Statistical Analysis Plan

9. STATISTICAL CONSIDERATIONS

This section outlines the general statistical methods contributing to the clinical trial design and planned analyses. In general, baseline will be defined as the last assessment prior to or on the day of oocyte retrieval. Descriptive statistics for continuous data will include the number of Subjects with an observation, mean, standard deviation, median, minimum, and maximum. Summaries of changes from baseline will include only Subjects who have both a baseline value and the corresponding value at the time point of interest. Descriptive statistics for categorical data will include frequency and percentage.

9.1. Analysis Sets

9.1.1. Stimulated Analysis Set (SAS)

The stimulated analysis set comprises all screened Subjects that receive any dose of gonadotropins for the purpose of abbreviated controlled ovarian stimulation. Subjects will be analyzed agnostic of treatment assignment.

9.1.2. Intention-to-Treat Analysis Set (ITT)

The intention-to-treat analysis set comprises all Subjects. Subjects will be analyzed according to planned treatment.

9.1.3. Full Analysis Set (FAS)

The full analysis set comprises all Subjects with at least one COC exposed to either Fertilo or MediCult IVM. Subjects will be analyzed according to planned treatment.

9.1.4. Safety Analysis Set (SAS)

The safety analysis set comprises all Subjects in the full analysis set that also receive at least one embryo transfer. Subjects will be analyzed according to actual treatment received.

9.2. Analyses Supporting Primary Objective(s)

The primary endpoint is cumulative live birth after at most two embryo transfers within six months from oocyte collection.

9.2.1. Statistical Model, Hypothesis, and Method of Analysis

The primary objective of this study is to serve as a two-part safety evaluation for a First In Human

(FIH) study of Fertilo. In the first part of the study, an observational analysis of 20 treatments will be performed. All treatments will utilize Fertilo and will be performed at one of two centers. The primary intention of this phase of study is observational, to determine rates associated with the primary and secondary endpoints as well as safety objectives. As such, there is no comparator for evaluation of superiority or non-inferiority and analysis will be restricted to descriptive statistics. Statistical methods for the study are primarily descriptive, in order to establish frequency rates of the primary and secondary objectives. Data will be compared to historical precedents and to site-specific outcomes to contextualize findings. Rate data is measured and aggregated as a mean and standard deviation for measurements whose distribution is considered normal. Per patient significance of formation rates (M2, Cleavage, Euploid) will be investigated using Linear Regression analysis using treatment and patient dependent categorical values measured against historical and site-specific controls not generated in this study.

For the second phase of the study, a limited pilot of 20 patients will be prospectively randomized to intervention (Fertilo) or control (MediCult IVM) and treated in an unblinded RCT. This phase will be limited to a single study center in Peru only. For the comparative evaluation phase, outcomes of both arms will be compared at the interim analysis point, considered completion of the first treatment cycle and embryo transfer for all 20 patients. Logistic regression comparing embryo outcomes will be utilized, along with unpaired t-test. It is expected that no less than 65 oocytes per arm are needed for adequate power to detect a rate difference at the oocyte maturation and blastocyst formation stage.

9.2.2. Primary Efficacy Estimand

The primary efficacy estimand is constructed using the framework of the five components set forth in ICH E9(R1). The treatment is the culture of one or more immature COCs in either Fertilo or MediCult IVM, fertilization, growth to the blastocyst stage, vitrification, and later transfer for reproductive purpose. The population is the full analysis set, defined as all Subjects with at least one COC exposed to either Fertilo or MediCult IVM. The following intercurrent events have been identified as potentially preventing the measurement of the primary endpoint: Having no mature oocytes after treatment with Fertilo or MediCult IVM, having no blastocysts of freezable quality, not receiving embryo transfer for any reason, and withdrawal from the study for any reason before the completion of all ongoing pregnancy assessments.

9.2.3. Handling of Intercurrent Events and Missing Data

Intercurrent events will be handled according to a composite variable strategy. For observations missing due to any one of the intercurrent events identified in Section 9.2.2, a negative value (i.e., ‘No’ or ‘no cumulative ongoing pregnancy’) will be imputed. For other types of missing data (e.g., if a subject misses their ongoing pregnancy visit), a negative value (i.e., ‘No’ or ‘no cumulative ongoing pregnancy’) will be imputed unless subsequent data indicate otherwise (e.g.; a live birth is later observed).

9.2.4. Sensitivity Analysis

No sensitivity analysis will be performed.

9.2.5. Supplementary Analysis

Additional supplementary analyses such as subgroup analyses will be described in the SAP.

9.3. Analysis Supporting Secondary Objective(s)

The following secondary endpoints for the second phase of the study will be analyzed in the same manner as the primary endpoint (Section 9.2): Cumulative biochemical pregnancy, cumulative clinical pregnancy, and cumulative ongoing pregnancy.

The remaining secondary efficacy endpoints are measured on both the subject- and COC-level and will be tested using two approaches, both under superiority hypotheses. The first approach will use the subject-level definition of each endpoint in a linear regression model. The model will include the endpoint as the outcome variable.

The secondary analyses will yield both a p-value and a corresponding 95% confidence interval. If the p-value is less than 0.05 (or equivalently, if the lower limit of the 95% confidence interval is greater than 0), the null hypothesis H_0 will be rejected in favor of the alternative hypothesis.

The estimand for the first approach is referred to as secondary estimand 1. For secondary estimand 1, the treatment is the culture of one or more immature COCs in either Fertilo or MediCult IVM®. The population is the full analysis set, defined as all randomized Subjects with at least one COC exposed to either Fertilo or MediCult IVM®. The population-level summary is the mean across all Subjects in the given treatment group. The patient-level outcome and intercurrent events depend on the endpoint being considered and are listed in the table below.

Intercurrent events for secondary estimand 1 will be handled according to a hypothetical strategy. Missing values will be imputed using a worst-case approach by imputing a value of 0%. No sensitivity analyses are prespecified for secondary analyses.

9.4. Safety Analyses

Safety data will be presented by summary tables and listings. Missing data will be treated as missing, except for causality, intensity, seriousness, and outcome of treatment-emergent adverse events (TEAE). A worst-case approach will be used: if causality is missing, the TEAE will be regarded as related; if the intensity is missing, the TEAE will be regarded as severe; if seriousness is missing, the TEAE will be regarded as serious; and if outcome is missing and no end date is present, the outcome will be regarded as ongoing. No formal hypothesis tests are planned for any safety endpoints.

9.4.1. Adverse Events

Adverse events will be collected from the time of informed consent until up to 6 weeks postpartum. Adverse events will be coded using MedDRA and the version of MedDRA will be documented. Adverse events occurring on or after the day of embryo transfer will be regarded as TEAEs. Adverse events will be reported in concordance with 21CFR 312.32.

TEAEs will be summarized overall as the number and percent of subjects reporting any TEAEs and the number of events reported for the following categories: all TEAEs, severe TEAEs, related TEAEs, TEAEs leading to discontinuation, SAEs, and deaths. TEAEs will be summarized by system organ class alphabetically and preferred term in decreasing order of frequency. TEAEs will be summarized by preferred term alone in decreasing order of frequency. Summary tables will be produced for the following: all TEAEs, TEAEs by relatedness, TEAEs leading to death, TEAEs by max severity, related TEAEs by max severity, serious TEAEs, TEAEs leading to discontinuation, and non-serious TEAEs with an incidence of more than 5% in either treatment group.

9.4.2. Adverse Events of Special Interest

The following safety events will be collected and summarized by treatment group using categorical summary statistics (number and percent): frequency and intensity of OHSS (mild, moderate, severe), clinical miscarriage rate, ectopic pregnancy rate, preterm delivery, hypertensive disorders of pregnancy (hypertension, pre-eclampsia, HELLP syndrome, and eclampsia), antepartum hemorrhage, gestational diabetes mellitus, fetal abnormalities, and congenital anomalies. Birth weight will also be collected and summarized as a continuous variable.

9.5. Interim Analyses

One interim analysis is planned to occur once all 40 subjects (from both the first and second phase of study) have completed at least one embryo transfer. During the interim, analysis of all available primary and secondary endpoints will be performed for both phases of the study. For subjects with missing data (due to not enough time elapsed for observation at time of the interim), data will not be inferred.

9.6. Sample Size Determination

The assumptions for the sample size calculation are based on the data provided in Piechota et al. 2023 as well as other published studies where MediCult IVM was used. This study is designed as a FIH analysis of safety, using primarily good prognosis infertile couples. Based on outcomes seen in previous studies, generally 20 patients or no less than 65 oocytes are required for determination of efficacy at the embryology level. Therefore, 20 subjects are considered for the first, single arm observational phase of study and 20 subjects (10 per arm) are considered for the second phase of study. This study is not powered for the primary endpoint and is designed to inform later powered trials.

9.7. Protocol Deviations

Protocol deviations will be rated as either important or not important. A list of important protocol deviations will be documented prior to database lock.