Cover page for Statistical Analysis Plan

Sponsor name	Ferring Pharmaceuticals A/S
NCT Number:	NCT03809429
Sponsor trial ID:	000304
Official title of trial:	A randomised, controlled, open label, parallel group, multicentre trial comparing the efficacy and safety of individualised FE 999049 (follitropin delta) dosing, using a long GnRH agonist protocol and a GnRH antagonist protocol in women undergoing controlled ovarian stimulation
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STATISTICAL ANALYSIS PLAN

A randomised, controlled, open label, parallel group, multicentre trial comparing the efficacy and safety of individualised FE 999049 (follitropin delta) dosing, using a long GnRH agonist protocol and a GnRH antagonist protocol in women undergoing controlled ovarian stimulation

Trial 000304

Investigational Product:	FE 999049, human recombinant follicle-stimulating hormone (rFSH), solution for subcutaneous injection
Indication:	Controlled ovarian stimulation for the development of multiple follicles in women undergoing assisted reproductive technologies (ART) such as an in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) cycle
Phase:	3b
Author:	[redacted]
Date of issue:	January 26, 2022
Version:	1.0

Change log

Version No.	Effective Date	Reason for the Change / Revision	Supersedes
1.0	See title page	First version	None

Review and Approval of Statistical Analysis Plan

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1 List of abbreviations

AMH	anti-Müllerian hormone
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ART	assisted reproductive technologies
ATC	Anatomical Therapeutic Chemical classification system
βhCG	beta unit of human chorionic gonadotropin
FAS	full analysis set
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
hCG	human chorionic gonadotropin
ICSI	intracytoplasmic sperm injection
IMP	investigational medicinal product
IU	international units
LH	luteinising hormone
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
OHSS	ovarian hyperstimulation syndrome
SAE	serious adverse event
SAP	statistical analysis plan
SOC	system organ class

2 Introduction

This document describes the planned statistical analyses for Trial FE 999049 000304 and is based on the final protocol version 3.0 dated 09DEC2020. This document do not introduce any changes to the statistical analyses described in the protocol. Some details of the analyses are clarified and some additional analyses are introduced.

The scope of the Statistical Analysis Plan (SAP) is the statistical analysis of data. It does not cover dataset content/structure or content and format of tables, listings, and figures. The latter is described with a similar level of detail as in the protocol.

3 Trial Objectives and Endpoints

3.1 Objectives

Primary Objective

- To evaluate the effect of individualised FE 999049 treatment on ovarian response in a long GnRH agonist protocol versus a GnRH antagonist protocol

Secondary Objectives

- To evaluate the effect of individualised FE 999049 treatment on other pharmacodynamic parameters in a long GnRH agonist protocol versus a GnRH antagonist protocol
- To evaluate the effect of individualised FE 999049 treatment on pregnancy rates in a long GnRH agonist protocol versus a GnRH antagonist protocol
- To evaluate the safety of individualised FE 999049 treatment in a long GnRH agonist protocol versus a GnRH antagonist protocol

3.2 Endpoints

Primary Endpoint

- Number of oocytes retrieved

Secondary Endpoints

- Proportion of subjects with cycle cancellation due to poor ovarian response or excessive ovarian response
- Proportion of subjects with blastocyst transfer cancellation after oocyte retrieval due to (risk of) OHSS
- Number and size of follicles on stimulation day 6 and end-of-stimulation
- Proportion of subjects with <4, 4-7, 8-14, 15-19 and ≥20 oocytes retrieved
- Number of metaphase II oocytes (only applicable for subjects where all oocytes are inseminated using ICSI) fertilisation rate as well as number and quality of embryos on day 3 and blastocysts on day 5 after oocyte retrieval

- Circulating concentrations of FSH, luteinising hormone (LH), estradiol, progesterone and inhibin B on stimulation day 6, end-of-stimulation and at oocyte retrieval
- Total gonadotropin dose and number of stimulation days
- Positive βhCG rate (positive serum βhCG test 13-15 days after transfer)
- Implantation rate (number of gestational sacs 5-6 weeks after transfer divided by number of blastocysts transferred)
- Clinical pregnancy rate (at least one gestational sac 5-6 weeks after transfer)
- Vital pregnancy rate (at least one intrauterine gestational sac with fetal heart beat 5-6 weeks after transfer)
- Ongoing pregnancy rate (at least one intrauterine viable fetus 10-11 weeks after transfer)
- Ongoing implantation rate (number of intrauterine viable fetuses 10-11 weeks after transfer divided by number of blastocysts transferred)
- Proportion of subjects with early OHSS (including OHSS of moderate/severe grade)
- Proportion of subjects with late OHSS (including OHSS of moderate/severe grade)
- Frequency and intensity of adverse events
- Technical malfunctions of the pre-filled injection pen

3.3 Post-trial Information

- Live birth rate and neonatal health, including minor/major congenital anomalies, at birth and at 4 weeks after birth

4 Trial design

This is a randomised, controlled, open label, parallel group, multicentre trial comparing the efficacy and safety of individualised FE 999049 dosing in first cycle patients aged 18-40 years undergoing controlled ovarian stimulation for IVF/ICSI following either a long GnRH agonist protocol or a GnRH antagonist protocol. The trial has been designed to describe potential differences in the mean number of oocytes retrieved with adequate precision.

Subjects will be screened within 90 days prior to randomisation for compliance with the inclusion and exclusion criteria. Randomisation may take place as soon as all eligibility criteria are met. Subjects will be randomised in a 1:1 ratio to controlled ovarian stimulation with FE 999049 in a long GnRH agonist protocol or to controlled ovarian stimulation with FE 999049 in a GnRH antagonist protocol. Randomisation will be stratified by centre and according to age (<35, 35-37 and 38-40 years). The subject will be informed of treatment allocation and when to start treatment by the delegated trial staff.

Subjects randomised to treatment with FE 999049 in the long GnRH agonist protocol will start down-regulation with GnRH agonist (triptorelin acetate, GONAPEPTYL) 0.1 mg/day subcutaneously in the mid-luteal phase (i.e. cycle day 21-24) of their menstrual cycle. Ovarian stimulation with FE 999049 will start after 14 days (±1 day) if the subject has experienced withdrawal bleeding and down-regulation criteria are fulfilled. If down-regulation is confirmed and ovarian stimulation is initiated, treatment with GnRH agonist is continued throughout the stimulation period. If the subject has not experienced withdrawal bleeding after 14 days (±1 day), a urinary pregnancy test is to be performed. If the subject is not pregnant and down-regulation is not confirmed, treatment with GnRH agonist will be continued and stimulation will be postponed until down-regulation is confirmed. Subsequent visit(s) for confirmation of down-regulation will be scheduled according to local practice. If down-regulation is not achieved after 28 days, treatment with GnRH agonist will be stopped and the subject will be withdrawn from the trial.

Subjects randomised to treatment with FE 999049 in the GnRH antagonist protocol will start stimulation with FE 999049 on day 2 or 3 of their menstrual cycle. To prevent a premature LH surge, a GnRH antagonist (cetrorelix acetate, CETROTIDE) 0.25 mg/day subcutaneously will be initiated on stimulation day 6 and continued throughout the stimulation period.

Subjects will have their individual FE 999049 dose determined on the basis of their AMH level at screening and their body weight on stimulation day 1. The daily FE 999049 dose will be fixed throughout the stimulation period. For subjects with AMH <15 pmol/L the daily FE 999049 dose is 12 µg, irrespective of body weight. For subjects with AMH ≥15 pmol/L the daily FE 999049 dose is on a continuous scale ranging from 0.19 to 0.10 µg/kg, i.e. dependent on actual AMH and body weight. AMH will be measured by Elecsys® AMH Plus Immunoassay (Roche Diagnostics). The maximum allowed daily FE 999049 dose is 12 µg. Subjects can be treated with FE 999049 for a maximum of 20 days, and coasting is not allowed.

During stimulation, subjects will be monitored by transvaginal ultrasound on stimulation day 1 and 6 and hereafter at least every second day. When the leading follicle reaches ≥15 mm, visits must be performed daily. Triggering of final follicular maturation will be done as soon as ≥3 follicles with a diameter ≥17 mm are observed. If there are <25 follicles with a diameter ≥12 mm, a single dose of hCG (OVITRELLE) 250 µg will be administered. If there are ≥25 follicles with a diameter ≥12 mm, the cycle will be cancelled. If it is judged by the investigator that ≥3 follicles with a diameter ≥17 mm cannot be reached, but 1 or 2 follicles with a diameter ≥17 mm are observed, the cycle may either be cancelled due to poor follicular development or triggering of final follicular maturation is to be induced, as judged by the investigator.

Oocyte retrieval will take place 36h (±2h) after triggering of final follicular maturation and the oocytes can be inseminated by IVF and/or ICSI. Fertilisation and embryo development will be assessed from oocyte retrieval to the day of transfer. For all subjects blastocyst transfer is performed on day 5 after oocyte retrieval. Subjects <38 years at randomisation will have single blastocyst transfer. Subjects ≥38 years at randomisation will have single blastocyst transfer if they have a good-quality blastocyst available, i.e. a blastocyst of grade 3BB or higher; otherwise they may have double blastocyst transfer. Remaining blastocysts may be cryopreserved and used by the subject after completion of the trial, in accordance with local guidelines and/or regulations. All procedures and assessments related to cryopreserved cycles will take place outside this protocol and will not be paid for by Ferring.

Vaginal progesterone tablets (LUTINUS) 100 mg three times daily will be provided for luteal phase support from the day after oocyte retrieval and until the serum βhCG test. Luteal phase support may be continued after a positive βhCG is obtained if this is local practice. A serum βhCG test is performed 13-15 days after transfer, clinical pregnancy will be confirmed by transvaginal ultrasound 5-6 weeks after transfer, and ongoing pregnancy will be confirmed by transvaginal or abdominal ultrasound 10-11 weeks after transfer.

Blood samples will be collected throughout the trial for the purpose of evaluating the endocrine profile assessed at screening, stimulation day 1, stimulation day 6, end-of-stimulation and at oocyte retrieval.

Post-trial Activities

Post-trial activities cover pregnancy and neonatal health follow-up. All subjects with an ongoing pregnancy will be followed till delivery to gather information on live birth rate. Furthermore, data will be gathered on neonatal health, including minor/major congenital anomalies, at birth and at 4 weeks after birth. These data will be reported separately.

4.1 Design Considerations

The trial will be open label as a double–blind design is not feasible due to the required GnRH agonist treatment prior to start of stimulation in a long GnRH agonist protocol. In contrast, in a GnRH antagonist protocol, the GnRH antagonist is only started during the mid-follicular phase of

the stimulation period. Prior to start of stimulation in a long GnRH agonist protocol, down-regulation needs to be confirmed based on ultrasound findings, thus neither patients nor the assessors in this trial can be blinded. The lack of blinding may result in different withdrawal patterns for the two protocols. To minimise this, only subjects without strong preference for one of the protocols will be included. No measures are taken to prevent investigators, delegated trial staff or subjects from knowing the treatment allocation.

Subjects with an AMH level >35 pmol/L at screening will be excluded from the trial as these subjects are likely to have polycystic ovaries and may have an increased risk of OHSS.

The primary endpoint of this trial will allow the comparison of distribution of the number of oocytes retrieved following each GnRH analogue protocol, and more specifically in subjects with low AMH (<15 pmol/L) and high AMH (≥15 pmol/L). Based on current knowledge about the difference in ovarian response following treatment with CHO-derived rFSH in either protocol an overall treatment difference of at least 1 oocyte is to be anticipated, the difference being smaller in subjects with low AMH than in subjects with high AMH. This treatment difference, especially in subjects with high AMH, will indicate whether the FE 999049 dosing algorithm remains safe and effective when applied in a GnRH agonist protocol.

4.2 Determination of Sample Size

The purpose of this trial is to gain clinical experience with the use of FE 999049 when applied in a long GnRH agonist protocol. The trial is descriptive and does not include any hypothesis testing. The sample size is based on achieving a reasonable precision in the estimate of the difference in mean number of oocytes retrieved between treatment with FE 999049 in a long GnRH agonist protocol versus treatment with FE999049 in a GnRH antagonist protocol.

In the ESTHER-1 trial, the standard deviation for the number of oocytes retrieved was 5.8 in the FE 999049 treatment group. Due to a potentially higher number of oocytes retrieved in the long agonist protocol, the standard deviation in this trial may be slightly higher. Assuming a standard deviation of 7.0, a sample size of 400 subjects (200 subjects per group) will give a 95% CI that ranges from -1.4 to +1.4 of the observed mean difference, i.e. the interval from the observed mean difference -1.4 to the observed mean difference +1.4 will with 95% probability include the true mean difference. This is regarded to be reasonably precise for describing the comparability of individualised FE 999049 dosing using the two protocols.

5 Subject Disposition

All subjects screened and randomised will be accounted for. All post-randomisation discontinuations will be summarised by time of, and reason for, discontinuation. Discontinuations will be classified as occurring before or after start of FE 999049 treatment. The number of subjects screened and not randomised will be presented.

6 Protocol Deviations

Important protocol deviations will be summarised and listed by subject. The impact of important protocol deviations on the trial results may be addressed in additional sensitivity analyses.

7 Analysis sets

7.1 Full Analysis Set

The FAS will consist of all subjects that were randomised, regardless of whether they started FE 999049 treatment or not. However, subjects that discontinued before starting FE 999049 treatment due to the COVID-19 pandemic (e.g. due to operational and/or societal issues) will be excluded from the FAS. In the analyses of efficacy endpoints subjects will be analysed according to the protocol to which they were randomised.

7.2 Safety Analysis Set

The safety analysis set will consist of all subjects that were randomised in the trial and were exposed to FE 999049. In all presentation of safety data subjects will be presented according to the protocol they were actually treated with.

8 Trial population

8.1 Demographics and Other Baseline Characteristics

Descriptive statistics of demographics and other baseline characteristics (body measurements, infertility history, menstrual history, reproductive history, smoking and alcohol habits, physical examination, and gynaecological examination) will be presented for the FAS. In addition, a summary of demographic characteristics will be presented by trial site.

8.2 Medical History and Concomitant Medication

Medical history and concomitant medication will be presented for the FAS. Medical history recorded at the screening visit will be coded using MedDRA and summarised. The version of MedDRA will be documented. Prior and concomitant medication will be summarised by ATC classification 1st level (alphabetically) and ATC classification 2nd level (in decreasing order of frequency). These medications will be tabulated separately for:

- Prior medication, i.e. medication taken exclusively prior to treatment (i.e. with stop date/time before date/time of 1st IMP administration)

- Concomitant medication, i.e. medication taken during the treatment period (i.e. medication that was not stopped before date/time of 1st IMP administration and not started after the end-of-trial visit)

If the timing of the dose of a concomitant medication cannot be established in relation to the administration of IMP, it will be considered as concomitant medication.

9 Treatment Compliance

Number of days with GnRH agonist and GnRH antagonist treatment will be summarised.

Compliance with progesterone treatment for luteal phase support will be summarised (number of days with treatment, completed according to instruction, number of tablets missed, and prematurely stopped due to menstrual bleeding).

10 Efficacy

10.1 General Considerations

The primary objective of the trial is to evaluate the effect of individualised FE 999049 treatment on ovarian response in a long GnRH agonist protocol versus a GnRH antagonist protocol. The primary endpoint is the number of oocytes retrieved.

The estimand of interest is the difference between FE 999049 treatment in the long GnRH agonist protocol versus treatment with FE 999049 in the GnRH antagonist protocol for all randomised subjects, assuming that all subjects start treatment with FE 999049. In the statistical analyses, subjects that discontinue after randomisation but before start of FE 999049 treatment (except for subjects that never started treatment due to the COVID-19 pandemic, which are not included in any analysis) will have their endpoint data imputed from subjects who started FE 999049 treatment in the same protocol, using a multiple imputation method. No imputation will be done for subjects who discontinue the trial after starting FE 999049 treatment. For these subjects, the actual data will be used and not be regarded as missing. Therefore, the number of oocytes retrieved will be regarded as zero and pregnancy outcomes will be regarded as negative.

The trial is descriptive and no formal hypothesis will be tested. The statistical analyses aims at estimating any potential differences between FE 999049 treatment when applied in the two different GnRH protocols. The results will generally be described as an estimated mean treatment difference or treatment ratio and an associated 95% confidence interval.

Since the trial is descriptive without any hypothesis testing, no corrections for multiplicity will be applied.

Analyses and descriptions for ovarian response endpoints and other pharmacodynamics endpoints will be based on the FAS. Analyses and descriptions for pregnancy endpoints will be based on the FAS but excluding subjects that had transfer cancellation due to the COVID-19 pandemic. Additional descriptions for pregnancy endpoints will be made based on the FAS (i.e. including also the subjects with transfer cancellation due to the COVID-19 pandemic). Analyses and descriptions of safety endpoints and exposure will be based on the safety analysis set.

Subjects in the FAS that did not start treatment with FE 999049 will not have any data after exposure to FE 999049. For these subjects, post-randomisation data will only be imputed for the purpose of statistical analysis, using multiple imputation techniques. In tables, figures and listings all post-randomisation data from these subjects will be missing. Therefore tables, figures and listings will be based on subjects in the FAS starting treatment with FE 999049, i.e. randomised and exposed subjects (i.e. same as the safety analysis set).

10.2 Primary Endpoint

The primary endpoint, number of oocytes retrieved, will be analysed using a negative binomial regression model with treatment (long GnRH agonist protocol or GnRH antagonist protocol), age

stratum (<35, 35-37, or 38-40 years), and AMH at screening (<15 pmol/L or ≥15 pmol/L) as factors. A logarithmic link function will be used for the mean in the model. From this model, the mean difference between the two protocols in number of oocytes retrieved will be estimated, and a 95% CI for the estimate will be calculated using the delta method. The analyses will be done using the multiple imputation method described below.

Age stratum is included as factor in the model since randomisation is stratified by age. AMH at screening is additionally included as a factor since AMH is known to influence the number of oocytes retrieved. Although randomisation is stratified by centre, centre and the interaction between centre and age stratum are not included in the model as all centres may not randomise subjects from all age strata, and therefore the inclusion of the interaction in the model is likely to give empty cells in the analysis. The potential influence of centre differences on the results will instead be investigated in a sensitivity analysis.

For subjects in the FAS who discontinue the trial after randomisation but before start of FE 999049 treatment, the number of oocytes retrieved will be imputed from subjects who started FE 999049 treatment in the same protocol, using the following multiple imputation method:

- First, a negative binomial regression model with age stratum and AMH at screening as factors will be fitted to data from subjects who started FE 999049 treatment for each protocol separately.
- In the second step, 1000 complete datasets will be constructed. For the construction of each complete dataset, a set of parameter values will be sampled from a normal distribution with characteristics determined by the estimated parameters and covariance matrix for the two models in the first step. Missing individual subject data will then be imputed from a negative binomial distribution with the sampled parameters.
- In the third step, each complete dataset will be analysed using the negative binomial regression model described in the first paragraph of this section.
- In the final step, the estimated mean differences and the standard deviations from the 1000 analyses will be combined using Rubin's formula:

$$m_{MI} = \frac{1}{1000}\sum_{i=1}^{1000} m_{i,} \quad SD_{MI} = \sqrt{\frac{1}{1000}\sum_{i=1}^{1000} {SD_i}^2 + \left(1+\frac{1}{1000}\right)\left(\frac{1}{1000-1}\right)\sum_{i=1}^{1000}(m_i - m_{MI})^2}$$

  Where m_i and SD_i are the estimated mean differences and standard deviations for the differences for the 1000 copies of the dataset (derived using the delta method), and m_{MI} and SD_{MI} are the pooled estimates.

- The mean difference in number of oocytes retrieved between the long GnRH agonist protocol and the GnRH antagonist protocol will be estimated as m_{MI}, and the 95% CI for the estimate will be calculated as $m_{MI} \pm 1.96 * SD_{MI}$.

The assumption underlying the above imputation procedure is that all randomised subjects could have started FE 999049 treatment, and that their outcome would not have differed in any systematic way from the subjects that started FE 999049 treatment.

For subjects who discontinue the trial after start of FE 999049 treatment but before oocyte retrieval the number of oocytes retrieved will be regarded as zero.

Supplementary analyses and descriptions for the Primary Endpoint

The primary analysis will be repeated based on subjects in FAS that started treatment with FE 999049, i.e. without using multiple imputations (this is the same as the safety analysis set).

The potential influence of centre differences on the results for the primary endpoint will be investigated by repeating the analyses of the 1000 complete datasets generated above, with the addition of centre and the interaction between centre and age strata as factors in the model.

Additional analyses will be made for two subgroups based on AMH at screening (<15 pmol/L or ≥15 pmol/L) and for the three age strata (<35, 35-37, or 38-40 years). These analyses will be based on the 1000 complete datasets used for the primary analyses and will use the same analysis model, except that age stratum will not be included as factor in the age strata analyses and AMH will not be included in the AMH subgroup analyses. The results of the subgroup analyses will be illustrated in a forest plot.

The distribution of number of oocytes retrieved will be described using the empirical distribution and approximated using kernel estimates.

10.3 Secondary Efficacy Endpoints

Proportion of Subjects with Cycle Cancellation due to Poor Ovarian Response or Excessive Ovarian Response

The proportion of subjects with cycle cancellation due to poor ovarian response or excessive ovarian response will be described. Additional descriptions will be made for the two subgroups based on AMH at screening (<15 pmol/L or ≥15 pmol/L) and for the three age strata (<35, 35-37, or 38-40 years).

Proportion of Subjects with Blastocyst Transfer Cancellation after Oocyte Retrieval due to (Risk of) OHSS

The proportion of subjects with transfer cancellation after oocyte retrieval due to (risk of) OHSS will be described. Additional descriptions will be made for the two subgroups based on AMH at screening (<15 pmol/L or ≥15 pmol/L) and for the three age strata (<35, 35-37, or 38-40 years).

Number and Size of Follicles on Stimulation Day 6 and End-of-stimulation

The follicle cohort on stimulation day 6 and end-of-stimulation will be summarised by treatment on the follicle level (number of follicles 8-9 mm, 10-11 mm, 12-14 mm, 15-16 mm and ≥17 mm) and on the subject level (total number of follicles, size of largest follicle, average follicle size, average size of three largest follicles, and number of follicles ≥8 mm, ≥10 mm, ≥12 mm, ≥15 mm and ≥17 mm).

The mean number of follicles ≥8 mm, ≥10 mm, ≥12 mm, ≥15 mm and ≥17 mm at end-of-stimulation will (in separate analyses) be compared between the long GnRH agonist protocol and the GnRH antagonist protocol using the same negative binomial regression model as used for the primary endpoint. The analysis will only include subjects in the FAS that started treatment with FE 999049, i.e. multiple imputations will not be used (this is the same as using the safety analysis set).

Proportion of Subjects with <4, 4-7, 8-14, 15-19 and ≥20 Oocytes Retrieved

The percentage of subjects with <4, 4-7, 8-14, 15-19 and ≥20 oocytes retrieved will be described in histograms. Additional descriptions will be made for the two subgroups based on AMH at screening (<15 pmol/L or ≥15 pmol/L) and for the three age strata (<35, 35-37, or 38-40 years).

Number of Metaphase II Oocytes

Oocytes undergoing ICSI will have their maturity stage assessed prior to insemination. The total number of metaphase II oocytes will be compared between the long GnRH agonist protocol and the GnRH antagonist protocol using the same analysis method as for the primary endpoint. This analysis will be based on subjects that had all their oocytes inseminated using ICSI. No imputation will be done for subjects having their oocytes inseminated by IVF, or a combination of IVF and ICSI, and consequently no imputation will be done for subjects not starting treatment with FE 999049. Therefore, multiple imputations will not be used and the analysis will be based on subjects in FAS that had all their oocytes inseminated using ICSI (this is the same as subjects in the safety analysis set that had all their oocytes inseminated using ICSI).

Fertilisation Rate

An oocyte is defined as fertilised if it is scored as 2 pronuclei on day 1 after oocyte retrieval. The total number of fertilised oocytes will be compared between the long GnRH agonist protocol and the GnRH antagonist protocol using the same analysis method as for the primary endpoint. The proportion of oocytes becoming fertilised will be summarised by treatment.

Number and Quality of Embryos on Day 3 after Oocyte Retrieval

The number and the quality of embryos on day 3 will be described. The number of embryos and the number of good-quality embryos will be compared between the long GnRH agonist protocol and the GnRH antagonist protocol using the same analysis method as for the primary endpoint. A good-quality embryo is defined as an embryo with ≥6 blastomeres and ≤25% fragmentation, or with embryo stage classified as compacting/compacted.

Number and Quality of Blastocysts on Day 5 after Oocyte Retrieval

The number and the quality of blastocysts on day 5 will be described, as well as the quality and number of blastocysts transferred.

The number of blastocysts and the number of good-quality blastocysts (grade 3BB or higher) will be compared between treatments using the same analysis method as for the primary endpoint.

Circulating Concentrations of FSH, LH, Estradiol, Progesterone and Inhibin B on Stimulation Day 6, End-of-stimulation and at Oocyte Retrieval

For each parameter, the concentrations on stimulation day 6, end-of-stimulation, and at oocyte retrieval will be described and compared for the long GnRH agonist protocol versus the GnRH antagonist protocol using analysis of variance models with treatment, age stratum, and AMH at screening (<15 pmol/L or ≥15 pmol/L) as factors. Multiplicative models will be used, i.e. the concentrations will be log-transformed before analysis. The results of the analyses will be back-transformed and presented as estimated geometric means for each parameter, and the mean ratios for long GnRH agonist protocol versus GnRH antagonist protocol with 95% confidence intervals.

The analyses will be based on subjects in the FAS starting treatment with FE 999049, i.e. multiple imputations will not be used (this is the same as using the safety analysis set). Values below the lower limit of quantification (LLOQ) will be estimated with LLOQ/2.

Total Gonadotropin Dose and Number of Stimulation Days

The total gonadotropin dose and the number of stimulation days will be described for each of the two protocols and for subgroups based on age strata and AMH at screening.

Number of stimulation days, total dose, and the average daily dose will be compared between long GnRH agonist protocol and the GnRH antagonist protocol using an analysis of variance model with treatment, age stratum, and AMH at screening (<15 pmol/L or ≥15 pmol/L) as factors. This analysis will be based on subjects in the FAS that started treatment with FE 999049, i.e. multiple imputations will not be used (this is the same as using the safety analysis set).

Pregnancy Rates

The percentage of subjects with positive βhCG, clinical pregnancy, vital pregnancy, and ongoing pregnancy will be described for each of the two protocols and for subgroups based on age strata and AMH. Pregnancy rates will be compared between the long GnRH agonist protocol and the GnRH antagonist protocol using a logistic regression model with treatment (long GnRH agonist or GnRH antagonist), age stratum (<35, 35-37, or 38-40 years), and AMH at screening (<15 pmol/L or ≥15 pmol/L) as factors. The results of this analysis (in terms of odds for pregnancy) will be converted into proportions, differences in proportions, and 95% CIs for differences in proportions using the delta method.

To account for subjects withdrawing from the trial before starting FE 999049 treatment, the multiple imputation method applied for the primary endpoint will be used, using a logistic regression model instead of a negative binomial regression model. Pregnancy data for subjects with pregnancies occurring before start of FE 999049 treatment will not be included in the analysis. These subjects are withdrawn and their data will be imputed.

Missing pregnancy assessments will be assumed to indicate a negative test. If, however, a subsequent test is positive, a previous missing assessment will be considered as positive (if e.g. the clinical pregnancy assessment is missing but the ongoing pregnancy assessment is positive, the missing clinical pregnancy assessment will be regarded as positive).

The main analyses of pregnancy rates will be based on subjects in the FAS, but excluding subjects that had transfer cancellation due to the COVID-19 pandemic. Additional analyses will be made for the FAS, and also for subjects in the FAS that started treatment with FE 999049, i.e. without using multiple imputations (i.e. the safety analysis set).

Implantation Rates

The implantation rate will be calculated as the number of gestational sacs 5-6 weeks after transfer divided by the number of blastocysts transferred.

Ongoing implantation rate will be calculated as the number of intrauterine viable fetuses 10-11 weeks after transfer divided by the number of blastocysts transferred.

The implantation rate and the ongoing implantation rate will be described for each of the two protocols and for subgroups based on age strata and AMH at screening.

11 Safety

11.1 OHSS

Proportion of Subjects with Early OHSS (including OHSS of Moderate/Severe grade)
Incidence of early OHSS will be tabulated by classification (mild, moderate, severe, moderate or severe) and grade (1, 2, 3, 4, 5). Early OHSS is defined as OHSS with onset ≤9 days after triggering of final follicular maturation. Note this includes OHSS with onset before triggering and OHSS with onset during stimulation where triggering is not performed.

Proportion of Subjects with Late OHSS (including OHSS of Moderate/Severe grade)
Incidence of late OHSS will be tabulated by classification (mild, moderate, severe, moderate and severe) and grade (1, 2, 3, 4, 5). Late OHSS is defined as OHSS with onset >9 days after triggering of final follicular maturation.

Proportion of Subjects with Any OHSS (including OHSS of Moderate/Severe grade) Incidence of any OHSS will be tabulated by classification (mild, moderate, severe, moderate and severe) and grade (1, 2, 3, 4, 5).

11.2 Adverse Events

Adverse events will be coded using MedDRA. The version of MedDRA will be documented. Adverse events will be grouped according to start of FE 999049 as follows:

- Pre-treatment adverse event, i.e. any adverse event occurring after signed informed consent and before start of FE 999049 treatment, or a pre-existing medical condition that worsens in intensity after signed informed consent but before start of FE 999049 treatment.
- Treatment-emergent adverse event, i.e. any adverse event occurring after start of FE 999049 treatment and before the end-of-trial visit, or a pre-treatment adverse event or pre-existing medical condition that worsens in intensity after start of FE 999049 treatment and before the end-of-trial visit.

Treatment-emergent adverse events will be presented in summary tables and listings. Pre-treatment adverse events will be presented in a listing only. Treatment-emergent adverse events will be summarised overall and tabulated by System Organ Class (SOC) and PT in decreasing order of frequency. The total number of subjects reporting an adverse event, the percentage of subjects with an adverse event, and the number of events reported will be presented.

Summary tables will be produced for the following: all adverse events, adverse events by causality (reasonable possibility / no reasonable possibility), adverse events leading to death, adverse events by intensity (mild / moderate / severe), adverse reactions[I] by intensity (mild / moderate / severe), serious adverse events, adverse events leading to discontinuation, adverse events with an incidence of ≥5% in any treatment group, and non-serious adverse events with an incidence of ≥5% in any treatment group.

11.3 Technical Malfunctions of the Pre-filled Injection Pen

The frequency of technical malfunctions of the administration pen will be tabulated and all technical malfunctions will be listed.

12 Post-trial Information

Live birth rate and neonatal health, including minor / major congenital anomalies, at birth and at 4 weeks after birth will be described. These data will be reported separately. Live birth and live at 4 weeks status will be analysed using the same method as for pregnancy endpoints.

[I] An adverse reaction is defined as an adverse event with reasonable possible causal relationship to IMP

13 Interim analyses

No interim analysis is planned.

14 Changes to the analyses described in the protocol

This SAP does not introduce any changes to the analyses already described in the trial protocol. A few additional details and clarifications have been added, and a few additional analyses have been added. These include the following:

- Addition of supplementary analyses based on subjects in the FAS starting treatment with FE 999049, i.e. analyses without the use of multiple imputations (this is the same as using the safety analysis set).
- Clarification that the analysis of number of MII oocytes will be based on subjects that had all their oocytes inseminated using ICSI, and subsequently multiple imputations will not be used.
- Addition of analyses for number of follicles, embryos, number of good-quality embryos, and number of blastocysts.
- Addition of analyses for number of stimulation days, total dose, and the average daily dose of FE 999049 treatment.