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Órgano de difusión científica de la Academia Mexicana de Cirugía
Fundada en 1933

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ORIGINAL ARTICLE

The clinical use of cryopreserved human skin allografts for transplantation[☆]



Francisco Martínez-Flores^{a,*}, María Chacón-Gómez^a,
Juan Antonio Madinaveitia-Villanueva^a, Araceli Barrera-Lopez^a,
Lucinda Aguirre-Cruz^b, Walter Querevalu-Murillo^c

^a Banco de Piel y Tejidos, Instituto Nacional de Rehabilitación, Secretaría de Salud, México, D.F., Mexico

^b Laboratorio de Neuroinmunoendocrinología, Instituto Nacional de Neurología y Neurocirugía «Manuel Velasco Suarez», Secretaría de Salud, México, D.F., Mexico

^c Coordinación de Donación de Órganos y Tejidos, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México, D.F., Mexico

Received 27 October 2014; accepted 6 May 2015

Available online 8 December 2015

KEYWORDS

Tissue bank;
Skin allografts;
Burns;
Biological recovery

Abstract

Background: The biological recovery of human skin allografts is the gold standard for preservation in Skin Banks. However, there is no worldwide consensus about specific allocation criteria for preserved human skin allografts with living cells.

A report is presented on the results of 5 years of experience of using human skin allografts in burned patient in the Skin and Tissue Bank at *Instituto Nacional de Rehabilitación*.

Material and methods: The human skin allografts were obtained from multi-organ donors, processed and preserved at -80°C for 12 months. Allocation criteria were performed according to blood type match, clinical history, and burned body surface.

Results: Up to now, the Skin and Tissue Bank at *Instituto Nacional de Rehabilitación* has processed and recovered $125,000\text{ cm}^2$ of human skin allografts. It has performed 34 surgical implants on 21 burned patients. The average of burn body surface was 59.2%. More than two-thirds (67.7%) of recipients of skin allografts were matched of the same to type blood of the donor, and 66.6% survived after 126 days hospital stay.

[☆] Please cite this article as: Martínez-Flores F, Chacón-Gómez M, Madinaveitia-Villanueva JA, Barrera-Lopez A, Aguirre-Cruz L, Querevalu-Murillo W. El uso clínico de aloinjertos de piel humana criopreservados con fines de trasplante. *Cirugía y Cirujanos*. 2015;83:485–491.

* Corresponding author at: Banco de Piel y Tejidos, Dirección Quirúrgica, Instituto Nacional de Rehabilitación, Secretaría de Salud, Calzada México-Xochimilco 289, Colonia Arenal de Guadalupe, C.P. 14389 México, D.F., Mexico. Tel.: +52 55 5999 1000; ext. 14802.

E-mail address: fcomartinez@inr.gob.mx (F. Martínez-Flores).

PALABRAS CLAVE

Banco de tejidos;
Aloinjertos de piel;
Quemaduras;
Recuperación
biológica

Conclusion: It is proposed to consider recipient's blood group as allocation criteria to assign tissue; and use human skin allografts on patients affected with burns over 30% of body surface (according the "rule of the 9").

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El uso clínico de aloinjertos de piel humana criopreservados con fines de trasplante

Resumen

Antecedentes: La recuperación biológica de aloinjertos de piel humana se considera que es el estándar de oro para los procesos de preservación de bancos de tejidos; sin embargo, no existen criterios de asignación para los aloinjertos de piel con elementos celulares vivos.

Reportamos la experiencia de 5 años en el uso de aloinjertos de piel humana en pacientes adultos quemados, del Banco de Piel y Tejidos, del Instituto Nacional de Rehabilitación.

Materiales y métodos: Los aloinjertos de piel humana fueron obtenidos a partir de donadores multiorgánicos, procesados y preservados en congelación a -80°C durante 12 meses. La asignación de los aloinjertos se realizó en referencia al grupo sanguíneo, historia clínica, grado de la quemadura, y la extensión con un promedio de 40% de superficie corporal afectada y con consentimiento informado.

Resultados: Hasta el momento el Banco de Piel y Tejidos ha procesado más de 125,000 cm² de aloinjertos de piel humana. Se han realizado 34 implantes quirúrgicos en 21 pacientes quemados. El promedio de superficie corporal quemada fue del 59.2%. El 67.7% de los receptores de aloinjertos de piel humana tuvieron el mismo grupo sanguíneo que el donante. El 66.6% de los receptores de aloinjertos de piel humana sobrevivió después de 126 días de estancia hospitalaria.

Conclusión: Se propone considerar el grupo sanguíneo del receptor como un criterio para la asignación de tejido; y el uso de aloinjertos de piel humana en pacientes con más del 30% de la superficie corporal quemada (de acuerdo a la «regla de los 9»).

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Background

Biological recovery is the gold standard in tissue preservation processes in tissue banks.¹ Quality control, health certification and the biological stability of allografts for transportation has a direct impact on the therapeutic result of a successful tissue implant for surgical reconstruction procedures or tissue replacement.

Over the past 5 years, the Skin and Tissue Bank at *Instituto Nacional de Rehabilitación* (BPYT-INR) has developed a technological research programme dedicated to the recovery, preservation and implantation of tissues for transplantation, such as: skin, deep dermis, cardiac valves, tendons and bone tissue. The primary focus of the processes is to keep the cellular elements of these alive and rich in growth factors.

In the specific case of human skin allografts, various preservation processes have been described, including gamma radiation and sterilisation using chemical methods.^{1,2} However, biological recovery with controlled chemical disinfection has recently been accepted as the method with the best therapeutic results.³⁻⁵ The method proposed and developed in the BPYT-INR, the BanPiel-INR

method, is based on preserving cellular viability and controlled isotonic disinfection in class 100 facilities. This enables allografts of viable skin to be obtained for the purpose of transplantation.⁶ However, there are no reports about the bank's experience with regard to the distribution or allocation criteria of live skin allografts in Mexico. From an immunological perspective, skin recovered from cadaveric donors is used as temporary covering with no established allocation criteria.

This article presents the first data of a skin recovery programme in the National Health Hospitals and Institutes of the Federal System in Mexico, and the clinical experiences of recipients.

Material and methods

Donors and tissues

Allografts of skin and other tissues were obtained from multi-organ donors with the informed consent of their families, in accordance with the *Ley General de Salud* (General Health Act) on organ and tissue donation for transplantation.

Table 1 Inclusion criteria for human skin allograft donation.

Inclusion criteria for skin donation candidates	Exclusion criteria for skin donation candidates
Brain death certification for cadaveric donor or certified loss of life certification	Hyperglycaemia + septicemia
Negative serology for human immunodeficiency virus types 1 and 2, hepatitis B, hepatitis C, human lymphotropic virus and luetic reaction a	Unknown cause of death
No significant history of diseases affecting the skin	History of drug abuse
No uncontrolled septic process	Recent tattoos, under 3 years
No clinical history of autoimmune disease	Skin lesion suspicious of neoplasia
Age range 18–75 (aged over 75 after evaluation)	Uncontrolled septicemia
Relative haemodynamic stability	

The protocol for harvesting tissues was authorised by the Internal Research Committee of the *Instituto Nacional de Rehabilitación* (Registration number INR-CII-14B-2011) and funded by the programme of the *Fondos Sectoriales de Investigación en Salud* (Sector Funds for Health Research) (Fosis Project: 2011-161624) as a multi-centre programme of hospitals generating organs and tissues for transplantation.

The inclusion criteria for skin donation are summarised in Table 1, and the current criteria of the European Association of Tissue Banks were used. The epidemiological data of all the donors were recorded, such as: age, gender, blood groups, Rh factor, time of aortic clamping in order to document the warm ischaemia time.

Surgical harvesting of skin allografts

The skin and tissues were harvested in the operating theatre immediately after organs were harvested for transplantation. After the surfaces were decontaminated for 30 min and sterile drapes placed, skin allografts were ablated of medium thickness (0.5 mm/7.5 cm wide), with dermatome (Acculan TIII, B Braun-Aesculap, Germany). The sheets of skin were kept in washing solution (Sol *BPYT-1, pH 7.5) for 30 min and then placed in transport medium (Sol *BPYT-2-Piel) with antibiotic–antimycotic (PPA Biotech, USA).

Storage in the BPyT

Once the tissue had been registered in the BPyT, the process of biological skin recovery took place according to

the protocol,⁶ and our facilities' standard Process Manual (PR-DQ17-ISO-9001-2008) certified 2012, and with health licence 14TR090120006.⁷ The skin allografts were processed according to the routine protocol in the BPyT-INR, and were frozen at -80°C in cryopreservation solution (*Sol Cryo-Piel). All the tissues were kept in quarantine until they were microbiologically, and health certified for use. The quality control certificates were issued to document bacterial growth, absence of human immunodeficiency virus type 1 and 2 (HIV-1, and 2), cytomegalovirus, hepatitis virus B and C, and *Treponema pallidum*. Identification of pathogens was based on nucleic acid tests (NATS), in real time modality (RT-PCR), using the protocols recommended by the manufacturer on the Rotor Gene 3000 platform (Corbett Research, Australia).

General skin allograft allocation criteria

The criteria for allocating tissues for burn patients are based on the general organ allocation criteria. Each patient was documented with: (1) signed informed consent for the use of an allograft, with live cellular elements; (2) request authorised by the manager of the burns unit, specifying the recipient's blood group and the volume requested, and (3) clinical history of the recipient.

The following were considered specific criteria for the allocation of human skin allografts: (1) compatibility with the recipient's blood group, (2) degree of burn and burned body surface area, and (3) the presence of associated surface infection.

Defrosting skin allografts

After the clinical history and the allograft request were received, gradual defrosting was scheduled of the human skin allografts in sterile conditions and laminar flow hood (Herasafe 120, Kendro; Germany). Once the tissues had been defrosted they were kept in a phosphate buffered saline solution (PBS-pH 7.5), with an antibiotic cocktail, taken from each recipient's individual regimen. In this last point, microbiological control samples were taken, to certify the tissue defrosting process, and to rule out any contamination process of the tissue.

Monitoring of recipient patients

The patients who had received grafts were clinically monitored in the burns intensive care unit. The clinical course and hospital stay of each of the recipients was documented. The recipients were followed up as outpatients every 6 months, in order to assess the implant areas long term.

Data analysis

The data were gathered on an Excel MS-Office matrix and descriptive analysis measures were analysed to understand the recipient population.

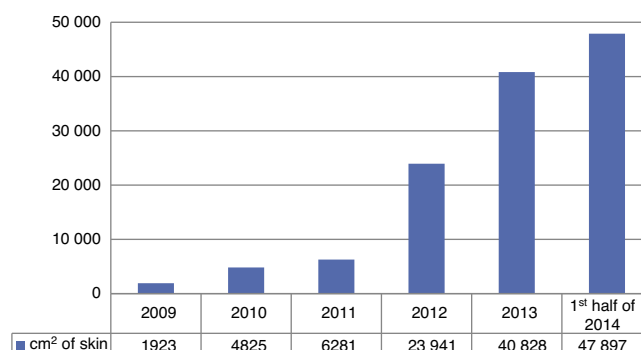


Figure 1 Volume of skin processed in the Skin and Tissue Bank at *Instituto Nacional de Rehabilitación*.

Results

Volume of skin processed using the method of the Skin and Tissue Bank at *Instituto Nacional de Rehabilitación*

During the period between 1 January 2009 and 31 December 2014, 140,351 cm² of human skin allografts, of medium thickness (0.5 mm) were harvested, recovered and frozen in our facilities. Quantitative measures were used for the preliminary analysis of cell viability, based on the metabolism of vital insoluble fluorophores per cm² of each batch of sheets, which demonstrated the presence of live cells in all the tissue, in a percentage greater than 75%. The processing volume of human skin allografts per year is illustrated in Fig. 1, which shows a volume in 2009 of 1923 cm², 4825 cm² in 2010, 6281 cm² in 2011, 23,941 cm² in 2012, 40,828 cm² in 2013, and 62,553 cm² in 2014.

Recipients of human skin allografts preserved in the Skin and Tissue Bank at *Instituto Nacional de Rehabilitación*

During the abovementioned period, a total of 34 requests were received for tissues with a range of 238–6000 cm², and requests were received for a total of 57,937.5 cm² of skin. The distribution by institution was 97.1% in the *Centro Nacional de Atención a Quemados* (CENIAQ) (National Burns Centre), and 2.9% in the Plastic Surgery Department at Hospital General de México. Allocations of human skin allografts for surgical implant were made from 2011, and were: 6 in 2011, 4 in 2012, 17 in 2013, and 7 in the first half of 2014 (Fig. 2).

Epidemiological characteristics of human skin allograft recipients

Of the total number of recipients ($n=21$), the age range was from 1 to 52, with a mean age of 25 and a mode of 29 (Fig. 3). Distribution by gender was 44.2% for female recipients and 55.8% for male recipients.

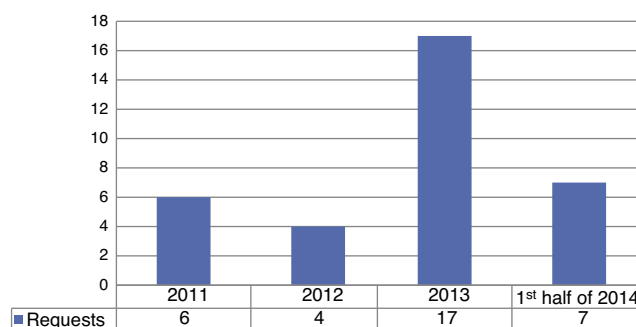


Figure 2 Number of surgical human skin allograft implants preserved in the Skin and Tissue Bank.

Tissue distribution based on blood group and Rh factor

Taking blood group and Rh factor as allocation criteria, there were 34 requests in total, of those 23 were of the same blood group (68%), 10 requests were compatible (29%) and one request was not compatible with the Rh factor (3%) (Fig. 4).

Distribution of tissue based on diagnosis and the patient's burned body surface area

The most common diagnoses for admission to the human skin allograft distribution were burns caused by direct flame 76.1% ($n=16$), 14.5% ($n=3$) scalds, and the remaining 9.4% other burns. The distribution of human skin allografts in relation to recipients with large burned body surface area was as follows: 21 patients (range from 29% to 86% of burned body surface area) with a mean of 56.4% of burned body surface area, and a mode of 60% of burned body surface area (Fig. 5).

Identification of surface microorganisms in the human skin allograft recipients

It was found that 66.6% ($n=21$) of the recipients were reported as colonised, and 33.4% were not colonised at the time the allografts were applied. The frequency of microorganisms is shown in Table 2.

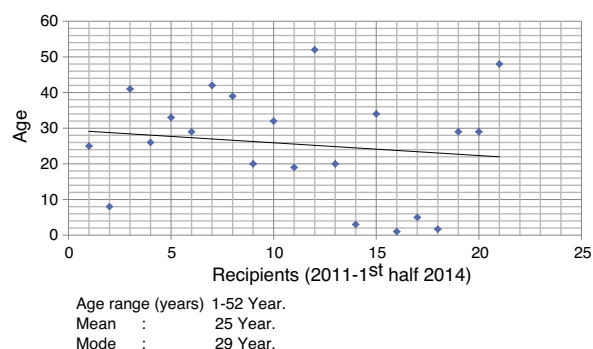


Figure 3 Age distribution of human skin allograft recipients.

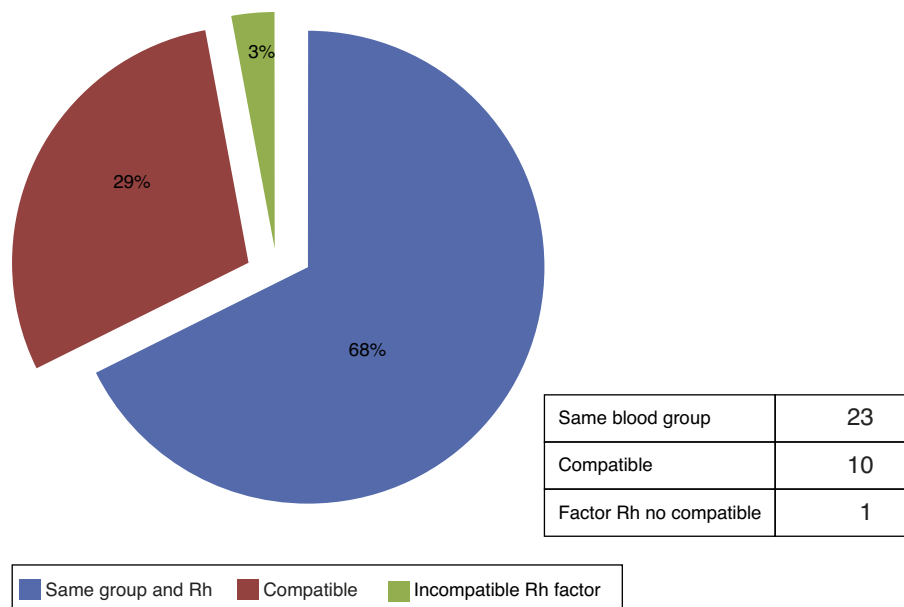


Figure 4 Graph of human skin allograft allocation by blood group.

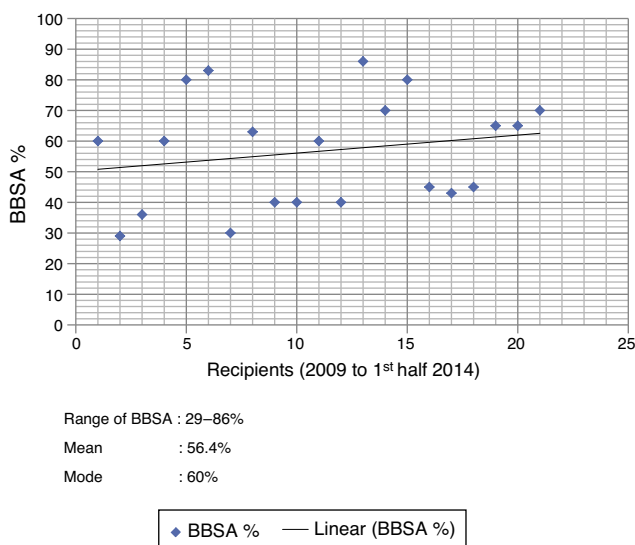


Figure 5 Distribution of human skin allografts and the burned body surface area of the recipient. BBSA, burned body surface area.

Table 2 Microorganisms identified in the human skin allograft recipients.

Microorganism identified	Release number	Frequency percentage (%)
<i>Pseudomona</i> sp.	10	25
<i>Acinetobacter</i> sp.	5	12
<i>Candida</i> sp.	5	12
<i>Staphylococcus aureus</i>	2	5
<i>Acinetobacter baumani</i>	2	5
Did not develop	7	17

Clinical course of the human skin allograft recipients

During monitoring of the human skin allograft recipients, their clinical course was recorded as well as a physical assessment of the grafts. It was found that 76.1% of the human skin allograft recipients ($n = 14$) were alive one month after the human skin allograft was applied. And 23.9% ($n = 8$) died during the first month after application of the allograft (Table 3).

Discussion

Since it was founded in 2009, BPyT-INR has been the first to propose a recovery programme for tissues for transplantation. This programme is based on applied research processes towards meeting health needs. To date, the preliminary studies undertaken in a sample taken at random from each tissue batch have shown a range of 75–85% of live cells per cm^2 of human skin allograft, with negative molecular certificate and with negative microbiological cultures, which are essential as quality processes to ensure health safety. The volume of processed human skin allografts has increased annually, showing an upward trend for the period from 2009 to 2014 (Fig. 1). This growth trend could be due to

Table 3 Clinical course of the human skin allograft recipients.

	Number of patients	Mean burned body surface area (%)	Range of burned body surface area (%)
Alive	$n = 14$	50.07	29–83
Dead	$n = 8$	66.75	40–86

2 factors: (1) the increase in the number of donations as a result of the impact of promotion and education in encouraging a culture of skin and other tissue donation, which did not exist in our country until 2010 when the BPyT-INR launched their first skin donation campaign aimed at the general public via the mass media: television, radio, written press and the internet, and (2) the increased number of hospitals generating and harvesting tissues in the national health institutes and other organ and tissue donation programmes, as well as the participation of other institutions, such as the *Instituto Mexicano del Seguro Social* (IMSS), and the *Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado* (ISSSTE), and the incorporation of other state hospitals.

The clinical impact of human skin allografts for transplantation

Human skin allograft implantation in burn patients is the treatment of choice in the first 48 h after their accident⁸; however, other groups of researchers have described, in addition to its successful use, adding an individual antibiotic regime in patients with added infections.

Of the 14,100 burn accidents per year, 10% are classified as major burns, with third-degree burns, and affected body surface area over 30%. The 2 age groups most affected are children under 10, and adults over 60 with some form of disability. It is interesting that our data showed a mode age of 30 for tissue recipients, which implies that an economically active group is affected. Furthermore, there has been an upward trend in the use of human skin allografts which increased significantly in 2013. This information is important because the application of human allografts is a relatively new technique in Mexico; historically pig skin, amnion and irradiated skin were used for the treatment of burn patients.^{9–11} Our results show that the application of human skin allografts in burn patients started in 2011, and reached its peak of 47.2% in 2013.

The chief consumer of human skin allografts is the *Centro Nacional de Investigación y Atención a Quemados* (CENIAQ), with 97.1% of the 57,937.5 cm² of human skin allografts, requested in the period from 2011 to 2014. This increase is explained by the fortuitous national contingency situation in 2013, and because the main consumer of tissues was a national reference centre (CENIAQ). However, the allocation of tissues is limited to burns units of hospitals which have valid permission for organ and/or tissue donation or transplantation granted by COFEPRIS. This situation should be assessed according to Mexico's current legislation, without forgetting that the method for the biological recovery of tissues involves the risk of transmitting infectocontagious diseases, which can be identified with high sensitivity molecular tests, such as NATs,^{12–14} in order to minimise the risk of transmission; we have implemented these in our processes since the beginning of the programme.^{7,15,16}

The most frequent recipients of human skin allografts are men in the third decade of life, who have had a human skin allograft implanted surgically, with a mean body surface area of 59.2% of burned body surface area (Fig. 5). This information is consistent with that reported in the literature, and reaffirms the use of human skin

allografts in major burns patients as the treatment of choice.

The allocation of human skin allografts based on blood group

According to the reports in the literature, there is no consensus at all with regard to taking blood type (ABO system and Rh factor) into consideration when allocating human skin allografts, because they are considered temporary coverings. In this report, 68% of requests were made according to the recipient's blood group, 29% were for compatible groups; and there was only one case where an incompatible group was designated (Fig. 4), a different blood group was used in the latter case, because the recipient had a rare blood type (A Rh negative). The reason for taking this reference as an allocation criterion is based on the fact that the traditional processing methods, such as gamma radiation or chemical sterilisation, although demonstrated as safe procedures, have the disadvantage that cell viability is sacrificed. Considering that the method we use in our bank is biological recovery, this factor could be essential in implementing scientifically based use of human skin allografts according to the recipients' blood group, in order to minimise responses of incipient graft rejection.

It was found that 67% of the human skin allograft recipients were alive after discharge from hospital. And 32.3% of the human skin allograft recipients died during the course of their medical treatment. The burned body surface area of the surviving patients had a lower mean rate (50.07% burned body surface area) compared with the group of patients who died (66.75% burned body surface area). This suggests that patients with greater burned body surface area present a greater risk of death. However, a more detailed analysis is necessary of the conditions of the recipients, their clinical course, and their general condition on receiving the implant, and the presence of superimposed infections, since these factors are essential and decisive for prognosis.

Conclusions

According to our preliminary results, the human skin allografts processed in our bank are of high biological quality and highly safe, which means that they can be used safely in major burns patients. Considering that the major burns patient is kept immunosuppressed, and even when there is no consensus on the use of human skin allografts for transplantation, it is suggested that the blood type of the recipient should be taken as a criterion for tissue allocation, based on the presence of live elements in tissues processed by biological recovery. A more detailed medium and long-term follow-up study is necessary to determine the impact of the use of human skin allografts in major burns patients in order to establish their therapeutic efficacy, and the economic repercussions for the national health system.

Conflict of interests

The authors have no conflict of interests to declare.

Financing

This project was financed by the Consejo Nacional de Ciencia y Tecnología de México CONACYT, via the Fondos Sectoriales de Investigación en Salud (FOSIS-2011-161624).

María Chacón Gómez is a postgraduate fellow in the project FOSIS-2011-161624.

Acknowledgements

María Simona Ustoa, Pablo Adrián Vizcaíno Dorado, Karina Guzmán Martínez, Araceli Barrera López, Christian Lomeli Rodríguez, Bruno Escobedo, members of the technical and harvesting team of the BPyT, for their collaboration in identifying donors and in tissue processing.

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