NUMBER OF VOLUNTEERS AND STATISTICAL ANALYSIS PLAN

The primary endpoint:

ST/HR index during ETTs at the early and late follicular phases of the menstrual cycle

(μV/beats per minute (bpm))

The secondary endpoints:

1. Change in maximal exercise capacity with exercise and menstrual cycle (METs score)
2. maxımum ST segment depression (mm)
3. Relationship of blood pressure and heart rate changes with exercise and menstrual cycle
4. ST/HR slope (μV/beats per minute (bpm))
5. The change in high-sensitive cardiac troponin T after ETT (ng/L)
6. Hormone levels (estrogen, progesterone, FSH, LH) at the early and late follicular phases of the menstrual cycle

Definition of the endpoints:
The primary endpoint will be the ST/HR (heart rate) index (μV/bpm) in ETT. The ST/HR index (μV/bpm) is automatically generated by the General Electronic GE Healthcare T2100-ST Treadmill & CASE 6.73 Stress Test System. The secondary endpoints will be the change in hs cTnI between the before and after ETT, maximal exercise capacity (METs score), ST/HR slope (μV/bpm), maximal horizontal or down slope ST segment depression (mm) with exercise. Secondary end points other than cTnI are automatically generated by the General Electronic GE Healthcare T2100-ST Treadmill & CASE 6.73 Stress Test System.

The null hypothesis
There is no difference between in the ST/HR index (μV/bpm) between the menstrual phases.

The study power
The power is calculated to prove the alternative hypothesis in which there is a 50 % difference between the ETT ST/HR index (μV/bpm). In a paired study sample, with type I error of 5 % and

type II error of 20 %, the sample size is calculated as 34 patients.

The statistical tests

All statistical tests are performed using the Statistical Package for the Social Sciences 25.0 for Windows (SPSS Inc., Chicago, IL, USA). The sample size is determined using the G-power program, according to the study's effect size, type 1 error, and power. Type error is 5%, the power of the study is 80%, and effect size has not been previously reported in the literature. Assuming a 50% difference in the primary endpoint between the early and late follicular phases (effect size: 0.5), the number of premenopausal female patients needed to be enrolled is 34.

Kolmogorov-Smirnov or Shapiro-Wilk test is used to analyze the normality of the data. Numerical data with normal distribution are expressed as mean ± SD, parameters with skewed distribution are expressed as median (min-max or interquartile range (IQR)), while categorical data are expressed as percentages (%). A comparison of categorical data between the groups is made with the chi-square test. In addition, a comparison of numerical data before and after menstruation is made using the paired samples T-test and/or Wilcoxon test, depending on the distribution of the data. A 2-sided p-value of <0.05 is accepted as statistical significance.