AARHUS UNIVERSITY

Statistical Analysis Plan (SAP) for SnartForældre.dk/Aktivitet – a randomized controlled trial

Log of changes		
Date	**Changes**	**Reason/explanation**

Administrative

Title*:* A Preconception Cohort Study of Physical Activity, Fertility, and Spontaneous Abortion – including a randomized controlled trial

Acronym: SF/Aktivitet

Trial registration number:

Ethical Committee record number: 1-10-72-174-23

ClinicalTrials.gov identifier: NCT ID not yet assigned

Collaborators

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Contents

1. Overall aim

In the SnartForældre.dk/Aktivitet randomized controlled trial (RCT) we will test the effectiveness of being randomized to setting goals and receiving motivational counseling on physical activity (PA) among women trying to conceive and during the first trimester of the pregnancy if they conceive. We will further investigate whether PA is associated with fecundability and spontaneous abortion (SAB).

2. Objectives and hypotheses

2.1. Primary objectives

Specific primary objective 1:

- *Aim*: To investigate whether receiving information, setting goals to meet PA recommendations, and receiving feedback on PA will cause a change in PA.
- *Hypothesis:* Setting goals to reach PA recommendations, and receiving feedback on PA, will increase PA (especially light to moderate PA and will break up sedentary time).

Specific primary objective 2:

- *Aim:* To investigate whether meeting the current recommendations on PA is associated with fecundability.
- *Hypothesis:* Meeting the recommendations on PA in the preconception phase is positive for fertility.

Specific primary objective 3:

- *Aim:* To investigate whether meeting the current recommendations on PA is associated with SAB.
- *Hypothesis:* Meeting the recommendations on PA in the preconception phase and during early pregnancy reduces the risk of SAB.

2.2. Secondary objectives:

Specific secondary objective 1:

- *Aim:* To investigate if the number of daily steps, measured with an activity tracker, is associated with fecundability and SAB.
- *Hypothesis:* Higher number of daily steps is associated with higher fecundability and reduces the risk of SAB.

Specific secondary objective 2:

- *Aim:* To investigate whether meeting the current recommendations on PA is associated with different pregnancy and birth outcomes like gestational diabetes, preeclampsia, and birth weight.

- *Hypothesis:* Meeting the recommendations on PA in the preconception phase and during early pregnancy reduces the risk of gestational diabetes and preeclampsia.

3. Study population

3.1. Eligibility

Potential female participants must complete a screener and meet the following inclusion criteria:

- age 18-49 years
- in a stable relationship with a partner of the opposite sex
- trying to become pregnant ≤ 6 menstrual cycles
- not using any birth control
- not receiving fertility treatment

3.2. Recruitment

SnartForældre.dk/Aktivitet is part of the ongoing internet-based cohort study, SnartForældre.dk (SF), which recruits participants through online media such as Instagram and e-Boks(1, 2). E-boks invitations are planned for 2023-2025. The campaign consists of a letter, inviting females to participate in SF if they are trying to conceive. Enrollment in SF takes place via the study website www.SnartForældre.dk and online consent is obtained from participants. Among enrolled female participants, we will consecutively recruit 530 female participants for SnartForældre.dk/Aktivitet. Newly enrolled female participants in the SF cohort will be invited to participate in the Snartforældre.dk/Aktivitet after answering the baseline questionnaire. We will recruit participants nationwide and expect to do this within two years.

3.3. Withdrawal/lost to follow-up

If a randomized participant withdraws or is discontinued from the intervention, they will not receive more feedback on their PA-level. The number of participants lost to follow-up will be provided for both the control and intervention group. If possible, the reason for participants not completing the trial will be given.

4. Study methods and data sources

4.1. Study design

SnartForældre.dk/Aktivitet is a parallel two-arm RCT.

4.2. Data sources

Data in SF are obtained by self-administered online questionnaires. At enrollment, participants complete a baseline questionnaire regarding sociodemographic and behavioral factors as well as reproductive and medical history. Ten days after completing the baseline questionnaire, participants are asked to complete a food frequency questionnaire. Further, we ask participants to complete bimonthly follow-up questionnaires until they report a pregnancy or for up to 12 months. In each follow-up questionnaire, women report the date of the first day of their last menstrual period (LMP), whether they are currently pregnant, and whether they experienced a pregnancy loss (SAB, induced abortion, or ectopic pregnancy) since their last questionnaire. If they have had a pregnancy loss since the date of the most recent completed questionnaire, we also ask them to report the date of the loss as well as the number of completed weeks of pregnancy (gestational age) at loss. Additionally, we will link data from the self-reported questionnaires with data from the Danish National Patient Registry (DNPR) and the Danish Medical Birth Registry (DMBR) to ascertain pregnancy outcomes. The DNPR provides data on hospitalizations and outpatient visits including dates and gestational ages of SABs and induced abortions. The DMBR provides information on all live- and still-births. We use the ICD-10 codes shown in table 1 to ascertain abortive pregnancy outcomes in the registries (3).

For participants who have signed up for SnartForældre.dk/Aktivitet, we will collect data from wrist-worn activity trackers (Garmin VívoSmart 5) on PA (e.g., pulse rate, steps, distance, exercise, sleep, and burnt calories) before conception and during the first trimester of the pregnancy among participants who conceive within the 12 months of attempt time. We will use the an application from Fitrockr Health Solution to collect data from the activity trackers (4).

Table 1: Diagnosis codes used to identify pregnancies with abortive outcomes (ICD-10)

Code	Description
O00	Ectopic pregnancy
O01	Hydatidiform mole
O02	Other abnormal products of conception, including: - O020: Blighted ovum and non-hydatidiform mole - O021: Missed abortion - O022: Pregnancy of unknown location
O03	Spontaneous abortion
O04	Medically induced abortion before 12 full weeks of gestation
O05	Medically induced abortion after 12 full weeks of gestation
O06	Other induced abortion
O07	Failed attempted abortion

O08	Complications after abortion
Z321L (May not be ICD-10, initial diagnosis for O022)	Confirmed pregnancy with unknown location

4.3. Randomization

The participants will be randomized 1:1 to the intervention and the control group immediately after they have provided online written consent to participate.

4.4. Brief description of the intervention

The targeted physical activity level for participants in the intervention group is at least 30 minutes on average per day of moderate physical activity as recommended by The Danish Health Authority (5-7).

4.4.1.Control croup

The control group will not be able to receive any PA feedback from the Fitrockr application and only a minimum of PA feedback (i.e. daily steps, heart rate, intensity minutes, calories, body battery) on the Garmin device.

4.4.2.Intervention group

The intervention group will receive information material on the Danish national guidelines on PA, health benefits from PA, and suggestions on how to reduce sedentary behavior and increase daily activity. Based on their current PA level, they are asked to set personal goals to meet the PA recommendations and to split up sedentary behavior. They will receive a phone call two and five weeks after they received the activity tracker, to stimulate the participant's motivation to be physically active, to set goals for maintaining or increasing PA and to facilitate a revision of the goals if needed. The conversations will be inspired by motivational interviewing and the principles of this motivational technique. Further, we will stimulate the participant's motivation by sending notifications regularly through the Fitrockr application.

4.5. Outcome

4.5.1.Primary outcome 1 – PA

PA will be objectively measured continuously (24/7) using wrist-worn activity trackers with triaxial accelerometry. The measures of PA include active calories, active time, steps, heart rate, moderate-intensity and vigorous-intensity activity and type of activity, which all are recognized automatically by the activity tracker.

4.5.2.Primary outcome 2 – Time to pregnancy (TTP)

Fecundability, defined as the average per-cycle probability of conception (8), is measured as time to pregnancy (TTP). TTP will be measured in menstrual cycles using information on cycle length and date of last menstrual period (LMP) from baseline and follow-up questionnaires. For irregularly cycling women, we estimate cycle length based on baseline LMP date, expected date of next menses, and LMP recorded during follow-up. TTP is calculated by the following formula:

$$TTP_i = (attempts_{baseline} + (date_{LMP} - date_{baseline})) + 1^{cycle\ length}$$

in which TTP_i is the calculated time to pregnancy for individual *i.* $attempts_{baseline}$ is individual *i* cycles of pregnancy attempt at baseline, $date_{LMP}$ is individual *i* LMP date from the most recent follow-up questionnaire, $date_{baseline}$ is the date on which the baseline questionnaire was completed, and cycle length is individual *i* usual cycle length.

4.5.3.Primary outcome 3 – SAB

SAB, defined as an intrauterine pregnancy loss before week 22 of gestation, will be identified from both bimonthly follow-up questionnaires and registries. Using the CPR number, we will retrieve data from the DNPR and the DMBR to identify SABs and induced abortions occurring after the baseline enrollment date. For SABs identified from both registry and questionnaire data, we will use data from the DNPR (based on early ultrasound fetometry or LMP) to ascertain the time of SAB.

4.5.4.Secondary outcomes

We will obtain information on other pregnancy complications and birth outcomes from the DNPR and the DMBR.

5. Statistical analyses

All analyses will be performed using SAS software (SAS 9.4).

5.1. Baseline participants characteristics

We will perform descriptive analyses on exposure, covariate, and outcome data. If the data approximate normal distribution after visual inspection we will use means and standard deviation (SD), otherwise we will use medians and interquartile range (IQR) to describe baseline characteristics (e.g., age, educational attainment, and behavioral factors) of the participants. For categorical variables we will use n (%).

5.2. Analysis of the specific aims

5.2.1.Specific aim 1 – treatment effect

The treatment effect, i.e. receiving information, setting goals to meet PA recommendations, receiving feedback on PA and receiving motivational phone calls will be presented as mean differences between the control and intervention group with respect to:

1. The number of women meeting the PA recommendations at baseline and study end.
2. Change in PA from baseline to study end.

We will estimate the effect of the intervention using t-test statistics.

5.2.2.Specific aim 2 – PA and TTP

Data on TTP are reported in discrete cycles with menstrual cycle being the natural time unit for analysis. We will use discrete-time Cox models to estimate fecundability ratios (FR) and 95% confidence intervals for PA associated with TTP (9, 10). Female participants contribute with menstrual cycles to the analysis from study entry until they report a pregnancy or a censoring event (12 cycles of follow-up, cessation of pregnancy attempt, initiation of fertility treatment, study withdrawal, or loss to follow-up), whichever comes first. We will censor couples at 12 cycles, the typical amount of time after which couples seek medical treatment for infertility. To reduce bias from left truncation, we will use the Anderson Gill data structure to allow for delayed entry and account for variation in pregnancy attempt time at study entry (11, 12). The FR may be interpreted as the per-cycle probability of conception among the intervention group compared with participants in the control group.

5.2.3.Specific aim 3 – PA and SAB

When analyzing the risk of SAB, we will use the Cox proportional hazards regression models with gestational weeks as the time scale to estimate hazard ratios (HR) and 95% confidence intervals for PA associated with SAB. Female participants are followed from pregnancy start until the week of SAB or a censoring event (emigration, induced abortion, ectopic pregnancy, or 22 weeks of gestation), whichever comes first. The analysis will be stratified by weeks of

gestation (<8 vs. ≥8 weeks) to assess whether assumptions of proportionality of the HR over time are violated. We will perform sensitivity analyses using inverse probability of censoring weights to assess the extent to which results differ when not conditioning on pregnancy (13). The HR may be interpreted as the average per-week risk of SAB for the interventions group divided by the corresponding risk for the control group.

5.3. Statistical principles

The primary analysis is an intention-to-treat analysis including all participants as randomized.

5.4. Covariates and sensitivity analysis

The randomization will balance the distribution of covariates in the intervention and the control group and will thereby help control confounding. Further, we will use directed acyclic graphs to guide our understanding of potential confounding variables, causal intermediates, and collinear variables (14, 15). If compliance is low, we will conduct multivariable models adjusting for known or suspected confounders and compare crude vs. adjusted effect estimates. We will include age in all models because it is a key determinant of TTP and SAB (16, 17).

5.5. Multiple imputation

We will multiply-impute missing covariate data using fully conditional specification. We will impute binary variables using logistic regression, ordinal variables using cumulative logistic regression, nominal variables using generalized logistic regression, and continuous variables using predictive mean matching. We will apply logarithmic transformation for continuous variables that, by visual inspection, appear non-normally distributed and where the transformation yields a better fit. We will impute missing values ordered by missingness, i.e. variables with the lowest number of missing values will be imputed first. We will generate 20 imputed datasets, perform the analyses on each individual dataset, and combine the 20 parameter estimates and confidence intervals into one parameter estimate and confidence interval using Rubin's rule (19).

5.6. Power calculation

The power to investigate TTP with a sample size of 530 women, a significance level of 0.05, 12 months accrual time, 12 months follow-up and a median survival (not getting pregnant) of 6 month is 80% to detect a FR of 1.4. The power to investigate SAB risk with a sample size of 530 women, a significance level of 0.05, and 18% probability of SAB in the control group, is 80% to identify an HR of 0.6.

6. Results/table shells

Table shell 1: Baseline characteristics of participants

	All	Intervention group	Control group
N (%)			
Age, years median (IQI)			
Partner age, years, median (IQI)			
Sedentary time (minutes/week), median (IQI)			
Walking (minutes/week), median (IQI)			
Moderate physical activity (minutes/week), median (IQI)			
Vigorous physical activity (minutes/week), median (IQI)			
Last week's physical activity typical, %			
BMI (kg/m^2), median (IQI)			
Total Energy intake kcal/day, median (IQI)			
Alcohol intake (drinks/week), median (IQI)			
Caffeine intake (mg/day), median (IQI)			
Sugar sweetened beverages (servings/week), median (IQI)			
Daily supplement of folic acid, %			
Current smoker, %			
Sleep (hours/day), median (IQI)			
Education level, upper tertiary education, %			
Monthly household income >65,000 DKK, %			
Hours worked per week, median (IQI)			
Job situation, currently employed, %			
Pregnancy attempt time at study entry (>3 cycles), %			
Parous, %			
Previous pregnancy loss, %			
Regular menstrual cycles, %			
Intercourse frequency, ≥4 (%)			
Last method of contraception (%)			
Barrier method (%)			
Hormonal contraceptives (%)			
Withdrawal, other (%)			

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