

BIORESOURCE PAPER

Amniotic Fluid Cells Biobank for Research on Fetal Mesenchymal Stem Cells

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The research biobank "Anna Maria Ferraro Cutino" AOR-villa Sofia-Cervello, started in 2010 by a grant of the Sicilian Department of Health. Within the biobank are collected, characterized, stored and distributed amniotic fluids (AF) from amniocentesis performed for maternal age. Patients choose to donate to the biobank the first 3 ml of AF that cannot be used for prenatal diagnosis. AF and donor data are anonymously archived in a database, according to the standard ethical principles. Collateral biobanks are present to collect, storage and manage donor sera and DNA samples, to perform microbiological and genetic tests necessary for the unit release.

Keywords: amniotic fluid; biobanking; MSC; collateral banking; biospecimen

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(1) Bioresource overview

Project description

The research Biobank (BB) "Anna Maria Ferraro Cutino", Hematology for Rare Diseases of Blood and Hematopoietic Organs, AOR-Villa Sofia-Cervello, started in 2010 by a Grant of the Sicilian Department of Health for the improvement of Sicilian Cell and Tissue Banks.

Within the BB are collected, characterized, stored and distributed amniotic fluids (AF) from amniocentesis performed for maternal age. Donors are between the ages of 36 and 45 and they have no positive history for genetic diseases.

Women choose to donate to the BB the first 3 ml of AF that cannot be used for prenatal diagnosis and would therefore be eliminated.

The AF BB research project has been approved by the local ethics committee (prot. 128.01/12/2010).

Since 2014, AF BB is part of the Integrated Clinical-Biological Network for Regenerative Medicine (RIMEDRI) through an interactive platform realized by a Grant of Sicilian Productive Assets Department (<http://rimedri.ospedaliriunitipalermo.it>).

RIMEDRI's interactive platform in 2015 was certified ISO9001: 2008 by Bureau Veritas Italia.

In 2016 AF BB joined BBMRI-ERIC Italian Node (<http://www.bbmri.it/home>) with the name of BCSMF: bank of mesenchymal fetal stem cells.

AF are rich in fetal mesenchymal stem cells (FMSC) that are able to regenerate tissues (adipose, cartilage, bone), have anti-inflammatory and immunosuppressive properties, and a high proliferative potential [1].

For this reason, BCSMF is an important resource of FMSC that could be employed in regenerative medicine [2], autoimmune and chronic inflammatory diseases, allogenic bone marrow transplantation, in-utero transplantation [3].

The BCSMF is a research biobank and AF samples can be used for research only.

The main goals of biobank include:

- collection, storage and management of AF samples and data,
- collection, storage and management of donor sera and DNAs,
- sharing AF samples and data with the scientific community,
- sharing protocols and know-how [4].

To date, BCSMF has collected and stored 300 AF and related clinical data.

Classification 1

Human amniotic fluid.

Species

Human.

Classification 2

BCSMF collects, characterizes and cryopreserves AF samples and related clinical data. AF donor sera and DNAs of AFs are also stored.

Context**Spatial coverage**

Campus UOC Ematologia per le Malattie Rare del Sangue e degli Organi Ematopoietici, AOR "Villa Sofia-Cervello", Via Trabucco n.180, Palermo- Italia.

Latitude: 38 degrees, 9 minutes, 10.7568 seconds.

Longitude: 13 degrees, 18 minutes, 55.1592 seconds.

Temporal coverage

From 2010 to present, on-going with no fixed expiry date.

Temporal coverage for accessibility

NA.

(2) Methods

BCSMF strictly follows the standard operating procedures (SOPs) drafted in accordance with ISO9001, and SIGUCERT 2014 and CNT (Centro Nazionale Trapianti) 2014 guidelines.

Steps

AF donors, who undergo amniocentesis for maternal age, are selected by a gynecologist of Prenatal Diagnosis Unit, who shows the biobank aims and provides donation informed consent.

Samples are transfer in a refrigerated CE container keeping transport temperature between 10°–20°C; time between sampling and treatment is 30'.

For cryopreservation DMSO is directly added to the AF cell suspension at a 10% final concentration.

Samples are aseptically treated under a dedicated biobank laboratory A+ laminar flow hoods.

AF are frozen in a controlled rate freezer according to a profile suitable for stem cells, up to a final temperature of –140°C, and stored in nitrogen vapor dedicated dewars at –175°C.

AF underwent aerobic and anaerobic contamination test through seeding in specific media.

Donors underwent virological tests at amniocentesis and after 4 months (HIV1–2, HBsAg, HBeAg, HBaAg, HBeAb, HBcA, HCV, TPHA, VDRL, CMV). AF are quarantined in a dedicated dewar until the final test results.

Integrity of thawing AF cells is tested with the trypan blue method, the found vitality is 70%.

Donor serum is obtained by centrifugation at 1500g for 10'; two aliquots of 1ml are stored in –40°C biobank freezer with continuous monitored temperature and data logger.

Donor DNA is extracted from peripheral blood by phenol-chloroform method; quantification and purity evaluation are performed on spectrophotometer. DNA integrity is checked by 1% agarose gel sybersafe stained, in comparison with Lambda DNA marker. DNA is dissolved in sterile distilled water and stored in the –40°C biobanks freezer.

Prenatal Diagnosis Unit provides donor clinical data; microbiology laboratory of AOR Villa Sofia-Cervello provides donor virology and microbiological AF test for aerobic and anaerobic agents.

Samples encoded data are recorded in an Excell database. AF modules are scanned and stored on a biobank dedicated PC and on external safe informatics support whose access is limited to biobank manager and staff. All folders are stored in limited access fireproof cabinets.

FA cells, both fresh and after thawing, were cultured in MSC expansion serum free medium (STEM CELL-Voden); and after 8 days of culture they developed CFU-F colonies. At 15 days culture there was fibroblast growth, which became confluent at 28 days.

On expanded AF-FMSC, both fresh and after thawing, CD106, CD146 and CD105 mesenchymal markers, and CD45 and CD34 non-mesenchymal markers were evaluated, by flowcytometry analysis.

The flowcytometry analysis of expanded FA-MS, both from fresh and after thawing, revealed the presence of mesenchymal markers CD146 and CD105 and the absence of CD34 and CD45 hematopoietic/endothelial markers.

The fetal origin of expanded AF-FMSC, both from fresh and after thawing, was verified and confirmed by QF-PCR on DNA from expanded AF-FMSC and maternal mononucleates.

Stam potential of AF-FMSC, both from fresh and after thawing, was evaluated, before and after expansion, by expression analysis (RT-PCR) of Oct4 and Nanog genes. As control, cDNA from maternal peripheral blood was used.

The specific RT-PCR performed on the expanded AF-MS, both from fresh and after thawing, cDNA revealed the presence of both Oct4 and Nanog expression.

Stabilization/preservation

- AF in 10% DMSO (for cryopreservation).
- Peripheral blood in EDTA (for DNA extraction and serum collection).

Type of long-term preservation

- Frozen at –40°C (for DNA and serum preservation), DMSO and liquid nitrogen for AF.

All samples are stored in temperature-monitored freezers that are locked and accessible only by BCSMF staff.

Storage temperature

Freezer – 40°C (DNA, serum).

Liquid nitrogen –175°C (AF).

Storage length: until the AF samples are available and DNA samples damaged or broken.

Shipping temperature from patient/source to preservation or research use

10°–20°C for AF and peripheral blood samples.

Shipping temperature from storage to research use

Dry ice or liquid nitrogen for AF samples (during the carriage temperature must be below –150°C).

Quality assurance measures

BCSMF strictly applied standard operating procedures (SOPs) drafted according to UNI EN ISO9001, SIGUCERT 2014 and CNT (Centro Nazionale Trapianti) 2014 guidelines.

Main procedures to ensure the reproducibility and efficacy of processes, and the quality of biological samples:

- Controlled access to biobank and cryogenic room with personal badges and indoor camera.
- Management and data storage (database and privacy protection).
- Management of cryogenic room surveillance systems: back-up system with dedicated dewar, constant computerized monitoring and maintenance of liquid nitrogen levels, atmospheric monitoring and ventilation.
- Freezers and refrigerators with continuous digital temperature recording.
- Testing of AF sample contamination: aerobic and anaerobic tests.
- Donor virological fitness.
- Guarantee AF integrity.
- Keep up to-date databases and perform back-up.

Source of associated data

- Medical records.
- Laboratory Reports.
- Instrumental findings.

Ethics Statement**Ethics Committee**

Patients are given a consensus that has been approved by the local ethics committee (prot 128.01/12/2010).

BCSMF operates according to:

- Recommendations issued by the Oviedo Agreement, 1997 (Oviedo – Convention on Human Rights and Biomedicine 04 April 1997) and the Additional Protocol concerning Genetic Testing for Health Purposes (CETS n. 203), 2008 (Strasbourg, 27 November 2008).
- SIGU guidelines, 2014.
- Italian Data Protection Authority (Garante Privacy), Personal Data Protection Code, Legislative Decree No. 196, 30 June 2003, published in Official Gazette No. 174 of the Italian Republic, 29 July 2003.
- Italian Data Protection Authority (Garante Privacy), General Authorisation for the processing of genetic data, 12 December 2013, published in Official Gazette No. 302 of the Italian Republic, 27 December 2013.
- Italian Data Protection Authority (Garante Privacy), General Authorization to process personal data for scientific research purposes, 01 March 2012, published in Official Gazette No. 72 of the Italian Republic, 26 March 2012.
- Guidelines for the Cryobiological Room of a National Institute of Transplants (CNT) Italy, November 2014.

Constraints

N/A.

(3) Bioresource description**Object name**

Human amniotic fluid samples biobank and data.

Bioresource name

- Research Biobank of fetal mesenchymal stem cells.
- Bioresources acronym: BCSMF.

Bioresource location

Biobank Campus UOC Ematologia per le Malattie Rare del Sangue e degli Organi Ematopoietici, Azienda Ospedali Riuniti “Villa Sofia-Cervello”, Via Trabucco n.180, Palermo – Italia.

Bioresource contact

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Bioresource URL

In progress.

Identifier used

N/A.

Bioresource type

Human amniotic fluid biobank.

Type of sampling

Amniotic fluids (AF) from diagnostic amniocentesis.

Anatomical site

Amniotic cavity.

Disease status of patients/source

Patients who undergo amniocentesis performed for maternal age.

Clinical characteristics of patients/source

- General data.
- Anamnestic data.
- Data on pregnancy (age and reason of amniocentesis).
- Virological analysis.

Size of the bioresource

To date, 300 samples of amniotic fluid have been collected and stored.

Vital state of patients/source

All patients are alive at sampling.

Clinical diagnosis of patients/source

Not clinical features, amniocentesis performed for maternal age.

Pathology diagnosis

NA.

Control samples

NA.

Biospecimen type

Amniotic fluid (AF), mesenchymal fetal stem cells expanded from AF.

Release date

NA.

Access criteria

Access to database is restricted to limited number of BB member staff.

The access rules for BCSMF samples are the following:

- The biological specimens can be distributed only for research and only if in the BB two aliquots of the same sample are present.
- The use of biological specimens must be non-profit.
- The distribution of biological specimens must be authorized by the biobank manager.
- In the resulting scientific publication, the authors guarantee to mention the source of the samples, to thank for the service, to send a copy of the publication.
- Research project have to be in agreement with BCSMF mission, and have a wide scientific significance.
- Sample distribution will be free, except for direct shipping costs.

(4) Reuse potential

Public and/or private scientific organizations, wishing to use FA samples, have to make an application on a specific form issued by the BB, to provide all the required data and to specify the purposes of the research for which they plan to employ FA samples.

All requests will be submitted to the attention of biobank Evaluation Committee, consisting of biobank manager and quality manager, who verifies the interest and the impact of the project on the scientific community.

If the request is not assumed appropriate, applicants will be aware about the reasons of the refusal.

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Competing Interests

The authors have no competing interests to declare.

Author Roles

Schillaci G and Damiani G performed amniocentesis.

Schillaci G, Damiani G, Disma R and Giangreco A recruited patients.

Sacco M manage sample database.

Garofalo GM is the biobank quality manager.

Maggio A is the UOC Director, revised the manuscript.

Fecarotta E and Piazza A, collected and processed samples.

Renda MC is the biobank manager, drafted the manuscript.

Cristian C is biobank technological systems manager.

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