

Embryo development of fresh versus vitrified metaphase II after intracytoplasmic sperm injection (ICSI): a sibling-oocyte study

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Registration date 29/05/2009	Overall study status Completed	<input type="checkbox"/> Protocol
Last Edited 21/12/2009	Condition category Pregnancy and Childbirth	<input type="checkbox"/> Statistical analysis plan
		<input checked="" type="checkbox"/> Results
		<input type="checkbox"/> Individual participant data

Plain English summary of protocol
Not provided at time of registration

Contact information

Type(s)
Scientific

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Additional identifiers

Protocol serial number
CVG02092008

Study information

Scientific Title

Embryo development of fresh versus vitrified metaphase II after intracytoplasmic sperm injection (ICSI): a prospective randomised active-controlled parallel group sibling-oocyte study

Study objectives

Non-inferiority trial in order to evaluate the effectiveness of the oocyte vitrification procedure, effectiveness being defined by fertilisation rate after intracytoplasmic sperm injection (ICSI) per warmed oocyte.

Ethics approval required

Old ethics approval format

Ethics approval(s)

Local medical ethics committee (Clinica Valle Giulia, Roma) approved on the 1st September 2008

Study design

Randomised active controlled parallel group trial

Primary study design

Interventional

Study type(s)

Treatment

Health condition(s) or problem(s) studied

Intracytoplasmic sperm injection cases, oocyte vitrification

Interventions

After oocyte denudation, MII oocytes with normal morphology were randomly allocated to fresh ICSI insemination or to vitrification procedure. If pregnancy was not obtained a subsequent ICSI cycle was performed with warmed oocytes of the same cohort. In both groups, 3 oocytes were inseminated per cycle by ICSI procedure.

The vitrification and warming procedures were performed according to Kuwayama and co-Authors (2005). Commercial kits were used (Vitrification and Warming KIT, Kitazato BioPharma Co, Japan).

The vitrification procedure was performed at room temperature (RT). Oocytes were equilibrated in the equilibration solution (ES) containing 7.5% ethylene glycol (EG) and 7.5% dimethylsulfoxide (DMSO) in HEPES buffered basic culture medium M-199 with 20% synthetic serum substitute (SSS). To perform the equilibration gradually, the oocytes were first placed in a 20 microlitre drop of M199+20%SSS and, immediately, after mixed with a second 20 microlitre drop of ES. After 3 minutes incubation, a third 20 microlitre drop of ES solution was mixed. Finally, the oocyte were moved in a pure drop of 20 microlitre ES and incubated for an additional 6 - 9 minutes. The oocytes (1 to 3, contemporaneously) were then transferred in 1 ml of vitrification solution (VS) containing 15% EG, 15% DMSO and 0.5M sucrose in M199+20%SSS for 1 minute. The oocytes were then placed on the Cryotop strip in a single small drop of VS. Much care was driven to re-aspirate, as much as possible, the excess of VS in such way to leave just a thin layer around each oocyte. The Cryotop was then immediately submerged into liquid nitrogen. Finally, the plastic cap was pulled over the Cryotop inside the liquid nitrogen and the sample was stored submerged in liquid nitrogen.

The first step of warming procedure was performed at 37°C. The cap was removed in liquid nitrogen and the cryotop was immediately submerged in 1 ml of warming solution containing 1.0 M sucrose in M199+20%SSS. After 1 minute, oocytes were placed in 1 ml solution containing 0.5 M sucrose, and incubated at RT for 3 minutes. Finally, the oocytes were washed at RT for 6 minutes in 2 different dishes containing 1 ml basic medium M199+20%SSS each, and transferred into 1 ml culture media. Degenerated oocytes were removed from the cohort.

The surviving oocytes were co-cultured at 37°C (6% CO₂ and 5% O₂) for exactly 2 hours before ICSI.

Intervention Type

Other

Phase

Not Applicable

Primary outcome(s)

Non-inferiority in fertilisation rates calculated per warmed and per injected oocyte, assessed 16 - 18 hours post-treatment (ICSI).

Key secondary outcome(s)

1. Pronuclear morphology
2. Embryo development, assessed 42 - 44 hours post-treatment
3. Patient's baseline characteristics
4. Clinical outcomes

Completion date

10/03/2009

Eligibility

Key inclusion criteria

Between September 2008 and February 2009 consecutive patients not older than 42 years of age, presenting greater than 6 normal appearing metaphase II (MII) oocytes and undergoing ICSI treatment with ejaculated sperm in the Centre for Reproductive Medicine GENERA in Rome.

Participant type(s)

Patient

Healthy volunteers allowed

No

Age group

Adult

Sex

Female

Key exclusion criteria

1. Female partner older than 42 years old
2. Less than 6 normal appearing MII oocytes retrieved
3. Surgically extracted spermatozoa
4. Very severe oligoasthenoteratozoospermia (motile sperm count less than 500,000/ml after preparation)
5. Patients enrolled in our polar body biopsy programme

Date of first enrolment

02/09/2008

Date of final enrolment

10/03/2009

Locations

Countries of recruitment

Italy

Study participating centre

G.EN.E.R.A.

Rome

Italy

00197

Sponsor information

Organisation

G.EN.E.R.A. - Clinica Valle Giulia (Italy)

ROR

<https://ror.org/05aq4y378>

Funder(s)

Funder type

Hospital/treatment centre

Funder Name

G.EN.E.R.A. - Clinica Valle Giulia (Italy)

Results and Publications

Individual participant data (IPD) sharing plan

IPD sharing plan summary

Not provided at time of registration

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article	results	01/01/2010		Yes	No