Statistical Analysis Plan

SALSTER

SALpingectomy for STERilisation

2025-01-11

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Revisions

Version	Description of Changes	Date
1.0	Original SAP	2023-03-22
1.1	1.2 Randomisation detail added. 2.2.1 Clarification about the non-randomised questionnaire population added. Data retrieval set 2 formulated.	2023-07-09
2.0	6 Sub-study AMH section was added	2024-01-11

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1 STUDY DETAILS

1.1 Study Objectives

This registry-based randomised controlled trial (R-RCT) will study the safety of laparoscopic salpingectomy for sterilisation compared with tubal ligation. The underlying rationale is the potential of opportunistic salpingectomy to reduce the incidence of high grade serous ovarian cancer. The specific aim is to analyse if the risk of complications and hormonal side effects do not increase beyond pre-defined non-inferiority margins after salpingectomy compared with tubal ligation.

The primary objectives are to test the hypotheses that salpingectomy compared with tubal ligation for laparoscopic sterilisation,

- does not increase the risk for complications perioperatively and up to eight weeks postoperatively beyond a pre-defined non-inferiority margin.
- does not cause earlier menopause beyond a pre-defined non-inferiority margin, assessed as age at onset of natural menopause.

The secondary objectives are to compare the two randomisation groups regarding the secondary outcomes listed under 3.3.

In a nested trial, ovarian function will be evaluated comparing the mean difference of anti-Müllerian hormone (AMH), assessed preoperatively and one year after surgery. A separate SAP will be written for the AMH nested trial.

In the long term, the ovarian cancer outcome will be pooled with data from the Hysterectomy and OPPortunistic SAlpingectomy (HOPPSA) trial in an individual participant data (IPD) meta-analysis. A separate SAP will be written for the IPD meta-analysis.

The present statistical analysis plan (SAP) concerns the analysis of the peri-operative, short and intermediate terms outcomes in SALSTER. Separate SAPs will be made for the long-term outcomes.

1.2 Study Design

SALSTER is a national registry-based randomised controlled non-inferiority trial with parallel groups. Women <50 years wishing laparoscopic sterilisation are randomised to either salpingectomy or tubal ligation in proportion 1:1, using permuted blocks with random sizes of either two or four, while stratifying for center. The Swedish National Quality Register of Gynecological Surgery (GynOp) is used for inclusion, randomisation, and follow-up. Blinding is attempted but not guaranteed.

1.3 Treatment Groups

The patients are allocated to undergo salpingectomy (intervention group) or tubal ligation (control group).

1.4 Sample Size

A minimum of 968 women should be randomised according to the calculations of sample sizes for the two primary outcomes:

Primary short-term outcome: *complications up to eight weeks post-operatively*
Complications (mild and severe) to laparoscopic tubal ligation is registered in GynOp at a rate of 13.6% in the period of 2010-2017. An increase of 3% is estimated after salpingectomy. If

the non-inferiority margin is defined as +10%, the upper limit of the two-sided 95% CI (α=0.05) for the difference between the salpingectomy group and the tubal ligation group shall not be above the +10% with a probability of 80% (β=20%). To demonstrate non-inferiority, 411 women per randomisation group are needed (based on a two-sided Farrington-Manning test). For protection against a 10% loss to follow-up, the target sample was determined at 914. The interim analysis revealed that 5% of randomised women interrupted their participation. For protection against this loss, the target was increased to 968.

Primary long-term outcome: *age at onset of natural menopause*
Age at menopause on a Swedish population level is reported to be in mean 51.5 years (SD 3.0). A decrease of one year is estimated after salpingectomy. If the non-inferiority margin is defined as two years, the upper limit of the two-sided 95% confidence interval (α=0.05) for the difference between the salpingectomy group and the tubal ligation group shall not be above two years with a probability of 80% (β=20%). To demonstrate non-inferiority, 143 women per randomisation group are needed (two-sided non-parametric permutation test for comparison of two means). Considering exclusion of women without a natural menopause (30%), 5% of randomised women interrupting participation before the eight-weeks questionnaire, and 15% loss during the 20 years long follow-up, approximately 572 women are needed for recruitment.

2 STUDY POPULATIONS

2.1 Definition of Randomised Study Populations

2.1.1 Intention-To-Treat (ITT) Population.

All randomised subjects will be included in the ITT-population.

2.1.2 Full-Analysis-Set (FAS) Population

All randomised subjects who did not have an eligibility violation or actively withdrew consent will constitute the basis for the FAS-population according to the ITT principle. A requirement for inclusion in the FAS population is that the subject started the allocated surgical intervention.

2.1.3 Per-Protocol Population

The per-protocol population is defined from the FAS population as the subjects who were randomised to and underwent the allocated surgical intervention. The population differ depending on which outcome is being analysed.

For the first primary outcome *Any complication up to eight weeks post-operatively*, subjects with an intended salpingectomy that was converted to tubal ligation due to a perioperative complication, will still be included in the per-protocol population.The reason is to avoid exclusion of a subject with a true complication related to the allocated intervention. The same principle is applied when the same scenario occurs at an intended tubal ligation.
If a conversion of the allocated procedure NOT related to a perioperative complication occurs, the subject is excluded from the per-protocol population.
The surgical route is not part of the intervention, and conversion to laparotomy will thus not conflict with the inclusion of a subject to the per-protocol population.

For the second primary outcome *Age at onset of natural menopause*, the per-protocol population will be defined by those who underwent the allocated intervention, regardless of conversion to laparotomy for any reason.

2.2 Definition of Non-randomised Populations

2.2.1 Non-randomised Questionnaire Population

All eligible individuals who have not consented to randomisation but to follow-up questionnaires.
This population will be followed and analysed as observational data in separate publications.

2.2.2 Background Population

All individuals who underwent a sterilisation procedure (code LGA or LBE with diagnosis code Z30.2) and were registered in GynOp during the study period of SALSTER.

3 STUDY VARIABLES

3.1 Baseline Variables

3.1.1 Demographics and Baseline Characteristics

Standard variables registered in GynOp; present bleeding pattern, coitus pain, previous pregnancies, miscarriages, extrauterine pregnancies and parity, gynecological diseases, including previous salpingitis and previous surgery, weight, length, BMI, smoking habits, present or previous diseases, medication, ASA-classification.
Specific study variables added to GynOp; age at menarche, duration of breast feeding, previous and present use of hormonal contraceptives, previous Chlamydia infection, and scale for menopausal symptoms.
Specific operative study variables are the number and sizes of trocars, and instruments used.
Variable details are specified in Appendix 1 (Data retrieval set 1).

3.2 Primary Outcome Variables

3.2.1 First Primary Outcome Variable (Short term)

First primary outcome is *Any complication up to eight weeks post-operatively* (as per definition in GynOp, dichotomous). This outcome includes peri- and post-operative outcomes registered by the physician, as well as complications up to eight weeks post-operatively, assessed by the physician after the patient's questionnaire.
Variable details are specified in Appendix 2 (Data retrieval set 2).

3.2.2 Second Primary Outcome Variable (Long term)

Second primary outcome is *Age at onset of natural menopause*. This outcome is based on the bleeding pattern reported in every-other year questionnaire up to menopause or the age of 55. Details of planned analyses for this outcome will be reported in a separate SAP that will be published before data retrieval.
Variable details will be specified in a subsequent appendix before data retrieval.

3.3 Secondary Outcome Variables

3.3.1 Secondary Peri-operative Outcome Variables

Operative time (continuous, min)
Perioperative blood loss (continuous, ml)
Length of hospital stay (continuous, days)
Variable details are specified in Appendix 1 (Data retrieval set 1).

3.3.2 Secondary Outcome Variables in the Short term (up to eight weeks)

Severe complication (as per definition in GynOp, dichotomous)
Complication classified according to Clavien-Dindo (1-5, categorical)
Type/site of complication (descriptive)
Variable details are specified in Appendix 2 (Data retrieval set 2).

3.3.3 Secondary Outcome Variables in the Intermediate term (at one year)

Complications any, severe (dichotomous)
Complication classified according to Clavien-Dindo (1-5, categorical)
Subsequent surgery on uterus, salpinges and/or ovaries (dichotomous)
Pregnancy (dichotomous)
Satisfaction with surgery (1-5, ordinal scale)
Type/site of complication (descriptive)
Variable details will be specified in Appendix 3 (Data retrieval set 3).

3.3.4 Secondary Outcome Variables in the Long term (3-30 years)

Secondary outcome variables in the long term will be retrieved from GynOp as well as from Swedish health registers and national quality registers.
Age at the start of the perimenopausal state (continuous, years)
Length of the perimenopausal state (continuous, months)
Change in menopausal symptom score from baseline (continuous, scale units)
Use of menopausal hormone therapy at any time during follow-up (dichotomous, and continuous, months)
Subsequent surgery on uterus, salpinges and/or ovaries (dichotomous)
Pregnancy (dichotomous)
Secondary expressions of oestrogen deficiency (e.g. cardiovascular events, osteoporotic fractures, cognitive disorders, dichotomous) will be analysed if there is a difference in ovarian function in other previous outcome variables.
Variable details will be specified in a subsequent appendix before data retrieval.

3.4 Safety Variables

All primary and the majority of secondary outcomes are safety outcomes and described under 3.2 and 3.3.

4 STATISTICAL METHODOLOGY

4.1 General Methodology

The two primary analyses measure different outcomes (*Any complication up to eight weeks post-operatively* and *Age at onset of natural menopause*) at different time points and will be published in separate articles. As they also test two different hypotheses, we will refrain from adjusting the 5% significance level for multiplicity.

Missing data on the primary outcomes will be replaced with multiple imputation using fully conditional specification in the main analysis. In addition, a complete case analysis will be conducted as a sensitivity analysis. If both analyses of the two primary outcomes demonstrate non-inferiority, a common conclusion on the safety of the intervention can be inferred. However, the long period between these analyses will entail separate conclusions on complications and age at menopause, in a temporal order.

The non-inferiority hypothesis tests will be performed as one-sided test, with a significance level of 2.5%. This is equivalent to the upper limit of a two-sided 95% confidence interval for the difference between salpingectomy and tubal ligation, being lower than the non-inferiority margin. Results will be presented as the difference between groups with a two-sided 95% CI.

The secondary outcomes will be evaluated using two-sided test, with the arms being equal with respect to the outcomes as null hypothesis. All confidence intervals presented will be 95% and two-sided.

All statistical analyses will be made using R as statistical software (1).

4.2 Patient Disposition and Data Sets Analysed

The number of subjects included in each of the ITT, FAS and per-protocol populations will be summarised for each treatment group and overall. The ITT-population will only be used for analysis of baseline characteristics to evaluate that the randomisation procedure has been properly conducted resulting in comparable groups. The number and percentage of subjects randomised and treated will be presented. Subjects who completed the study and subjects who withdrew from study prematurely will also be presented with a breakdown of the reasons for withdrawal by treatment group for the per-protocol and for the FAS populations. Analyses will be performed on both the per-protocol and FAS populations. For non-inferiority design, the “per protocol” analysis will be the primary.
The main analyses for the primary outcomes will be on the per-protocol population with imputation for missing data and the sensitivity analysis will be on the per-protocol population with a complete case set.
Analyses on the FAS population will also be with imputed data and with a complete case set.

The characteristics of the background population in the GynOp register (2.2.2) will be reported exclusively for demographic and operative descriptive data, to evaluate the generalizability of the SALSTER results.

4.3 Protocol Violations/Deviations

Deviations from allocated surgical procedure are expected. The main reasons are
-to refrain from salpingectomy or tubal ligation when a severe adhesion state is likely to cause complications
-to perform salpingectomy despite allocated tubal ligation, when unexpected tubal or ovarian pathology is detected peri-operatively.
These protocol deviations will be summarised.
Any surgical procedure (e.g. peritoneal and ovarian biopsy) added to the allocated intervention will be registered but not considered a protocol deviation. Unilateral oophorectomy performed due to ovarian pathology, is not a protocol violation but may impact on outcomes related to ovarian function. A subsequent SAP on menopausal-related outcomes will deal with these issues.
Oophorectomy performed due to complicated bleeding will be registered as a complication.

4.4 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarised by treatment group for the ITT and FAS populations and analysed according to the methods described in section “General Methodology” above.

4.5 Prior and Concomitant Medications

Any hormonal treatment for gynaecological indication prior to surgery will be registered as baseline variable and reported per randomisation group. Any post-operative hormonal treatment with a gynaecological indication will be registered and considered as a co-intervention affecting menopausal symptoms and possibly causing treatment induced changes in bleeding pattern. Age at natural menopause will not be possible to evaluate in these subjects and they will not be included in the analysis of this outcome. Details will be presented in the SAP for the primary outcome *Age at natural menopause.*

4.6 Primary Analyses

4.6.1 First Primary Outcome Analysis (Short term)

Primary analysis: The number and percentage of *Any complication up to eight weeks post-operatively* will be presented by treatment groups. The primary effect measure will be the difference in risk complication between the groups. To account for the lack of independence introduced by the stratification of the randomisation, the primary analysis will estimate the difference in the complication risk between the two randomised groups with a 95% CI using a generalised estimation equation (GEE) with logistic link function, marginalised over centre. An exchangeable covariance will be used. The two-sided 95% CI of the marginal risk difference will be estimated from the GEE-model using the delta method. Non-inferiority will be declared if the upper limit of the 95% CI does not exceed the non-inferiority margin of 10%. For fitting the GEE model, the function *geeglm* from the R package *geepack* will be used, while the CI for the marginal group difference will be estimated from the GEE model using the function *avg_comparisons* from the R package *marginaleffects*.
As a sensitivity analysis, the unadjusted 95% CI for the difference in complications will be calculated according to Ferrington-Manning.
Furthermore, unadjusted risk ratio (RR) and adjusted RR with 95% CI will also be calculated in secondary analyses using a GEE Poisson model with robust standard errors.

4.6.2 Second Primary Outcome Analysis (Long term)

A detailed SAP for the long term outcome *Age at onset of natural menopause* will be written in due time.

4.7 Secondary Analyses

Secondary outcome variables are given in section 3.3. The secondary peri-operative outcomes, which are all continuous variables with an expected positively skewed distribution, will be presented as median and quartiles, mean and SD, minimum and maximum by treatment group. The outcomes will be tested using proportional odds models, adjusted for centre as fixed effects. Odds ratios (ORs) with 95% CI will be presented. Unadjusted tests will be performed as sensitivity using Fisher's non-parametric permutation tests.

Dichotomous short and intermediate terms secondary outcomes will be presented as numbers and percentages. Their primary effect measure will be relative risks, estimated from GEE Poisson regression model with robust standard errors. Furthermore, risk differences between groups and their 95% CI will be presented.

Ordinal short- and intermediate term secondary outcomes will be presented as numbers and percentages. The outcomes will be tested using proportional odds models, adjusted for centre as fixed effects. Odds ratios with 95% CI will be presented. Unadjusted tests will be performed as sensitivity using Fisher's non-parametric permutation tests.

4.8 Safety analyses

Separate safety analyses are not applicable, since the outcomes are safety variables.

4.9 Exploratory analyses

4.9.1 Subgroup analyses

Subgroup analyses for the primary outcome *Complications* will be conducted for the following variables: age, BMI, previous salpingitis, adhesion status, previous abdominal surgery.

4.9.2 Interaction analyses

Interactions of age, BMI, previous salpingitis, adhesion status, previous abdominal surgery will be analysed.

4.9.3 Other exploratory analyses

Association between number and sizes of trocars, and the outcomes *Complication* and *Satisfaction* respectively, will be analysed per treatment group.

5 INTERIM ANALYSIS

One interim analysis for safety has been performed. The interim analysis was considered a futility analysis (2) and no adjustment of alpha level will be performed to account for this.

6 SUB-STUDY ON ANTI-MÜLLERIAN HORMONE (AMH)

6.1 Study details

6.1.1 Study objectives

The objective is to test the hypothesis that salpingectomy for sterilization compared with tubal occlusion, does not increase the risk of ovarian impairment beyond a pre-defined limit, one year after surgery.

6.1.2 Study design

This is a multi-center, non-inferiority trial nested within the R-RCT SALSTER. Women enrolled in SALSTER across six gynecological departments (Central Hospital Karlstad, Örebro University Hospital, Eksjö Hospital, Kungsbacka Hospital, Danderyd Hospital, Helsingborg Hospital, and Borås Hospital) between 2019 and 2023 were invited to participate in this sub-study. Serum AMH levels were measured both at baseline and one-year post-surgery.

6.1.3 Treatment groups

Patient allocation was determined by the original SALSTER randomization: salpingectomy (intervention group) or tubal occlusion (control group).

6.1.4 Sample size

The non-inferiority margin for the difference in change of serum levels of AMH (expected reduction) was defined as 0.2 µg/L. Thus, non-inferiority will be declared if the upper limit of

the two-sided 95% CI for the difference in change between the two groups (intervention group - control group) does not exceed 0.2 µg/L. Assuming a SD of change in AMH level of 0.45 (µg/L) and a true difference in reduction of AMH between groups of 0 (i.e. no difference in change), 81 patients are needed per randomization group to reach a power of 80% (β=20%). Originally, the loss to follow-up was expected to be 20%, consequently 204 patients was the target in this nested trial.

6.2 Study populations

6.2.1 Intention to treat population

All randomized subjects with a baseline blood sample taken will be included in the ITT-population.

6.2.2 Per-protocol population

The per-protocol population is defined from the ITT population and includes all subjects that underwent the allocated surgical intervention. Any ovarian surgery during the sterilization procedure or during the period up to the one-year postoperative sampling of AMH, will exclude the subject.

6.2.3 As treated population

Subjects in the ITT population not following the allocated treatment will be analyzed “as treated”.

6.3 Study variables

6.3.1 Baseline variables

Already present in GynOp: age, weight, length, BMI, ASA-classification, autoimmune diseases, employment, smoking habits, length of hormonal contraception, parity, gynecological diseases and symptoms, and prior abdominopelvic surgery.

Specific study variables added to GynOp: age at menarche, menstruation pattern, duration of breast feeding, previous and present use of hormonal contraceptives, previous Chlamydia infection, and scale for menopausal symptoms.

6.3.2 Descriptive covariates

Specific operative study variables: per-operative bleeding, conversion to other operative route, per-operative complications, operative time.

6.3.3 Outcome variables

The primary outcome is absolute change in AMH. Secondary outcomes are relative change in AMH and level of AMH one year after surgery. All outcomes are continuous. AMH is measured in µg/L.

6.4 Statistical methodology

6.4.1 General methodology

The non-inferiority hypothesis tests will be performed as one-sided tests, with a significance level of 2.5%. This is equivalent to the upper limit of a two-sided 95% confidence interval (CI) for the difference between salpingectomy and tubal occlusion, being lower than the non-inferiority margin. Results will be presented as the difference between groups with a one-sided 97.5% CI.

The large loss to follow-up (a second sample not taken) is considered to be completely at random and a complete case analysis is chosen as the primary analysis.

Sensitivity analyses will handle missing data on the primary and secondary outcome (applies thus to the 1-year postoperative sample of AMH). Multiple imputation will be applied using fully conditional specification (MICE) with m = 50 datasets and an initial seed generated by the *char2seed* function from the R package *TeachingDemos,* with the string “SALSTER” as argument. AMH will be imputed using partial mean matching from a pool of 10 donors. Imputation will be conditioned on the following variables; group (salpingectomy/no salpingectomy), age at surgery, log of baseline AMH, BMI, smoking habits at baseline, hormonal contraception prior to surgery, log of operative time, number of days to 1 year visit (imputed as 365 days for those without a 1 year visit), log of AMH at 1 year, smoking at 1 year, and hormonal treatment at 1 year (yes/no).

6.4.2 Patient disposal and data sets analyzed

The number of subjects included in each of the ITT, per-protocol and as treated populations will be summarized for each treatment group

6.4.3 Protocol violations/deviations

See section 4.3

For this substudy, any ovarian surgery during the sterilization procedure or during the period up to the second blood sampling is considered a protocol violation and subjects are excluded from the per-protocol population. Any treatment causing ovarian failure, such as chemo- or radiotherapy during the time interval for blood sampling are considered violations as well. Salpingectomy after a procedure of tubal occlusion is not a protocol violation.

6.4.4 Baseline variables and descriptive covariates

Baseline variables and descriptive covariates will be analyzed and presented as numbers and percentages, mean and SD, median and quartiles, minimum and maximum by treatment group.

6.4.5 Prior and concomitant medications

Any hormonal treatment for gynecological indication prior to surgery will be registered as baseline variable and reported per randomization group.

6.4.6 Primary analysis

The primary analysis will be per-protocol. The mean, standard deviation (SD), median and quartiles, minimum and maximum of absolute change in AMH levels in serum from pre-

operatively to one year postoperatively will be presented by treatment group. The primary effect measure will be the difference in absolute change between the groups. The primary analysis will estimate the difference in the absolute change between the two randomized groups with a 95% CI using an analysis of covariance (ANCOVA) model adjusted for the covariates center (as a fixed effect), baseline AMH, age at surgery, and time from surgery to the 1-year postoperative sampling of AMH. Age at surgery, and time from surgery to the 1-year postoperative sampling of AMH will be entered into the model using restricted cubic splines with three knots placed at the 10^{th}, 50^{th} and 90^{th} percentile of the corresponding distribution. Non-inferiority will be declared if the upper limit of the 95% CI does not exceed the non-inferiority margin of 0.2 µg/L.

The time from baseline to 1-year postoperative sampling may for some individuals exceed one year, mainly due to the difficulties to visit the hospital during the Covid-19 pandemic. Differences in time to postoperative sampling will be compared between groups to rule out a systematic error using a LOESS plot with time to postoperative sampling on x axis and an indicator variable for group (tubal occlusion group = 0 and salpingectomy = 1) on the y axis.

The sensitivity analysis using multiple imputation will apply the same methods and adjustments as described above (center, age at surgery, baseline AMH, and time from baseline to the 1-year postoperative sampling of AMH).

A complementary two-sided 95% CI for the mean difference in absolute change in AMH, also from the per-protocol population, will be constructed using an analysis only adjusted for center, applying the same method as in the primary analysis.

Complementary analyses will also be conducted on the ITT and as-treated populations:

The analysis of the ITT population will be carried out, using ANCOVA models, adjusted for the same variables as in the primary analysis (center, age at surgery, baseline AMH, and time from surgery to the second sampling of AMH).

The analysis of the as-treated population will be carried out using ANCOVA models, adjusted for the same variables as in the primary analysis (center, age at surgery, baseline AMH, and time from surgery to the second sampling of AMH).

6.4.7 Secondary analyses

Secondary outcome variables are relative change in AMH and level of AMH one year after surgery and will be presented as mean and SD, median and quartiles, minimum and maximum by treatment group. The outcomes will be tested between the groups using ANCOVA models, with center, age, baseline AMH, and time from surgery to the second sampling of AMH as covariates. Both a complete case analysis and an analysis after multiple imputation will be conducted. The difference in mean relative change and level of AMH one year after surgery will be presented with 95% CI.

7 REVISIONS

7.1 In this first revision of the statistical analysis plan a randomisation detail concerning block sizes has been added. A clarification about the non-randomised questionnaire population has been added. Data retrievel set 2 has been worked out. An application to GynOp was submitted 7 July 2023 and will be handled by GynOp's steering committee 24 August.

7.2 A description of the sub-study on anti-Müllerian hormone (AMH) was added.

8 REFERENCES

1. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria; 2021. Available from: https://www.R-project.org/

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