

Clinical Trial Protocol

Iranian Registry of Clinical Trials

21 Jun 2026

Comparison of fertilization percentage, embryo quality, pregnancy and implantation rate, between two sperm separation procedures (density gradient centrifugation with surface markers- density gradient centrifugation) in infertile men intra-cytoplasmic sperm injection candidate

Protocol summary

Summary

Objectives: Comparison of fertilization, embryo quality, pregnancy and implantation rates between two sperm separation procedures, density gradient centrifugation (DGC) and Magnetic-Activated Cell Sorting (MACS), followed DGC (MACS-DGC) in infertile men candidates for intra-cytoplasmic sperm injection (ICSI). Design: Infertile couples with primary male factor who referred to Isfahan fertility and infertility center infertility were included in this study. These individuals were informed about the study and were grouped into study (MACS-DGC) and control (DGC). Setting and conduct: In the day of ICSI operation, a total of 113 infertile individuals were randomly assigned (in a 1:1 proportion) to MACS-DGC and DGC groups. In study group, the oocytes were inseminated with sperm selected by MACS-DGC processing. In DGC group, oocytes were inseminated with sperm selected by DGC method (based on viability and morphology). Major Inclusion and Exclusion criteria: Inclusion criteria: Primary male factor infertility (according to the WHO criteria, 2010); with mean age of 20-45. Exclusion criteria: Females factor infertility based on WHO criteria (2010); age higher than 35 years; less than 6 oocytes and poor quality oocyte. Intervention: In study group, all oocytes were inseminated with sperm selected with DGC following MACS processing (separation of intact spermatozoa from those which present apoptotic markers), while in the control group, only routine sperm preparation procedure (DGC) were used. Main outcome measures (variables): In this study, fertilization, embryo quality, implantation and pregnancy rates were compared between two groups. The number of embryos transferred to each patient on day 3 was based on female age, number of previous failed cycles

and quality of embryos at the time of embryo transfer and final decision made by the gynecologist. Clinical pregnancy was defined by ultrasonography findings showing at least one embryo with a fetal heartbeat, 5 weeks after transfer. Implantation rate was defined by the number of observed gestational sacs per number of transferred embryos.

General information

Acronym

IRCT registration information

IRCT registration number: **IRCT201610317223N8**

Registration date: **2017-08-22, 1396/05/31**

Registration timing: **retrospective**

Last update:

Update count: **0**

Registration date

2017-08-22, 1396/05/31

Registrant information

Name

Mohammad Hossein Nasr-Esfahani

Name of organization / entity

Royan Institute (Royan Institute for Biotechnology)

Country

Iran (Islamic Republic of)

Phone

+98 31 9501 5688

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mh_nasr@med.mui.ac.ir

Recruitment status

Recruitment complete

Funding source

Expected recruitment start date

2015-04-21, 1394/02/01

Expected recruitment end date

2016-03-10, 1394/12/20

Actual recruitment start date

empty

Actual recruitment end date

empty

Trial completion date

empty

Scientific title

Comparison of fertilization percentage, embryo quality, pregnancy and implantation rate, between two sperm separation procedures (density gradient centrifugation with surface markers- density gradient centrifugation) in infertile men intra-cytoplasmic sperm injection candidate

Public title

Comparison of fertilization percentage, embryo quality, implantation and pregnancy rate, between two sperm separation procedures in infertile men intra-cytoplasmic sperm injection candidate

Purpose

Health service research

Inclusion/Exclusion criteria

Inclusion criteria: Primary male factor infertility (according to the WHO-2010); with mean age of 20-45.
Exclusion criteria: Females factor infertility based on WHO criteria (2010); age higher than 35 years; less than 6 oocytes and poor quality oocyte

Age

From **20 years** old to **45 years** old

Gender

Both

Phase

N/A

Groups that have been masked

No information

Sample size

Target sample size: **113**

Randomization (investigator's opinion)

Randomized

Randomization description

Blinding (investigator's opinion)

Single blinded

Blinding description

Placebo

Not used

Assignment

Parallel

Other design features

Secondary Ids

empty

Ethics committees

1

Ethics committee

Name of ethics committee

Ethical Committee of Royan Institute

Street address

Royan Institute, Banihashem St, Resalat Ave, Tehran, Iran

City

Tehran

Postal code

Approval date

2017-01-07, 1395/10/18

Ethics committee reference number

IRI.ACECR.ROYAN.REC.1395.156

Health conditions studied

1

Description of health condition studied

Male factor infertility

ICD-10 code

N46

ICD-10 code description

Male infertility

Primary outcomes

1

Description

Fertilization rate

Timepoint

16-18 h post insemination/injection

Method of measurement

View of 2 pronucleuse with invert microscope, the ratio of fertilized oocytes (2PN) by the total number of injected metaphase II oocytes, multiplied by 100

2

Description

Implantation rate

Timepoint

4 weeks after transfer

Method of measurement

The number of observed gestational sacs per number of transferred

3

Description

Chemical pregnancy

Timepoint

18 days post-ICSI

Method of measurement

Measurement of Beta HCG level in serum

4

Description

Clinical pregnancy

Timepoint

5 weeks after embryo transfer

Method of measurement

ultrasonography findings showing at least one embryo with a fetal heartbeat

5

Description

Quality of embryo

Timepoint

On day 3 after oocyte retrieval

Method of measurement

Using a three-point scoring system including fragmentation rate, number of blastomere, shape and size

Secondary outcomes

1

Description

Perinatal mortality (fetal or neonatal death)

Timepoint

At 20 completed weeks of gestational age and later, during childbirth, or up to 7 completed days after birth

Method of measurement

Through ultrasound

2

Description

Miscarriage (The spontaneous loss of a clinical pregnancy)

Timepoint

Before 20 completed weeks of gestational age (18 weeks after fertilization)

Method of measurement

Through ultrasound

Intervention groups

1

Description

In study group, total of oocytes were inseminated with sperm that selected with DGC following MACS processing (Separate intact spermatozoa from those that present apoptotic markers by magnetic particles conjugated with Annexin V, which has a high affinity for Phosphatidylserine)(MACS-DGC group). For MACS procedure, semen samples were washed twice with 1X buffer and diluted to ten million sperm per ml. Then, each semen sample was mixed with one hundred microliter of microbeads solution and then, this mixture was incubated for 15 min at room before being loaded to MACS column. Annexin positive sperm was retained within the column while annexin negative sperm were allowed to pass the column.

Category

Other

2

Description

In control group (DGC group), oocytes were inseminated with sperm selected by Density gradient centrifugation method (based on viability and morphology). Density gradient centrifugation was prepared by laying 2 ml of 80% PureSperm into a sterile 15ml conical centrifuge tube. Then, 2 ml 40% PureSperm suspension was slowly layered over lower layer. Subsequently, semen sample were washed (5 min, 3000 rpm) with VitaSperm medium were overlaid on the 40% or upper layer. The layer was centrifuged for 20 min at 1000rpm. The supernatant was aspirated, pellet was resuspended, and washed twice with VitaSperm. After injection of sperm into the oocyte to embryo formation at day 3, events such as fertilization, cleavage and embryo formation occur that these phenomena are in vitro. After 3 days, embryos were transferred into the uterus and then assessed implantation and pregnancy in vivo condition.

Category

Other

Recruitment centers

1

Recruitment center

Name of recruitment center

Isfahan Fertility and Infertility Center

Full name of responsible person

Mohammad Hossein Nasr- Esfahani

Street address

Fertility and Infertility Center, Salman Farsi St, Isfahan, Iran

City

Isfahan

Sponsors / Funding sources

1

Sponsor

Name of organization / entity

Royan Institute

Full name of responsible person

Abdolhossein Shahverdi

Street address

Royan Institute, Banihashem St, Resalat Ave, Tehran, Iran

City

Tehran

Grant name

Grant code / Reference number

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

Yes

Title of funding source

Royan Institute

Proportion provided by this source

Public or private sector

empty

Domestic or foreign origin

empty

Category of foreign source of funding

empty

Country of origin**Type of organization providing the funding**

empty

2**Sponsor****Name of organization / entity**

Isfahan Fertility and Infertility

Full name of responsible person

Mohammad Hossein Nasr- Esfahani

Street address

Fertility and Infertility Center, Salman Farsi St,
Isfahan, Iran

City

Isfahan

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

Yes

Title of funding source

Isfahan Fertility and Infertility

Proportion provided by this source**Public or private sector**

empty

Domestic or foreign origin

empty

Category of foreign source of funding

empty

Country of origin**Type of organization providing the funding**

empty

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

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Web page address**Sharing plan****Deidentified Individual Participant Data Set (IPD)**

empty

Study Protocol

empty

Statistical Analysis Plan

empty

Informed Consent Form

empty

Clinical Study Report

empty

Analytic Code

empty

Data Dictionary

empty