

# Clinical Trial Protocol

## Iranian Registry of Clinical Trials

13 Jun 2026

### The effect of probiotics on fertility in women with recurrent implantation failure based on Th1 to Th2 ratio

#### Protocol summary

##### Study aim

Determining the effect of probiotics on fertility in women with recurrent implantation failure based on Th1 to Th2 ratio.

##### Design

Before administering the drug, heparinized blood is taken from the patients, and PBMC are isolated. Then, flow cytometry technique will be used to count the number of Th1 and Th2 cells. In order to investigate the secretion of cytokines, ELISA will be used. QRT-PCR method will be used to measure transcription factors. After performing these steps according to the protocol, the probiotic dose (2 tablets per day) is adjusted based on the ratio of Th1 to Th2. At this stage and after the treatment protocol, all the factors that were measured before prescribing the drug will be measured again to determine the effects of the probiotic drug on the immune system of the patients. Finally, after 9 months, in order to check the number of babies born by people with RIF, we will continuously monitor the people whose  $\beta$ -HCG test was positive after taking the medicine with the help of gynecologists.

##### Settings and conduct

The study will be conducted in Valiasr International Hospital, Tabriz. Subjects will receive probiotic pills according to the protocol.

##### Participants/Inclusion and exclusion criteria

Patients will be selected only from Azerbaijan people. Patients who have experienced at least 3 consecutive implantation failures.

##### Intervention groups

The studied groups are including 3 groups of patients with RIF who receive probiotics, patients who do not receive any type of treatment despite the history of RIF problem as a control group and a group of healthy women with a history of successful pregnancy and delivery are also considered as a control group.

##### Main outcome variables

The frequency of Th1 and Th2 cells The expression level of cytokines (IL-4, IL-10,IFN $\gamma$  ,TNF $\alpha$ ) and transcription

factors (T-bet , GATA3) Secretory levels of cytokines (IL-4, IL-10,IFN $\gamma$  ,TNF $\alpha$ ) Pregnancy rate

#### General information

##### Reason for update

##### Acronym

RIF

##### IRCT registration information

IRCT registration number: **IRCT20160422027520N20**

Registration date: **2023-01-23, 1401/11/03**

Registration timing: **prospective**

Last update: **2023-01-23, 1401/11/03**

Update count: **0**

##### Registration date

2023-01-23, 1401/11/03

##### Registrant information

##### Name

Mehdi Yousefi

##### Name of organization / entity

Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

##### Country

Iran (Islamic Republic of)

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yousefime@tbzmed.ac.ir

##### Recruitment status

**Recruitment complete**

##### Funding source

##### Expected recruitment start date

2023-02-20, 1401/12/01

##### Expected recruitment end date

2024-08-22, 1403/06/01

##### Actual recruitment start date

empty  
**Actual recruitment end date**  
empty  
**Trial completion date**  
empty

**Scientific title**  
The effect of probiotics on fertility in women with recurrent implantation failure based on Th1 to Th2 ratio

**Public title**  
The effect of probiotics on fertility

**Purpose**  
Treatment

**Inclusion/Exclusion criteria**  
**Inclusion criteria:**  
Patients from East Azerbaijan Having at least three implantation failures Do not have any type of immunotherapy in their medical history. Having regular menstrual cycles  
**Exclusion criteria:**  
Patients under 18 years old and over 41 years old  
Patients with coagulation problems. Patients with intrauterine anomalies. Having chromosomal abnormalities

**Age**  
From **18 years** old to **40 years** old

**Gender**  
Female

**Phase**  
2

**Groups that have been masked**

- Participant
- Data analyser

**Sample size**  
Target sample size: **50**

**Randomization (investigator's opinion)**  
Randomized

**Randomization description**  
At first, the sample size of 50 patients with RIF that met our inclusion criteria was considered as the total sample size. 'Random allocation rule' method was used for randomization, where numbers were randomly assigned to cards on a random order of one to 50. Even numbers were assigned to the treatment group and odd numbers to the control group (who did not receive any treatment). In this method, the balance will be reached at the end of the study in the number of people assigned to each group. Each card was placed in sealed and opaque envelopes to hide the random assignment.

**Blinding (investigator's opinion)**  
Double blinded

**Blinding description**  
The participants and the person who analyzed the results were blinded in this study. Participants were aware of the study before being randomized and informed consent was obtained from them. It should be noted that the person analyzing the results had no other role in this study.

**Placebo**  
Not used

**Assignment**  
Parallel  
**Other design features**

**Secondary Ids**  
empty

## Ethics committees

### 1

**Ethics committee**  
**Name of ethics committee**  
Ethics committee of Tabriz University of Medical Sciences  
**Street address**  
Tabriz University of Medical Sciences , Daneshghah st, Tabriz,  
**City**  
Tabriz  
**Province**  
East Azarbaijan  
**Postal code**  
6446-14155  
**Approval date**  
2023-01-21, 1401/11/01  
**Ethics committee reference number**  
IR.TBZMED.REC.1401.798

## Health conditions studied

### 1

**Description of health condition studied**  
Recurrent implantation failure  
**ICD-10 code**  
N98.9  
**ICD-10 code description**  
Complication associated with artificial fertilization, unspecified

## Primary outcomes

### 1

**Description**  
The frequency of Th1 and Th2 cells  
**Timepoint**  
Before and after interference  
**Method of measurement**  
By using flow cytometry

### 2

**Description**  
Investigating the expression level of transcription factors T-bet and GATA3  
**Timepoint**  
Before and after interference  
**Method of measurement**  
By using Quantitative Real time PCR

### 3

#### **Description**

Examining the expression level of cytokines IL-4, IL-10, IFN $\gamma$ , TNF $\alpha$

#### **Timepoint**

Before and after interference

#### **Method of measurement**

By using Quantitative Real time PCR

### 4

#### **Description**

Investigating the secretion of cytokines IL-4, IL-10, IFN $\gamma$ , TNF $\alpha$

#### **Timepoint**

Before and after interference

#### **Method of measurement**

using sandwich ELISA method.

## **Secondary outcomes**

### 1

#### **Description**

Pregnancy rate

#### **Timepoint**

After positive beta-hCG test, clinical pregnancy was evaluated at 5-6 week

#### **Method of measurement**

Examining the formation of gestational sac using Sonography method

## **Intervention groups**

### 1

#### **Description**

Intervention group: probiotic tablets (2 tablets per day) orally

#### **Category**

Treatment - Drugs

### 2

#### **Description**

Control group: receive no intervention

#### **Category**

Treatment - Drugs

## **Recruitment centers**

### 1

#### **Recruitment center**

##### **Name of recruitment center**

Infertility center at Valiasr Hospital

##### **Full name of responsible person**

Dr. Mehdi Yousefi

##### **Street address**

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## **Sponsors / Funding sources**

### 1

#### **Sponsor**

##### **Name of organization / entity**

Tabriz University of Medical Sciences

##### **Full name of responsible person**

Mehdi Yousefi

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#### **Grant name**

#### **Grant code / Reference number**

#### **Is the source of funding the same sponsor organization/entity?**

Yes

#### **Title of funding source**

Tabriz University of Medical Sciences

#### **Proportion provided by this source**

100

#### **Public or private sector**

Public

#### **Domestic or foreign origin**

Domestic

#### **Category of foreign source of funding**

*empty*

#### **Country of origin**

#### **Type of organization providing the funding**

Academic

## **Person responsible for general inquiries**

#### **Contact**

##### **Name of organization / entity**

Tabriz University of Medical Sciences

##### **Full name of responsible person**

Mehdi Yousefi

##### **Position**

PhD in Medical Immunology, Associate Professor

##### **Latest degree**

Ph.D.

**Other areas of specialty/work**

Immunology

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**Person responsible for updating data****Contact****Name of organization / entity**

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Sanam Dolati

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**Other areas of specialty/work**

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**Sharing plan****Deidentified Individual Participant Data Set (IPD)**

Undecided - It is not yet known if there will be a plan to make this available

**Study Protocol**

Undecided - It is not yet known if there will be a plan to make this available

**Statistical Analysis Plan**

Undecided - It is not yet known if there will be a plan to make this available

**Informed Consent Form**

Undecided - It is not yet known if there will be a plan to make this available

**Clinical Study Report**

Undecided - It is not yet known if there will be a plan to make this available

**Analytic Code**

Undecided - It is not yet known if there will be a plan to make this available

**Data Dictionary**

Undecided - It is not yet known if there will be a plan to make this available