but no gestational sac visualized); clinical pregnancy rate (defined as confirmation of gestational sac on ultrasound); ongoing pregnancy rate (defined as confirmation of a gestational sac with at least one fetal pole with a fetal heartbeat); live birth rate (defined as delivery > 23 weeks gestation); Cumulative pregnancy rate; Miscarriage rate; Multiple pregnancy, defined as twins or higher-order gestations

Semen characteristics will be examined in relation to:
Changes in semen analysis from baseline diagnostic sample
An advanced semen analysis panel
Time from last ejaculation to oocyte retrieval

All data that is collected for the trial is clinical data that would be collected and stored in the electronic medical record as part of routine clinical care. In addition to collecting outcome data from this initial IVF cycle, we will collect data regarding subsequent IVF cycles and outcomes for any of the frozen embryos created but not transferred in this cycle.

Procedure
Intervention
Participants will be randomized to EA more than 48 hours (routine care) or less than 24 hours from the day of their partner's oocyte retrieval.

Randomization
We will use computer-generated block randomization in REDCap to randomize participants in a 1:1 ratio to the EA period more than 48 hours (routine care) or less than 24 hours. Randomization will be stratified by whether the patients receive PGT-A. Research staff will call participants on stimulation day 6 to 8 to notify participants which treatment arm they were randomized to. The participant can withdraw from the study at any time if desired or will have an opportunity to not continue with the treatment arm they were randomized to. We anticipate that most of the crossovers will occur due to participants wishing to be in the alternate EA period or not being able to produce a sample in the window assigned.

The purpose of this study is to examine semen parameters with IVF/ICSI when male factor infertility patients abstain from ejaculation more than 48 hours (routine care) or less than 24 hours. Information from this study would allow us to provide evidence-based recommendations to future patients who need IVF/ICSI.

B. Statistical Considerations

Sample Size Justification
Our sample size calculation is based on data from Boston IVF from 2013 to 2018 for patients with less

than 3 million total motile sperm post prep for IVF/ICSI patients. The mean number of total motile sperm is 38 million ± 24 which will be our control group. We hypothesize that the mean number of total motile sperm at the time of retrieval will increase to 48 million. In order to achieve 80% power to detect the specified difference using a two-sided alpha of 0.05, we will need 90 evaluable participants per arm. We anticipate that approximately 15% of participants will drop out or crossover, and we will inflate the sample size by another 15% to account for a non-normal distribution. Thus, we aim to randomize 117 participants per arm for a total of 234 participants.

Data Analysis
We will conduct both intention-to-treat and per-protocol analysis. Both intention-to-treat and per-protocol analyses are noted in the RCT reporting guidelines (CONSORT) as valid approaches. For this study, we will perform an intention-to-treat analysis because it is the gold standard for RCTs, even with crossover or withdraw. We also will perform a per-protocol analysis. While this analysis can introduce bias, it is a valid statistical approach.

Descriptive data will be presented as a proportion, mean with standard deviation or median with interquartile range. Comparisons will be made using Chi-square or Fisher's exact test for categorical variables and parametric or non-parametric tests for continuous variables based on data distribution. Log-binomial regression will be used to estimate risk ratios and 95% confidence intervals for the primary and secondary outcomes. While we anticipate that randomization will balance the distribution of measured and unmeasured potential confounders in the two study arms, if this is not the case, we will assess the influence of potential confounders as needed. All data will be analyzed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). All tests will be two sided and a P value <0.05 will be required to confer significance.

C. Subject Selection

Inclusion Criteria

- Male Age 18 – 50 years and female age < 38 years
- First IVF cycle
- Abnormal semen parameters (at least 1 of the following on 2 semen analysis with at least one being in the past 12 months)
    - sperm concentration < 10 million/mL
    - unprocessed semen analysis with < 10 million motile sperm or processed semen analysis with < 3 million motile sperm
    - < 2 % normal forms (strict Kruger morphology)
- Female AMH > 0.7 and/or and day 2-4 FSH < 12

Exclusion Criteria

- Donor sperm
- Males with sperm concentration < 200,000/mL
- Utilization of a gestational carrier
- Frozen donor egg
- Female morbid obesity: BMI > 40
- History of recurrent pregnancy loss (≥2 spontaneous abortions)
- Preimplantation genetic testing – (M or SR) Monogenetic disorders or chromosomal rearrangement
- The use of non-ejaculated sperm (testicular sperm extraction)

B4. POSSIBLE BENEFITS

We do not know whether participants in the study will gain any direct benefit from participation. However, we hope that this study will provide data to inform how we can improve treatment for future patients with male factor infertility.

The potential benefits to the field, as a whole, are enormous. This is a clinical conundrum that is encountered daily by infertility physicians and the results of this study have the potential to change clinical practice.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

There is no clinical risk to the patient as the procedures followed are standard clinical practice.

EA > 48 hours is routine care.

The risks of EA < 24 hours:

- Lower semen volume
- Less total sperm

As there is no good evidence to guide clinical practice and these patients constitute a large volume of our practice, potential benefits far outweigh the potential risks. Given that all patients in the RCT are to be treated by ICSI, the number of sperm needed for treatment will not be compromised by EA less than 24 hours.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment

This study will utilize an opt-out recruitment method. First-time IVF/ICSI patients will be identified, and eligibility will be determined per the study inclusion and exclusion criteria prior to the start of the IVF cycle. All eligible participants will be patients of Boston IVF physicians. All Boston IVF physicians will have the option to exclude any of their patients from the study.

Eligible participants will have the study introduced by the medical care team (see script for medical care team). The medical care team will also have access to general study information (see handout for medical care team). Eligible participants will be given information on study details (see study details for participants) as well as an ejaculatory calendar/survey (see survey and calendar) that can be used if the participant enrolls in the study (see study timeline). The standard of practice for patients at Boston IVF is to receive communication via email, access many online documents, and enter the information into the online patient portal. To be consistent with this method of patient contact, participants will receive a recruitment email from the research team. The medical care team will inform eligible participants that they will receive a recruitment email from the research team at Bivfresearch@bostonivf.com that explains the study followed by a phone call from the research team to see if eligible participants are willing to participate. Eligible participants can choose to reply back to Bivfresearch@bostonivf.com to opt-out from receiving any more contact in regards to this study. Eligible participants who meet inclusion and exclusion criteria whom do not get information regarding the study from the medical care team will also receive a recruitment email.

Consent

We are requesting a HIPAA waiver for pre-screening and recruitment. For the study intervention, we are requesting a waiver of documentation of consent. Participants will be informed in the recruitment

email the study is completely voluntary. The day following the recruitment email, eligible participants will receive a phone call from a member of the research team to discuss the study and obtain verbal consent if the eligible participant is interested in participating in the study (see verbal consent). If the participant does not answer, a voicemail will be recorded followed by a second phone call the following day. The recruitment and consent process will occur between the pre-IVF period and before stimulation day 4 of the IVF cycle. If a participant consents to enrollment, we will make a note in the treatment plan for their IVF cycle. Throughout the study, we will let participants know that they have the option to stop participating at any time and that their care would not be affected by that decision.

We anticipate that a proportion of participants may desire to crossover to the other assigned EA period. If this occurs, participants will be instructed to email or call the research team. These patients would be included in the per protocol analysis. The study team will access the patients' medical records for up to 24 months following randomization in order to follow the clinical/pregnancy outcomes. These outcomes are recorded as part of routine clinical care.

Subject Protection
This is a completely voluntary study. Participants may withdraw from the study at any point; they also may choose to not continue with their randomized EA period. Participants will be advised that if they withdraw or choose to cross over to the other treatment arm their clinical care will not be influenced in any way.

B7. STUDY LOCATION

Privacy
Verbal, informed consent will be obtained at a location of their choosing.

Physical Setting
All patient recruitment will occur at Boston IVF and through email and phone. Participants will be notified of their intervention via phone during the randomization process. Participants will complete a survey at a location of their choosing or at Boston IVF.

B8. DATA SECURITY

All electronic data will be kept in a restricted-access folder on the Boston IVF secure server behind the firewall. Any paper generated as a result of this study will be kept in a locked office belonging to a member of the study team.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☐ Yes ☐ No

Is the BIDMC PI the lead investigator of the multi-site study? ☐ Yes ☐ No

B10 Dissemination of Research Results

We will share the final results of the study to participants who would like the results and provide their email in the survey.

Study results will be presented nationally and internationally at conferences and submitted for peer review publication. We will acknowledge the patients in future publications.

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