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Efficiency of aseptic open vitrification in ultraviolet-sterilised Liquid Nitrogen and hermetical cryostorage of human oocytes

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19/07/2010

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No longer recruiting

Registration date
30/07/2010

Overall study status
Completed

Last Edited
07/02/2012

Condition category
Pregnancy and Childbirth

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[X] Results
[_] Individual participant data

Plain English summary of protocol
Not provided at time of registration

Contact information

Type(s)
Scientific

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Study information

Scientific Title
Efficiency of aseptic open vitrification in ultraviolet-sterilised Liquid Nitrogen and hermetical cryostorage of human oocytes: a prospective randomised study between fresh versus vitrified /warmed sibling oocytes.

Study objectives
The objective of the present study is to demonstrate that ultraviolet (UV) sterilisation of Liquid Nitrogen (LN2) is not detrimental and can be safely used for aseptic vitrification of human oocytes. In addition, in this study, we also describe a system to avoid hypothetical contamination during cryostorage, by enclosing the vitrified oocytes in a sterile device for hermetic isolation inside cryobanks. With this article we wish to report for the first time a safe method for aseptic open vitrification and hermetical cryostorage of human oocytes in accordance with the European directives on tissue manipulation. In order to assess the safety of this kind of vitrification/storage procedure we performed a randomised comparison between fresh and vitrified/warmed sibling-oocytes on infertile couples coming to our centre for intracytoplasmic sperm injection (ICSI) treatment.

Ethics approval required
Old ethics approval format

Ethics approval(s)
The institutional medical ethics committee of GynePro Medical Center approved on the 16th of March 2008

Primary study design
Interventional

Study design
Single centre prospective randomised study

Study type(s)
Treatment

Health condition(s) or problem(s) studied
Intracytoplasmic sperm injection (ICSI), aseptic open oocyte vitrification in UV-sterilised liquid nitrogen, hermetical cryostorage

Interventions
After retrieval oocytes were denuded and then evaluated to assess their nuclear maturation stage. The oocytes that had released the first polar body (metaphase II - MII) underwent a strict selection by morphological features (zona pellucida thickness, perivitelline space size, oocyte shape, cytoplasm colour and granularity, presence of vacuoles and first polar body morphology). Immediately after decumulation and quality evaluation, the high quality MII oocytes were put in progressively-numbered culture droplets and randomised for ICSI; the supernumerary sibling MII oocytes were vitrified.Since at the beginning of this study the Italian IVF law allowed the injection of maximum three oocytes (Benagiano and Gianaroli, 2004), between April 2008 and 8 April2009 three MII oocytes were randomised for ICSI and the supernumerary sibling oocytes were vitrified. Randomisation was performed by a different embryologist to the operator who performed oocyte denudation using a specific software (www.randomizer.org). Since 9 April 2009 - due to changes in the Italian law (Benagiano and Gianaroli, 2010) - the number of oocytes to randomise for ICSI or vitrification has been defined following our centres guideline based on: female age at oocyte recovery and semen parameters, but generally not more than 6 oocytes are injected.

Only the first warming cycle per patient was included in the study: from the beginning of the study to 8 April 2009 maximum 3 random warmed oocytes were injected by ICSI; from 9 April 2009 the number of warmed oocytes to inject has defined following our centres guideline.

LN2 sterilisation via UV irradiation was performed by administration of 660,000 µW/cm2.

Cryotop (Kitazato BioPharma Co, Fuji-Shizuoka, Japan) oocyte vitrification was performed at room temperature in a 'home made' solution comprising 15% dimethylsulphoxide (DMSO- D 2438 Sigma Aldrich, Steinheim, Germany), 15% ethylene glycol (EG 10.246-6 Sigma Aldrich) and 0.5 mol/L sucrose (Sigma Aldrich), after a gradual initial equilibration of 15 minutes in a solution comprising 7.5% DMSO and 7.5% EG (Kuwayama et al, 2005, Rienzi et al., 2009). For the ultra-rapid cooling, the Cryotops - containing 1-2 oocytes- were plunged into UV-sterilised LN2 . and closed with their plastic caps. Then, the Cryotops of each patient were enclosed in 'home made' hermetical aluminium cylindric containers (high security goblets). which can contain up to 6 Cryotops each. These goblets had been previously submerged vertically in LN2 in order to avoid the infiltration of LN2 and checked for an inner temperature of -196° C at the end of UV-sterilisation process. The Cryotops were inserted into the 'high security goblets' taking care to keep the Cryotop strip containing the oocyte in the nitrogen vapour phase above the LN2. Finally, the goblets were hermetically closed with sterilised caps and polipropilene adhesive tape (Scotch® 3M Italia, Pioltello MI, Italy).

Before the rapid warming, the hermetical goblets containing the Cryotops were opened into the UV-sterilised LN2. The caps of the Cryotops were removed in the LN2, and each open carrier was submerged in 1 mL of warming solution containing 1 M sucrose at 37° C. Then, the oocytes were incubated at room temperature for 3 minutes first in 0.5 M and subsequently in 0.25 M and finally washed for 4 minutes in basic medium (PBS D8662 Sigma Aldrich, supplemented with 20% EHSA Conception Tecnologies, San Diego CA, USA) before culture. Warmed oocytes were considered to have survived in absence of negative characteristics: dark or contracted ooplasm, vacuolization, cytoplasmic leakage, abnormal perivitelline space, cracked zona pellucida. After 1-2 hours post-warm culture the surviving oocytes were inseminated by ICSI.

Intervention Type
Other

Phase
Not Applicable

Primary outcome(s)
1. Oocyte fertilisation rate
2. Embryo cleavage rate
3. Top-quality embryo rate

Key secondary outcome(s)
No secondary outcome measures

Completion date
30/05/2010

Eligibility

Key inclusion criteria

1. Women enrolled in the vitrification programme at GynePro medical centre undergoing ICSI with ejaculated spermatozoa
2. Not older than 41 years
3. At least six mature-Methaphase II oocytes at retrieval
This study compares the outcome of 31 warmed ICSI cycles performed from January 2009 to May 2010 with the outcome of fresh sibling oocytes ICSI performed from April 2008 to March 2010

Participant type(s)
Patient

Healthy volunteers allowed
No

Age group
Adult

Sex
Female

Key exclusion criteria
1. Women older than 41 years old
2. Women with less than six mature-MII oocytes at retrieval
3. Patients with male partners with testicular spermatozoa or severe oligoastenoteratozoospermia (motile sperm count ≤ 500.000/mL after sperm preparation)

Date of first enrolment
01/04/2008

Date of final enrolment
30/05/2010

Locations

Countries of recruitment
Italy

Study participating centre
GynePro Medical Centers
Bologna
Italy
40137

Sponsor information

Organisation
GynePro Medical Centers (Italy)

ROR
https://ror.org/03segdh23

Funder(s)

Funder type
Hospital/treatment centre

Funder Name
GynePro Medical Centers (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	01/10/2011		Yes	No