

# Clinical Trial Protocol

## Iranian Registry of Clinical Trials

13 Jun 2026

### Effects of calcium ionophore on human oocyte in vitro maturation outcome, fertilization rate and embryo development

#### Protocol summary

##### Study aim

Determination of the effects of calcium ionophore on human oocyte in vitro maturation outcome, fertilization rate and embryo development

##### Design

In this controlled clinical trial, participants are divided into two groups of intervention and control. In this study, immature oocytes unable to be used for ICSI will be used in this study after getting participants consent. Then, after approval by the ethics committee of Shahid Beheshti University of Medical Sciences, patients who undergo ovulation stimulation for ICSI are enrolled in the study. Sample size includes 210 oocytes and 150 embryos that obtained from study participants.

##### Settings and conduct

Germinal vesicle stage (GV) oocytes enter the two groups: intervention group (Treatment with calcium ionophore) and control group (Group without calcium ionophore) After 24-28 hours, in IVM-derived MII oocytes, nuclear maturation rate, cytoplasm maturation rate, cytoplasm oxidative stress level, meiotic spindle structure, apoptosis rate and MPF and MAPK genes expression will be evaluated. Subsequently, MII oocytes from IVM in the treatment group with ionophosphorus calcium and the control group will be under microinjection with sperm. fertilization rate, cleavage rate and embryo quality will be evaluated.

##### Participants/Inclusion and exclusion criteria

Inclusion criteria: Approval of the Ethics Committee of Shahid Beheshti University of Medical Sciences, women age between 20-40 years. Sperms have a normal parameter according to WHO criteria  
Exclusion criteria: Endometriosis, Polycystic ovarian syndrome, Chromosomal abnormalities

##### Intervention groups

1. Treatment with calcium ionophore: Each oocyte is placed in a 30-ml drop of ionophore calcium A23187 for 15 minutes, then washed in two 30-microliter drops of the culture medium. 2. Group without calcium ionophore

(control)

##### Main outcome variables

Glutathione level, expression of MPF and MAPK, meiotic spindle structure, ROS level, expression of BAX, BCL2 and caspase-3 genes, fertilization rate, embryo quality, cleavage rate

#### General information

##### Reason for update

##### Acronym

##### IRCT registration information

IRCT registration number: **IRCT20140707018381N4**  
Registration date: **2018-08-03, 1397/05/12**  
Registration timing: **registered\_while\_recruiting**

Last update: **2018-08-03, 1397/05/12**

Update count: **0**

##### Registration date

2018-08-03, 1397/05/12

##### Registrant information

##### Name

Ahmad Hosseini

##### Name of organization / entity

Shahid Beheshti University of Medical Science

##### Country

Iran (Islamic Republic of)

##### Phone

+98 21 2235 9153

##### Email address

ahmad-hosseini@sbm.ac.ir

##### Recruitment status

**Recruitment complete**

##### Funding source

##### Expected recruitment start date

2017-10-23, 1396/08/01

##### Expected recruitment end date

2018-10-23, 1397/08/01  
**Actual recruitment start date**  
empty  
**Actual recruitment end date**  
empty  
**Trial completion date**  
empty  
**Scientific title**  
Effects of calcium ionophore on human oocyte in vitro maturation outcome, fertilization rate and embryo development  
**Public title**  
Effects of calcium ionophore on human oocyte in vitro maturation outcome  
**Purpose**  
Treatment  
**Inclusion/Exclusion criteria**  
**Inclusion criteria:**  
Approval of the Ethics Committee of Shahid Beheshti University of Medical Sciences The age of a woman is between 20-40 years old Sperm has a normal parameter based on WHO criteria  
**Exclusion criteria:**  
Endometriosis Poly cystic ovary syndrome Genetic disorders  
**Age**  
From **20 years** old to **40 years** old  
**Gender**  
Both  
**Phase**  
3  
**Groups that have been masked**  
*No information*  
**Sample size**  
Target sample size: **150**  
**Randomization (investigator's opinion)**  
Randomized  
**Randomization description**  
The sampling method will be two-stage sampling methods. In the first stage, non-probability sampling method is used and in the second stage, random allocation is performed using permuted block randomization between the two intervention and control groups.  
**Blinding (investigator's opinion)**  
Not blinded  
**Blinding description**  
**Placebo**  
Not used  
**Assignment**  
Parallel  
**Other design features**  
  
**Secondary Ids**  
empty  
**Ethics committees**

**1**  
**Ethics committee**  
**Name of ethics committee**  
Ethics Committee of Shahid Beheshti University of Medical Sciences  
**Street address**  
Faculty of medicine, Arabi Ave, Daneshjo street, Velenjak, Tehran  
**City**  
Tehran  
**Province**  
Tehran  
**Postal code**  
1985717443  
**Approval date**  
2017-09-28, 1396/07/06  
**Ethics committee reference number**  
IR.SBMU.MSP.1396.416

## Health conditions studied

**1**  
**Description of health condition studied**  
Infertility  
**ICD-10 code**  
N97.9  
**ICD-10 code description**  
Female infertility, unspecified

## Primary outcomes

**1**  
**Description**  
Oocyte cytoplasmic maturation  
**Timepoint**  
After 24-28 hours, the culture was incubated at 37 ° C and 5% CO<sub>2</sub>.  
**Method of measurement**  
Cell Tracker Blue staining for oocyte cytoplasmic maturity

**2**  
**Description**  
Oocyte oxidative stress  
**Timepoint**  
After 24-28 hours, the culture was incubated at 37 ° C and 5% CO<sub>2</sub>.  
**Method of measurement**  
Evaluation of the free radical oxygen (ROS) in cytoplasmic by staining with H<sub>2</sub>DCFDA

**3**  
**Description**  
Meiotic spindle structure  
**Timepoint**  
After 24-28 hours, the culture was incubated at 37 ° C and 5% CO<sub>2</sub>.  
**Method of measurement**

Evaluation of mitotic spindle structure by staining with anti-tubulin antibody

#### 4

##### **Description**

Apoptosis rate

##### **Timepoint**

After 24-28 hours, the culture was incubated at 37 ° C and 5% CO<sub>2</sub>.

##### **Method of measurement**

Determination of apoptosis rate by evaluating expression bax, bcl2 and caspase-3 gene by Real Time-PCR

#### 5

##### **Description**

Nucleation maturation

##### **Timepoint**

After 24-28 hours, the culture was incubated at 37 ° C and 5% CO<sub>2</sub>.

##### **Method of measurement**

Determining the oocyte nucleus maturity by evaluating expression of MPF and MAPK by Real Time-PCR

## **Secondary outcomes**

#### 1

##### **Description**

Fertilization rate

##### **Timepoint**

16-18 hours after ICSI

##### **Method of measurement**

Divide the total number of oocytes fertilized to the total oocytes under ICSI

#### 2

##### **Description**

Cleavage rate

##### **Timepoint**

The third day after ICSI

##### **Method of measurement**

Split the total number of cleavage embryos divided into fertilized embryos

#### 3

##### **Description**

Embryo quality

##### **Timepoint**

The third day after ICSI

##### **Method of measurement**

Grading of embryo morphology based on the number and size of the blastomeres and the rate of fragmentation

## **Intervention groups**

#### 1

##### **Description**

Intervention group: Treatment group with calcium ionophore: Each oocyte is placed in a 30-ml droplet of ionophore calcium A23187 for 15 minutes, then washed in two drops of 30 µL of the culture medium. The oocytes are then culture in an ecobar for 24-28 hours.

##### **Category**

Treatment - Drugs

#### 2

##### **Description**

Control group: Group without treatment with ionophore calcium

##### **Category**

Treatment - Drugs

## **Recruitment centers**

#### 1

##### **Recruitment center**

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

Taleghani hospital

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

Elham Fazeli

###### **Street address**

Taleghani hospital, Arabi Ave, Daneshjo street, Velenjak, Tehran

###### **City**

Tehran

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Tehran

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1985711151

###### **Phone**

+98 21 2243 2560

###### **Email**

Elhamfazeli15@yahoo.com

## **Sponsors / Funding sources**

#### 1

##### **Sponsor**

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

Shahid Beheshti University of Medical Sciences

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

Marefat Ghaffari

###### **Street address**

Faculty of medicine, Arabi Ave, Daneshjo street, Velenjak, Tehran

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###### **Province**

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1985717443

###### **Phone**

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**Email**

Mghaffarin@yahoo.com

**Grant name****Grant code / Reference number****Is the source of funding the same sponsor organization/entity?**

Yes

**Title of funding source**

Shahid Beheshti 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**

Shahid Beheshti University of Medical Sciences

**Full name of responsible person**

Elham Fazeli

**Position**

PhD candidate

**Latest degree**

Master

**Other areas of specialty/work**

Reproductive Biology

**Street address**

Faculty of medicine, Arabi Ave, Daneshjo street, Velenjak, Tehran

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**Person responsible for scientific inquiries****Contact****Name of organization / entity**

Shahid Beheshti University of Medical Sciences

**Full name of responsible person**

Elham Fazeli

**Position**

PhD candidate

**Latest degree**

Master

**Other areas of specialty/work**

Reproductive Biology

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

Shahid Beheshti University of Medical Sciences

**Full name of responsible person**

Elham Fazeli

**Position**

PhD candidate

**Latest degree**

Master

**Other areas of specialty/work**

Reproductive Biology

**Street address**

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

Yes - There is a plan to make this available

**Study Protocol**

Yes - There is a plan to make this available

**Statistical Analysis Plan**

Yes - There is a plan to make this available

**Informed Consent Form**

Yes - There is a plan to make this available

**Clinical Study Report**

Yes - There is a plan to make this available

**Analytic Code**

Yes - There is a plan to make this available

**Data Dictionary**

Not applicable

**Title and more details about the data/document**

All interventions in the patient's file will be recorded and archived in the Taleghani hospital. All data obtained from this study will be published with regard to the privacy and confidentiality right of the the information of

research participants.

**When the data will become available and for how long**

Get started 6 months after publishing study results

**To whom data/document is available**

The findings of this study will be accessible to all individuals.

**Under which criteria data/document could be used**

To improve pregnancy outcomes in infertility clinics

**From where data/document is obtainable**

Elham Fazeli Email: Elhamfazeli15@yahoo.com

Tel:00989112448530

**What processes are involved for a request to access data/document**

Data that release in the published article will be available to other people according to the journal protocol that published article (free or purchase). In order to access data that not published in the paper, it is required to obtain the correspond author's approval and then approval of the research deputy of Shahid Beheshti University of Medical Sciences is obligatory.

**Comments**