ARRIVE Guidelines 2.0 – Key 10 Items Summary

1） Study design

The SD rats were randomly divided into two groups: the model group and the treatment group. A self-control study was conducted. According to the different experimental intervention measures applied to the uterine horns in each group, they were respectively labeled as the control group, the model group, the ethanol + normal saline group, and the ethanol + i-PRF injection group.

2）Sample size

This study involved a total of 50 female rats and 5 male rats. Among them, 30 female rats were randomly divided into the model group and the treatment group（n=15 ) (refer to the references below); the blood of the other 20 female rats was used for the preparation of i-PRF or platelets, and the uterine tissues were used for the cultivation of endometrial stromal cells.

reference： Xia L, Meng Q, Xi J, Han Q, Cheng J, Shen J, Xia Y, Shi L. The synergistic effect of electroacupuncture and bone mesenchymal stem cell transplantation on repairing thin endometrial injury in rats. Stem Cell Res Ther. 2019 Aug 7;10(1):244.

3）inclusion and exclusion criteria

The inclusion and exclusion criteria for the animals were determined before the start of the experiment and were strictly followed before data analysis. This study set the following criteria in advance:

Inclusion criteria: 8-10-week-old female rats

Exclusion criteria: Failure to collect data due to rat death during or after the surgery, and abnormal data values.

4）Randomization

The SD rats were randomly divided into two groups using a random number table method.This study employed a self-control design. For each rat, the uterine horns on both sides were treated with i-PRF and given physiological saline as the control treatment respectively. The allocation plan was stored in an opaque envelope sealed according to the animal numbers. After anesthetizing the rats and positioning them, the sealed box was opened to obtain the specific allocation plan for each rat, and then the i-PRF treatment was immediately carried out.

This study employed a self-controlled design. Firstly, the rats were randomly divided into two groups, and the uterine horns of each rat were randomly subjected to intervention treatment on one side, while the other side was treated with normal saline as a control. These designs were aimed at minimizing potential interfering factors to the greatest extent.

5）Blinding

Allocation: The random sequence of the rats was generated by LC and sealed for storage. LC did not participate in the subsequent experiments. NS opened the sealed envelope after the animal was anesthetized to obtain the group information for the surgery. HM placed the rats in different cages, marked them, and was unaware of the groups.

Experiment implementation: Since the operators of the i-PRF and saline control surgeries could not implement the blind method, HM was responsible for daily care and was also unaware of the groups.

Outcome: XL encoded the tissue samples and hid the group information. YZ and MZ were unaware of the groups and performed H&E, immunohistochemistry, WB and other tests on the histological samples. NS did not participate in the evaluation and measurement.
Data analysis: Statistical analysis was completed by ZZ and MZ. After all the statistical analysis was completed, JD would reveal the blind and convert the group codes to the actual treatment names.

6）Outcome measures

The main indicator of endometrial thickness in this study increased after i-PRF treatment, and the expression of related indicators such as VEGF and LIF also increased. The embryo implantation efficiency of the pregnancy test increased.

7）Statistical methods

All values are presented as mean ± standard deviation (SD). Data were analyzed by ANOVA, followed by Bonferroni's test for multiple comparisons using the SPSS program (SPSS 12.0; SPSS, Inc., Chicago, IL, USA). A P value <0.05 was considered statistically significant. The collected data were analyzed using GraphPad Prism, ver.8.4.0 software. All results were presented as mean ± SEM. The software compared the means of samples using *t* test between 2 groups and one-way analysis of variance (ANOVA) test among multiple groups. P-value of < 0.05 was considered statistically significant.

8） Experimental animals

All experimental studies were carried out in accordance with the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University and the Experimental Animal Centre of Xinjiang Medical University, China [Approval ID: IACUC-JT-20230321-71]. Adult female Sprague-Dawley (SD) rats (purchased form the Laboratory Animal Center of Xinjiang Medical University, Urumqi, China) aged 8-10 weeks (200-280g)were housed in the Experimental Animal Centre of Xinjiang Medical University under a 12-hour light-dark cycle, allowing free access to food and water. After one-week adaption, the rats were used in the experiment.

9） Experimental procedures

Group A: Inject 95% ethanol into one uterine horn and normal saline into the other uterine horn for comparison. Group B: Inject 95% ethanol into both uterine horns. 14 days later, the rats in Group A were randomly divided into two groups. One group prepared i-PRF from whole blood and collected uterine samples simultaneously; the other group mated with male rats. In Group B, i-PRF was injected into one uterine horn and normal saline was injected into the other uterine horn for comparison. 14 days later, the rats were randomly divided into two groups. One group collected uterine samples, and the other group mated with male rats.

10）Results

Administration of i-PRF enhanced the average thickness to 489.43 ± 20.14 μm, which is significantly higher than ethanol treated endometrium (115.16 ± 25.52 μm, n=5,P<0.05). The results showed that the number of embryos in the Ethanol+i-PRF(3.875 ± 3.907) group was significantly higher than that in the Model group （0, P<0.05）, but there was no statistical difference compared to the control group（5 ± 3.464，P>0.05）.