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Evaluation of blastocyst survival following vitrification and thawing using two methods - Fast Freeze™ Kit based on S3 and the Vit. Kit®

Submission date	Recruitment status	[X] Prospectively registered
16/01/2012	No longer recruiting	[_] Protocol
Registration date	**Overall study status**	[_] Statistical analysis plan
01/03/2012	Stopped	[_] Results
Last Edited	**Condition category**	[_] Individual participant data
22/01/2019	Urological and Genital Diseases	[_] Record updated in last year

Plain English summary of protocol

Background and study aims
Modern and fast methods of cryopreservation, namely vitrification, are a preferred method for freezing human embryos for in vitro fertilisation (IVF). From all methods of vitrification, DMSO is the most often used cryoprotectant (the substance used to protect the embryo from freezing damage). Several DMSO-based kits are available (e.g. Vit Kit), and have shown to be effective. However, DMSO is rather toxic and has generated an overall concern amongst embryologists. A new simple safe method of vitrification has been reported – the S3 method, which is DMSO-free. Furthermore, it is an affordable technique, uses the current laboratory equipment and is easy to learn. This technique has already been used in some clinics, and pregnancy rates ranged between 59% and 88%. The aim of this study is to compare the survival rate of embryos frozen with the Global® Fast Freeze Kit based on S3 and the Vit Kit® method. Furthermore, a slight modification of the S3 rapid freezing method is tested.

Who can participate?
Blastocysts (embryos left to develop until day 5 or 6), derived from abnormal or rejected embryos or from embryos donated for scientific research

What does thte study involve?
Blastocysts are randomly allocated to one of four groups. Group 1 undergo a fast freeze procedure using Global® Fast Freeze media with a -100oC step. Group 2 undergo a fast freeze procedure using Global® Fast
Freeze media and a direct plunge into liquid nitrogen. Group 3 undergo a vitrification procedure using the
Vit Kit®. Group 4 do not undergo vitrification. Blastocyst survival and quality are assessed.

What are the possible benefits and risks of participating?
The results will show which technique is the best to be used in daily practice. There are no risks of participating since the embryos would be discarded if not used for the study.

Where is the study run from?
Leuven Institute of Fertility and Embryology (Belgium)

When is the study starting and how long is it expected to run for?
March 2012 to February 2013

Who is funding the study?
Leuven Institute of Fertility and Embryology (Belgium)

Who is the main contact?
Prof. Stephan Gordts

Contact information

Type(s)
Scientific

Contact name
Dr Stephan Gordts

Contact details
Leuven Institute for Fertility and Embryology (LIFE)
Tiensevest 168
Leuven
Belgium
3000

Additional identifiers

EudraCT/CTIS number

IRAS number

ClinicalTrials.gov number

Secondary identifying numbers
N/A

Study information

Scientific Title
Evaluation of blastocyst survival following vitrification and thawing using two methods - Fast Freeze™ Kit based on S3 and the Vit. Kit®: a randomised controlled study

Study objectives
To evaluate and compare the survival rate of vitrified-warmed blastocysts submitted to the Global® Fast Freeze Kit based on S3 (LifeGlobal, Guelph, Canada) and the Vit. Kit® protocol (Irvine Scientific, Santa Ana, CA). Furthermore, a slight modification of the S3 rapid freezing

protocol was tested. The trialists expect to observe differences in terms of morphology, kinetics and cell survival on the three groups of blastocysts submitted to the different cryopreservation protocols.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Not provided at time of registration

Study design
Single-centre randomised controlled study

Primary study design
Interventional

Secondary study design
Randomised controlled trial

Study setting(s)
Hospital

Study type(s)
Other

Participant information sheet
Not available in web format, please use the contact details to request a patient information sheet

Health condition(s) or problem(s) studied
Reproductive medicine

Interventions
Group 1
Global® Fast Freeze and Thawing media based on S31 blastocyst at the time is removed from the incubator and transferred with a micropipette to previously made drops of vitrification solutions. The blastocyst is then transferred to a drop of solution 3 and immediately loaded in a 0.25 or 0.30 ml previously labelled freezing straw with solution S3, attached to a straw holding and aspiration device. The blastocyst is kept in a column of S3 solution, between two air bubbles. The straw is then sealed with an appropriate heat sealer and held vertically together with a thermocouple probe, to be lowered into the mouth of the liquid nitrogen tank. Once the temperature reaches -100 degree C, the straw is held still for 2 (0.25 cc straws) or 3 minutes (0.30 cc straws), before it is plunged into liquid nitrogen and stored in the cryotank.

Group 2
Some of the blastocysts are cryopreserved with the method described above, but with slight modifications. Once sealed, the straw is plunged directly into liquid nitrogen, without the "intermediate step of holding the straw for 2 or 3 minutes at -100 degree C. The remaining steps remain unchanged and thawing is performed similarly to the procedure described above.

Group 3: Vit Kit® media (Irvine Scientific)
One blastocyst at the time is removed from the incubator and transferred to a equilibration solution drop, where it should stay for 5-15 minutes, until it shrinks and returns to its size. Then the blastocyst is transferred consecutively to 4 drops of vitrification solutions, at intervals of 5 seconds. Using a cryotip attached to a 1ml syringe, the blastocyst is aspirated. The cryotip is sealed in both ends and the cover sleeve is slid back to protect the tip. The covered cryotip is then plunged directly into liquid nitrogen. Thawing is done in a water bath at 37°C for 3 seconds and the content of the cryotip is then expelled and mixed with 1ul of thawing solution for 1 minute. Subsequently the blastocyst is placed in another drop of thawing solution for 1 minute, then on 2 drops of dilution solution and finally the blastocyst is washed 3 times. The blastocyst is then transferred to pre-equilibrated culture dish containing drops of Global® media.

Group 4 (control group: non vitrified day 5 and day 6 blastocysts)

Intervention Type
Other

Phase
Not Applicable

Primary outcome measure
1. Survival assessment by vital staining
2. Assessment of cell number and survival rate following vitrification
3. Thawing and culture overnight is performed with the Life-dye TM and propidium iodide (Life-dead cell staining kit; Biovision, CA, US). Briefly blastocysts are incubated in a buffer media with 1ul of each staining solutions for 15 min in the dark, at 37°C.

Secondary outcome measures
1. Differences in terms of morphology, kinetics and cell survival on the three groups. Digital images of each blastocyst (day 5 or day 6) are acquired before the vitrification procedure, immediately after thawing and after overnight culture, using a digital still camera (Octax, Microscience Gmb H, Bruckberg, Germany) mounted on an inverted optical microscope (TE2000-S, Nikon Eclipse, Japan), with a thermal control microscope stage (MS100, Linkam, Surray, UK).
2. Assessment of blastocyst morphology quality is done according to the classification developed by Gardner & Schoolcraft 1999 and re-expansion is assessed following overnight culture.

Overall study start date
01/03/2012

Completion date
01/02/2013

Eligibility

Key inclusion criteria
1. Blastocysts derived from 1PN and 3PN embryos (abnormal embryos)
2. Blastocysts derived from biopsied and rejected embryo
3. Blastocysts derived from too fast or too slow embryos (that were neither transferred nor cryopreserved)
4. Blastocysts derived from culture of embryos (days 2 and 3) donated for scientific research

Participant type(s)
Other

Age group
Other

Sex
Both

Target number of participants
100

Key exclusion criteria
Still useful embryos and normal embryos not donated for scientific research

Date of first enrolment
01/03/2012

Date of final enrolment
01/02/2013

Locations

Countries of recruitment
Belgium

Study participating centre
Leuven Institute for Fertility and Embryology (LIFE)
Leuven
Belgium
3000

Sponsor information

Organisation
Leuven Institute for Fertility and Embryology (LIFE) (Belgium)

Sponsor details
Tiensevest 168
Leuven
Belgium
3000

Sponsor type

Industry

ROR
https://ror.org/012rp6f89

Funder(s)

Funder type
Industry

Funder Name
Leuven Institute for Fertility and Embryology (LIFE) (Belgium)

Results and Publications

Publication and dissemination plan
Not provided at time of registration

Intention to publish date

Individual participant data (IPD) sharing plan

IPD sharing plan summary
Not provided at time of registration