

# INVITED SPEAKER LECTURES

## BIOBANKING AND BANKING FOR RESEARCH: INTERACTION WITH TISSUE BANKING

### THE ETHICS OF RESEARCH CONSENT

*Martha Anderson, Musculoskeletal Transplant Foundation.*

#### Introduction:

The use of donated human tissues, organs and other body parts is critical to the development of new pharmaceuticals, medical devices and products, as well as to the on-going training of medical professionals. Unlike donation for transplantation, donation for research is far less regulated and less-well organized. In 2011, the AATB published standards for Non-Transplantable Anatomic Donation Organizations (NADO) that, for the first time, established specific guidelines for handling non-transplantable tissues (NTT). Many individuals who wish to donate for transplant upon their death are precluded from doing so, due to advanced age or disease processes; research donation provides them an opportunity to contribute to science and mankind.

In the U.S, tissue banks and recovery agencies (organ, eye and tissue) provide NT organs and tissues to researchers. A variety of uses and users include:

- Internal Use: Tissue Banks require NTT to conduct new product development and processing validation.
- Academic Use: Academic centers use NTT in research projects such as evaluating the impact of processing techniques on tissue quality, development of new surgical techniques and training medical professionals.
- Medical Industry Use: Bio-banks, Bio-skills labs and pharmaceutical and medical device companies use human specimens to train surgeons in new surgical techniques and to test new medical devices and pharmaceuticals.
- Non-medical Use: Search and Rescue teams use NTT for training cadaver dogs; the military and automotive industries use specimens for testing safety equipment.

Ethical issues associated with research tissues abound. They include:

- Obtaining adequate, legal authorization for research from a donor or the next-of-kin of a recently deceased person
- Using donated tissues and organs for research rather than transplant
- Use of human tissues for non-medical use
- Approval process for non-medical and medical use
- Use of human specimens by for-profit entities
- Eliminating the cost of funeral expenses in exchange for research donation.

#### Background:

For purposes of this abstract and presentation, I will discuss how the Musculoskeletal Transplant Foundation (MTF) and our subsidiary the International Institute for the Advancement of Medicine (IIAM) address these ethical issues.

Specimen Type	Use	Number of Specimens Provided
Organ	Pharmaceutical Research, Other Medical Device Research & Development	830
Anatomic Tissue	BioSkills labs, BioBanks, Medical Device R&D	1450
Anatomic Tissue	Internal (MTF) Research & Quality Assurance	4500
Anatomic Tissue	Academic Research	50

MTF and IIAM provide NT tissues and organs to a variety of entities. On an annual basis, we provide approximately the following number of research specimens:

#### **Donor authorization process**

On average, 89% of MTF donors give consent or authorization for “transplant, medical education and research”. When tissue is rejected for transplant (18% of recovered donors), the donor chart is reviewed and if medical education and/or research authorization has been given, we will endeavor to place that tissue for research, either internally or externally. Approximately 800 donors per year are potential research donors.

IIAM handles NT organs, tissues and whole body donors (WBD) that are not suitable for transplantation. WB donation is often coordinated with a dying patient and/or his/her family during the final phase of life. Nursing Homes, Hospices, Funeral Homes etc. are the primary referral sources. Many NADOs in the US allow patients to pre-register their desire to be a WBD; in these instances, the patient and/or family fills out a consent form, medical/social history form and is pre-authorized for donation prior to death. In other instances, the NADO is contacted at or near the time of death and evaluates the potential donor’s suitability. Research donor criteria is much less restrictive than transplant criteria. Donor authorization is obtained by tissue bank staff who also conduct a medical/social history interview. Authorization for non-medical uses (e.g., automotive safety testing, search and rescue training) is specifically documented.

#### **Determining appropriate use of NT Tissues and organs**

Although there is no mandatory allocation system for NTT in the U.S., it is MTF and IIAM’s philosophy that the priority for placement of donated organs and tissues should be:

1. Transplant
2. Internal research for MTF’s R&D and QA activities
3. Academic or non-profit medical research and surgical training
4. For-profit medical research and/or surgical training

All research entities must execute an application that specifies exactly what types and amounts of specimens are requested and a Biomaterials Transfer Agreement (BMTA). The BMTA includes a wide range of responsibilities of parties including:

- providing a clear explanation of the proposed uses of the donated specimens
- the requirement for IIAM to de-identify the specimens and donor-related documentation
- requirement that all specimens be tested for infectious diseases prior to use
- prohibition against re-selling any specimens to a third party without prior written permission, and
- appropriate disposal of specimens following completion of the project.

Each request for tissue is reviewed by senior staff at MTF, a medical director and other relevant departments such as Processing and QA. MTF’s Ethics Committee reviews all non-medical requests such as cadaver dog search and rescue training or the use of specimens in the development of military and automotive safety equipment, and requires supplemental consents from donor families for these types of research. Additionally, the MTF Donor Family Council is available to review research protocols and specific research requests. Because these specimens come from deceased donors, Internal Review Board (IRB) review and approval is not necessary, which can dramatically speed the review process.

#### **Financial questions**

Tissues from MTF are provided to academic researchers gratis; researchers who receive specimens from IIAM are charged a service fee that covers the costs associated with operating that business unit, with for-profit researchers typically being charged a higher service fee than those from non-profit agencies.

One controversial area of WBD is the practice of advertising free cremation to anyone who becomes a WBD. With WBD, there is no need for funeral services such as embalming, but it is the practice of WBD tissue banks to return ‘cremains’ to the family following donation. NADOs often cremate a small portion of a body, and return those remains to a family for their memorial service. This practice could be seen as an incentive for a family member to donate their elderly loved one’s body to avoid funeral expenses. Advertising by some WBD tissue banks underscores this concern:

***“With cremation prices being so high and the need for body donors to support medical sciences so critical, the lasting impact of whole body donation makes so much sense. So please do look at all of your options. There is high cost cremation, low cost cremation and then there is free cremation. We are sure you will agree that while low cost is pretty good, a free service is hard to beat. Cremation prices are always covered by XYZ when donating your body to science.”***

The AATB’s new standards for Non-Transplant Anatomic Donation Organizations (NADOs) specifically preclude any monetary inducement:

**NT-D3.000 monetary compensation**

Monetary inducement or other valuable consideration, including goods or services, shall not be offered to a donor, Authorizing Person, the donor’s estate, or any other third party, except that the NADO may reimburse responsible third parties for costs directly associated with a donation and may reimburse Living Donors for costs associated with an acceptable donation, including compensation for restoration of lost earnings when directly attributable to donation, if and as authorized by law. Donors or their families should not be required to pay for any expenses related to the Acquisition of NAM for education and/or research.

**Conclusion:**

Donation for research offers potential donors and their families an additional opportunity to contribute to science and mankind. Tissue Banks, medical teaching institutions, pharmaceutical and medical device companies depend on human specimens for new medical education and training, product development and quality assurance validation studies. Ethical issues in the field of Research Donation are similar to those faced by transplant tissue banks including: Donor Authorization, allocation and financial incentives. A Non-Transplant Anatomic Donation Organization should have systems in place to assure that ethical policies are implemented and followed.

## **SPECIFIC CHALLENGES OF DONATION, PROCESSING AND CLINICAL APPLICATION IN PAEDIATRICS**

### **20 YEARS OF EXPERIENCE ON BONE ALLOGRAFTING IN CHILDREN AND ADOLESCENTS**

*Dr. R. Huguet Carol; Dr. F. Torner Rubies; Dr. Alex Muset Lara, Hospital Sant Joan de Déu. Barcelona, Spain*

We can consider that in children and adolescents, there are currently 2 main groups of indications for the use of bone allografts in orthopedic surgery and traumatology.

- The reconstruction of bone defects in limb salvage for cancer surgery.
- The performance of spinal arthrodesis

Limb salvage surgery in the treatment of malignant bone tumors has now become the therapeutic option in most cases. This surgery requires several reconstructive techniques and the use of bone allografts on numerous occasions.

Allografts in orthopedic cancer surgery can be non-structural allograft used for filling cavities, or structural used to replace large bone defects secondary to bone resections.

We may use in 4 ways structural bone allografts in children and adolescents:

- Intercalary allografts
- Osteoarticular Allografts
- Composite allograft-prosthesis
- Composite allograft-vascularized bones.

We can find a high rate of complications by the use of structural allografts in some series published. Among the complications of allografts we can find nonunion, infection or allograft failure, complications that in some cases could be increased by the use of radio and chemotherapy.

In recent times, we have experimented with the composite use of vascularized bone and allograft, joint to a stable osteosynthesis that could significantly reduce these complications.

We consider that the use of structural allografts in children remains a valid option of treatment that may be permanent in some cases and in others allows growth and preserve bone stock during skeletal maturation.

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## THE CHALLENGES OF MUSCULOSKELETAL TISSUE TRANSPLANT IN CHILDREN

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From 1987, 4661 musculoskeletal allografts have been managed in our bone bank. Cortical allografts are mainly used for surgical treatment of paediatric bone sarcomas. Increase in survival combined with the demanding functional requirements of these young patients challenge the surgeon with limb salvage and reconstruction procedures. Since the early '80s, it has been well known that conservative limb surgery for bone sarcomas does not reduce the possibilities of survival. Thus, in most centres with experience, conservative limb surgery is the first treatment option. This approach, however, is much more disputed in the case of child patients. Conservative surgery in children is a challenge for the orthopaedic surgeon because of the small size of the patients and, the possibility of secondary limb length discrepancies and functional requirements.

Surgery is carried out in two steps. First, there is en bloc resection. The second step is reconstruction. The different types of limb salvage surgery can be classified by location of the tumour: Joint resections or diaphysis resections. According to the implanted material, reconstructions can be: Biological (allografts or autografts), non-biological (megaprosthesis or expandable prosthesis) or combination of prosthesis and allograft. Growth plate represents a temporary barrier to the tumour spread. When the tumour does not affect the articular end of the bone, it is possible to conserve the joint. This has numerous advantages for the patient. In the case of paediatric patients, the advantages are even greater, because the long-term problems of prosthetic reconstruction in children can be avoided and because conservation of the growth cartilage makes it possible to avoid many subsequent problems. Loss of the joint (as occurs, by definition, under any articular resection) can later lead to a significant degree of functional deficit. It was for this reason, to avoid loss of the joint, that Professor Cañadell developed, in 1984, a technique to allow conservation of the articulation and with it conservation of a great part of growth potential: epiphysiolysis before excision of the tumour. When the joint surface needs to be resected in children, an osteoarticular allograft can be used for reconstruction. This reconstruction method can be used as a temporary solution, while the patient is still growing.

Chemotherapy and radiation therapy have a negative influence on the healing of the allograft. The average consolidation time for a metaphyseal osteotomy is 6.5 months. In contrast, for a diaphyseal osteotomy, consolidation takes on average 16 months. Cortical bone allografts are associated with a higher rate of complications than those of cancellous bone. Stabilisation of the graft must be sufficiently secure to allow consolidation. Depending on the location, there are different techniques: Kirschner wires, osteosynthesis plates, intramedullary nails, etc. At the metaphyseal level, it has been demonstrated that consolidation takes place quite easily whichever method is used, and so complex and laborious syntheses are not necessary; crossed Kirschner wires are sufficient in most cases. The younger the patient, the faster is the consolidation of the allograft.

When possible, preservation of the growth cartilage will avert the problem of later dissymmetry. Note that graft osteosynthesis (if this method of reconstruction is used) must respect the physis if we wish growth to continue. For tumours that are near to or even in contact with the physis, epiphysiolysis prior to resection can conserve the greater part of potential for growth. Finally, if resection implies loss of the growth cartilage, we can employ a reconstruction method with an implant somewhat longer (2 or 3 cm at the most) than the resected piece, thereby diminishing the final dissymmetry. Sometimes, a complication can be made into an opportunity for elongation, for example, when a graft or insert has to be removed due to infection or fracture. Elongation can be through the patient's healthy bone, or the reconstruction material can be substituted for a larger version and elongation applied through the soft parts.

These grafts are usually extracted such that a part of each tendon is left, for example, the insertion of the gluteal tendons, rotator cuffs or patellar tendons, for soft tissue reconstruction. In special locations, such as foot bones, allografts can also be used for reconstruction after resection of tumours.

### Conclusion:

In the last few years it has become clear that in most paediatric cases of bone tumour it is possible to use reconstructive surgery with limb preservation and thereby improve these patients' functionality and quality of life.

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## **SKIN GRAFTING FOR BURNS – THE PARTICULAR CHALLENGES OF GROWING CHILDREN**

*HERSON, M. Australia.*

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Quite familiar is the saying: “children are like small adults”. Therefore, it should come of no surprise that children, similar to any other age group, become highly vulnerable to infection and prone to intense fluid depletion at the loss of continuity of the skin envelope. The difference lies in the intensity – that is when age and ‘size’ matters and responses are more dramatic.

Unfortunately, children remain as the largest age group prone to burns, quite often a result of a minute of parental distraction in the home setting. Emotions become high, challenges in treatment are multiple, and end functional or esthetical results quite often concerning, if not disappointing.

Burns scar for life – and children who survive severe burns, become otherwise healthy adults who have the largest part of their lives ahead of them. To compound the problem, different from the adult population children are, inevitably emotionally immature and ...must grow. Whilst within time, some scarring may subside into acceptable aesthetic levels, hypertrophic scarring and keloids particularly around articular surfaces may conform into true armours which prevent normal development and function. Progressive restriction of movement occurs, leading to severe functional deformity if not growth impairment. The ultimate consequence is social isolation.

The medical experience in the 50's Korean War increased the understanding around fluid replacement in the hypovolemic shock phase – this was a milestone in improved survival rates. Additional gains derived from care in specialised Units and the advent of topical anti microbials. However, the next important step in the survival rate increase was the acknowledgment of the benefits of early removal of damaged tissue. This has changed the way burns are managed today, in particular large ones. However, aggressive removal of tissues bring the need to cover the newly opened wounds; as alternatives were looked into, banked human skin became the gold standard as temporary wound cover.

Skin allografts can be applied immediately after in depth wound cleaning or surgical debridement and become accepted as “own skin”. Once applied to the wound, the ensuing renewed barrier promotes modulation of the healing response, reduction in fluid loss and in bioburden. By protecting the exposed nervous terminals, pain is alleviated. Children, of all patient populations, can highly benefit from this almost collateral effect, as has been described by the Dutch in the use of glycerol processed allografts as coverage for deep, and painful, second degree burns.

Unfortunately, within a couple of weeks of transplantation, allogeneic tissues are recognized by the immunocompetent recipient as ‘non-self’, and rejection is triggered manifested as sloughing of the epidermal component and absorption of the dermal component. This apparent tissue dissolution has made surgeons to consider skin allografts to be, albeit the best possible, only temporary wound dressing alternative.

As burn victims survive the initial trauma, wounds start to heal. Wound closure can become complete either through spontaneous re-epidermisation of more superficial (1<sup>st</sup> degree) burns, or by permanent replacement of damaged skin by auto grafting. Left to Nature, deep wounds will heal through a combination of re-epidermisation from the healthy borders and most important, through wound contraction; the end result will involve various degrees of hypertrophic scarring and wound bed contracture.

Is allograft skin truly anything else but a ‘temporary wound cover’?

Increasingly, there is the recognition that this may not be all the truth. Despite the apparent liquefaction of the allograft as the manifestation of the rejection phenomena, there seems to be a degree of allo dermal collagen integration into the wound.

Areas treated with allografts have lower tendency to hypertrophic granulation tissue and following auto grafting seem to evolve in the long term, as more pliable and contour improved areas. Potentially, the increased elasticity leads to less contractures. Therefore, improved outcomes of healed burn wounds closure seem to have a direct correlation between the presence, or absence, of dermal components.

This being the case and in the premise that dermal elements of the allografted skin can become permanently incorporated to the wound bed, allogeneic skin grafts can be processed and its use expanded from the concept of a just very sophisticated 'like for like' temporary wound dressing into a regenerative matrix of the so welcome neo dermal component.

Biotechnology enhanced products will eventually supersede traditional banked split-thickness human skin allografts. However, for the moment being, allogeneic skin tissue remain as a most versatile alternative where expanding its use from mere wound dressings into providers of essential elements for dermal regeneration may be of particular value in growing children.

## CARDIOVASCULAR (I)

### REPORT FROM THE CONSENSUS MEETINGS OF EUROPEAN CARDIOVASCULAR TISSUE BANKS

Theo de By<sup>1</sup>, Roland Hetzer<sup>1</sup>, Robert Parker<sup>2</sup> and Carl MacDonald<sup>3</sup>

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The report with respect to the Consensus Meetings of European Cardiovascular Tissue Banks comes in four sections:

1. General purpose of the consensus meetings
2. The surgeon's evaluation of the importance of the cardiovascular tissue banking methods
3. Directory of European Cardiovascular Tissue Banks and overview of addresses world wide
4. Decontamination methods in cardiovascular banks, validation, science and consensus

#### 1. General purpose of the consensus meetings

Consensus meetings of cardiovascular tissue banks have been held since the 1990's. Its purpose is for cardiovascular tissue banks to compare and discuss the many different methods that are used for standardization. The methods discussed cover the entire range of the tissue bank process, from donor recruitment and selection criteria to thawing and implantation.

After an interval of some years, The Foundation of European Tissue Banks decided to revive these useful meetings. As a result, 2 meetings took place in 2011; one of these was entirely focused on prevention of contamination, microbiology detection methods and different decontamination processes. Thirty-six professional representatives of 22 cardiovascular banks contributed to the programs and discussions of those consensus meetings.

The result was a "cross fertilization" of insights, knowledge, evidence, experience and learning, and a certain degree of consensus has been achieved. Summaries of the meetings were made available to the participants and ensured progress in the consensus, aimed at continuous improvement of cardiovascular tissue banking methods.

The Foundation of European Tissue Banks intends to continue the meetings in 2012 and subsequent years.

#### 2. The surgeon's evaluation of the importance of the cardiovascular tissue bank methods

Unfortunately, some surgeons may take it for granted that cardiovascular tissue grafts of good quality are available for those patients for whom a homograft is the implant of choice.

Demonstrating the experience with the treatment of endocarditis patients and right ventricular outflow tract reconstruction in infants and children at the German Heart Institute in Berlin, it becomes clear that a good communication between surgeons and the cardiovascular tissue bankers contributes to a better understanding of the surgical needs and a continuous improvement of methods and specifications issued by the tissue bank. For the treatment of active endocarditis, infecting the patient's heart valve(s), a combination of a treatment with antibiotics, combined with surgery is the effective method of choice. The specification and characteristics of the homografts used by the surgeon must be standardized, and predictable in terms of short- and long term outcome. The cases of endocarditis at the German Heart Institute show a great variety in morbidity. Most homografts, implanted in those patients, were tailored according to the patients' needs. As the homograft itself is not produced as an industrial product, both qualitatively and quantitatively, communication between tissue bankers and surgeons is of high importance. Besides standardizing information from tissue bank to surgeons, the feedback of the surgeons to the tissue banks is highly necessary for tissue bankers to improve methods where possible and to anticipate the surgeon's future needs. The Foundation of European Tissue Banks brings both together to achieve a consensus about the best methods for the benefit of our patients.

#### 3. Directory of European Cardiovascular Tissue Banks and overview of addresses world wide.

Bringing together of cardiovascular tissue bankers requires an overview of the organisations involved. Prior to and during the consensus meetings the need was felt to create a directory in which we could easily identify the different tissue bank organisations as well as colleagues in Europe and other parts of the world. For the first time we have been able to compose such a directory for publication.

The template for our Directory was generously provided by the EEBA, thus it follows more or less the same format. There is an overview per European country, including the tissue banks which have been identified.. In addition, the Directory contains specific statistics provided by 19 cardiovascular tissue banks in 10 countries. The numbers provided by the tissue banks cover the use of about 1700 hearts during the period 2007-2010. The responding banks issued 1486 homografts in 2010. Of these grafts 34% were aortic and 66% were pulmonary valves.

Statistics about donor age, microbiology and serology do give an insight in the dynamics influencing the results of the contributing tissue banks. The addresses of colleagues world wide have been listed, as far as we could trace them.

The Directory is clearly a first edition. We had to work with the data that were graciously made available by our colleagues. We realize that this edition is not yet optimal. The Foundation of European Tissue Banks strives to issue a second edition in 2012.

#### **4. Decontamination methods in cardiovascular banks, validation, science and consensus**

Comparing the methods of detection, decontamination and validation used by 17 European Cardiovascular Tissue Banks, it was learned that there is an existing wide spectrum of methods. At the Consensus Meetings, organized by the Foundation of European Tissue Banks, it became clear that there is a large variation both in use of antibiotics as well as in concentrations of these antibiotics and the time period over which these antibiotics are used. Some of these methods are supported with scientific studies, others with extensive validation methods as used in the pharmaceutical industry. Those cardiovascular banks that had not yet documented the rationale of their methods have certainly been able to benefit from the work done by their colleagues. It has become clear that even if methods are different, they may lead to the same desired result, namely an allograft without micro-organisms which could infect the recipient. The exchange of methods and results will be continued, a.o. by setting up an exchange of samples between cardiovascular banks and their microbiology laboratories, to further prove the effectiveness of the different decontamination methods.

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## **BRITISH ASSOCIATION FOR TISSUE BANKING CARDIOVASCULAR MICROBIOLOGY SURVEY 2010**

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### **Abstract:**

In 2010, the Cardiovascular Special Interest Group of the British Association for Tissue Banking (BATB) surveyed the microbiology testing, decontamination and evaluation practices of its members. This survey was subsequently extended to heart valve banks in Europe, North America and Australia. It addressed different aspects of microbiology protocols in detail:

- How banks tested the microbiological status of allografts both pre and post decontamination
- How banks decontaminated allografts
- How banks determined if allografts were suitable for clinical use based on microbiological data

The survey was circulated using an online survey tool, although written responses were also facilitated. It was carried out under the promise of anonymity to encourage participation.

Initially, fourteen responses were received. These were reviewed and summarised, and the outcome report was circulated to all participants in May 2011. Two further responses were received after this date, and the report updated accordingly in October 2011. Copies of the report have been circulated to all banks that participated in the survey, and may be obtained from the author on request.

The objective of the survey was to collate information on microbiology protocols used in different banks, so that banks could review the protocols used by their peers and determine where improvements in their own practises might be implemented. The collated data revealed considerable differences in decontamination, testing and acceptance protocols, even between banks operating in the same country. This highlights the need for close liaison between heart valve bankers, leading ideally to international harmonization of microbiology protocols to agreed best practice.

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## DEVELOPMENT OF UNIVERSAL CARDIOVASCULAR NOMENCLATURE

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### **Aim:**

The development of universal cardiovascular nomenclature is one of the current aims of ICCBBA.

### **Background:**

ICCBBA is an international, non-governmental, non-profit, information standards organization which manages the ISBT 128 standard - a global standard for the identification, labeling, and information transfer of human blood, cell, tissue, and organ grafts across international borders and disparate health care systems. The organization is staffed by eight, has a volunteer Board of Directors from around the world, and includes various technical advisory groups (TAGs) made up of 270+ volunteer experts. There are 3 TAGs which deal with tissues: North America Tissue TAG (NATTAG), European Tissue TAG (ETTAG) and Eye Bank TAG (EBTAG).

### **Methods:**

At the beginning of 2011 two Joint ETTAG-NATTAG Terminology Working Groups have been established to ensure a universal approach during revision of existing ISBT128 tissue graft terminology (Joint ETTAG-NATTAG Terminology Work Group for Cardiovascular Tissues and Joint ETTAG-NATTAG Terminology Work Group for Tendons). All the terminologies developed through Joint ETTAG-NATTAG Terminology Working Groups will be public and published on the ICCBBA website in a standards terminology document. The developed terminologies can be used for any coding system including ISBT 128. Therefore in order to ensure as global approach as possible the experts not only from Europe and North America but from other parts of the world (South America, Asia-Pacific region, Australia) were invited to participate in both groups. The groups work by consensus. Meetings are held through conference call and e-mail discussions. Anticipated time to complete each terminology is twelve months.

### **Results:**

To date, 14 individuals from 8 organizations were interested to offer their time and knowledge by taking part in cardiovascular project. They met 2 times by teleconference calls and started developing a consensus for class and attribute definitions of cardiovascular grafts.

### **Summary:**

The Joint ETTAG-NATTAG Terminology Work Group for Cardiovascular Tissues encourages everyone who has an interest in their work to participate in calls and meetings. To learn more, contact the ICCBBA office.

## REPRODUCTIVE

### **THE POWER OF DEMOGRAPHY. HOW DEMOGRAPHY DYNAMICS AFFECTS SOCIETY, POLITICS AND ECONOMY. THE PARTICULAR PROBLEM OF ART**

*J. de Mouzon, MD, MPH, EIM past chairman*

#### **Introduction:**

Access to assisted reproductive technology (ART) shows huge variations in the World, from a few cycles to several thousands per million in other countries. These differences can be viewed as inequity for couples suffering of infertility in those countries where access is low, and would need to be considered by health authorities and national policies. Many factors can play a role on the access, in particular, demography. But demography also interacts with economic and other societal aspects.

**METHODS:** ART data were obtained from World and EIM-ESHRE registries on ART, in which data are collected from established national or regional registers of various origin. Demographic data were obtained from the U.S. Census Bureau, Population Division, and the

CIA World fact book, in population section, through their website portal. Economic data were obtained in World development indicators (World bank). The analysis concern 2007 data from 52 countries participating in the ART registries. They belong to Europe, North and Latin America, Asia, Australia / New Zealand. Analyzed demographic indicators were the total fertility rate (total number of children per woman at the end of reproductive age), the mean women's age, the proportion of women of reproductive age (15-45 years) the women expectancy of life, infant mortality. The economic variables considered were the gross domestic product, total and per capita, the funding of ART cycles and the distribution of Public/Private Health Expenditure.

#### **Results:**

Human reproduction has to be considered in a general context of decreasing fertility rates, not only in developed countries, but also in developing ones, this being probably part of the demographic transition. However, the fertility rate is generally higher in countries with a low mean women's age, high proportion of women aged 15-45, a low life expectation, high infant mortality, and low GDP. On the opposite, access to ART is high in countries with high women's age, low proportion of women aged 15-45, a high life expectation, low infant mortality, and high GDP. ART access is also much higher when access is free or reimbursement important and in countries where the general balance between public and private health expenditure is in favour of public.

#### **Conclusions:**

Fertility decreases in all countries, and particularly in developed countries (<2.0 child / woman). Many demographic and economic factors play a role, but they do not explain all. Infertility treatments, and particularly ART, can correct partly this decrease. Clearly, demographic and economic factors are related to access to ART, and play a role in its inequity across the countries. Finally, it is also probable that other major factors, in particular cultural and religious, influence policy makers and consumers in making ART differently available across the world.

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## VIRAL TESTING OF PARTNER GAMETE DONORS – TIME FOR A CHANGE TO EU REQUIREMENTS?

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### Introduction:

The European Tissues and Cells Directive (EUTCD) 2006/17/EC, Annex III defines the requirements for partner donation (not direct use). The text states that donors (both members of the treated couple) of reproductive cells, which are processed and/or stored and reproductive cells that will result in the cryopreservation of embryos must be tested for HIV, Hepatitis B and C **at the time of donation** to assess the risk of cross-contamination.

### Background:

Many couples treated with medically Assisted Reproductive Technology (ART) receive more than a single transferral of gametes and embryos. In 2009 the European practices were discussed at a meeting of Competent Authorities for Tissues and Cells, with a view to establishing the level of compliance with the requirements of Annex III. The collated information from various Member States (MS's) of the EU revealed different interpretations and national practices with regard to the time frame and frequency of testing. Only a few MS's had implemented testing literally at each transferral event (i.e. 'each donation'). The majority reported testing every 6, 12 or 24 months e.g., Denmark interprets "donation" as comprising of a sequence of procurements events. A consecutive series of such events within the **same clinic**, and within the **same healthy and tested couple**, is regarded as part of one treatment regime and donation; so long as the treatment regime does not exceed a twenty-four month period.

The MS's also noted that testing at each time of donation is costly. Since a typical fertility treatment may extend over a 1 – 2 years period, with treatment intervals rarely exceeding 4 months, it is projected that each partner in the fertility treatment would have to be tested between 3 to 6 times. In addition, the high frequency of testing in presumed healthy couples, where the "risk behaviour" has been excluded by health professional interrogation, were seen as disproportionate compared with the risk reduction; where the latter being assessed as marginal or nil, therefore not adding appreciably to overall safety. However, the minutes of the Commission meeting reflect the Commission's view the Directive was clear, and that MS's not testing at 'each donation' were in infringement of the regulations.

On the above background, a Working Group in which participated three Competent Authorities (Denmark, Ireland and Belgium) and ESHRE (European Society of Human Reproduction and Embryology) was set up by the Commission in 2009. Its defined scope was to collect and analyse the available evidence-based data to formulate a modified proposal of a testing protocol – for partner donations - based on current scientific data. The working group was tasked to provide a comprehensive overview and a quantitative assessment of the potential risks related to quality and safety, if the testing frequency was reduced from once per donation, to once per year or once per 2 years. Areas of identified interest were the probability of infection of embryos, the potential or known cases for transmitting HIV, Hepatitis B and C during processing, preservation and cryo-storage, as well as the potential mix-up of gametes. In connection with this work, ECDC (European Centre for Disease Prevention and Control) was commissioned to perform a study and make a report, including risk assessments of various regimes of testing requirements for reproductive cells in partner donation, from 'each donation' to 'periodic testing' (e.g. every 12 or 24 months).

### Methodology:

The ECDC risk assessment was based on a model for the estimation of **residual risk (RR)** using available estimates of prevalence and incidence of the infections concerned. The residual risk is typically expressed as the probability of an infected donation being used or as the number of donations that need to be screened before one is missed. The residual risks under the different testing procedures were compared to estimate the change in risk.

A literature search of published studies on viral infections among MAR service patients and the risk of cross-contamination. Thereafter estimating the risk was based on the experience of the infection of the partner, when there is a test error or sero-conversion in the patient; infection of the non- partner in case of sero-conversion and mix up of gametes; infection of the non- partner in case of sero-conversion and transmission of virus during cryo-storage of embryos, as well as infection of the non- partner in case of sero-conversion and embryos infecting embryos.

### Results:

The residual risk of missing a case with the current testing requirements

- HIV: 17.83 undetected cases / million person years i.e. one case missed for every 56,101 tests
- HBV: 32.08 undetected cases / million person years i.e. one case missed for every 31,172 tests
- HCV: 267 undetected cases / million person years i.e. one case missed for every 3,745 tests

There was only a modest increase in the residual risk, when comparing the testing every 6 months, 12 months or 24 months with the base case of testing at each donation (which was assumed to take place in 4 month intervals)

The absolute numbers of additional cases missed when applying 24 month schedule were found to be:

- HIV: 0.46 more cases /million person years
- HBV: 1.93 more cases / million person years
- HCV: 8.6 more cases / million person years

The major contribution of risk comes from the prevalence, and this is practically removed in all of the potential scenarios by performing the first test itself.

Among ~14000 MAR service patients, including some patients found to be HBV-, HCV- or HIV –positive, the occurrence of cross-contamination in the ART facility, or the transmission of virus, and cross-contamination in storage, as well as horizontal or vertical transmission to a partner or neonate has never been documented. (Wingfield and Cottell, 2010).

Additional information in published journals have shown the risk of cross-contamination during processing, cryo-preservation and storage of reproductive cells to be negligible or non-existing.

Sero-conversion after tests of ~14.000 patients in Ireland were not seen in the study reported by Wingfield and Cottell. Risk of infecting the partner is much higher at home in an intimate relationship

We have never seen a sero-conversion in a viral negative ART patient. In 2010, 3 cases of gamete mix up were reported out of 42.000 cycles in the UK. Transmission of a virus through an embryo has never been seen. (Risk= never x 1 / 14000 x never)

We have never seen a sero-conversion in a viral negative ART patient. We have never seen transmission of virus between embryos & gametes in cryo storage. We have never seen transmission of a virus through an embryo. (Risk = Never x Never X Never)

We have never seen a sero-conversion in a viral negative ART patient. We have never seen airborne transmission of virus. We have never seen transmission of a virus through an embryo. (Risk = Never x Never X Never)

### What is the problem?

- Fertility clinics work with patients screened for infection
- For each couple it is known whether they are infectious or not
- More than 99 % of the patients seeking fertility treatment are free from HIV and Hepatitis virus
- All patients are tested e.g. ~100 % of patients in the normal ART program are free from HIV and Hepatitis virus

### Conclusion:

Based on the results presented at the meeting for national competent authorities (NCA's) for tissues and cells in June 2011 the NCA's group concluded that it was not needed to maintain the current testing requirements for partner donation as laid down in Annex III of Directive 2006/17/EC, seeing a testing regimen of up to 24 months would not add significantly to risk of transmission of infection. Taking this formally into account would require an amendment of the Directive, through a regulatory procedure. It is likely the proposed amendment to Directive 2006/17/EC may be formalised at the end of this year, with its transposition into national regulations taking a little longer.

From a financial perspective there are considerable cost savings in relation to testing for HIV, Hepatitis B and C for partner donors of reproductive cells in fertility treatment in Denmark and in the whole Europe.

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## SKIN AND AMNIOTIC MEMBRANE (I)

### CHALLENGES ON AMNIOTIC MEMBRANE - PROCESSING AND CLINICAL USE.

*Koller J., Comenius University School of Medicine and University Hospital Bratislava, Burn Department and Central Tissue Bank, Slovakia*

#### Introduction:

Human foetal membranes (HFM), from an anatomical point of view, represent two loosely connected membranes engulfing the human foetus during embryogenesis and organogenesis until delivery. The inner, thinner, strong and shiny membrane is the amnion (HAM), whereas the outer, thicker, less homogenous and dull membrane is the chorion (HCHM). Processing of HFM for use as a biological skin substitute/dressing was first described back in the 19th Century by Davis (1910) and Sabella (1913). Currently HFM, particularly HAM, are used as biological dressings or carriers for different purposes.

#### Material and Methods:

This presentation is aiming to review the relation between anatomical structure, biomechanical properties and content of various active substances included in HAM to functionality of HAM used in various clinical and therapeutic indications. In addition to approved methods of clinical applications of HAM, progress in research, particularly development of more sophisticated both somatic and embryonic in vitro cell culture methods, new horizons for enlarged use of HAM in research as well as clinical practices are going to be opened.

#### Current processing and clinical use:

Availability of HAM is good, as they are procured from living donors at childbirth. Donor screening and testing are provided according to current European regulations. Procurement is done either in clean environment such as delivery rooms, or under aseptic conditions during Caesarean sections at operating theatres. The procured material is transported in sterile containers to tissue establishments for processing. Processing is provided according to standard operative procedures applied in particular establishments. Preservation can be performed by air drying, freeze drying, deep freezing, or glycerolization. In case where clean non-sterile processing is provided, terminal sterilisation by irradiation, or other validated method is mandatory.

Current clinical use of HAM include treatment of burns, skin donor sites, different both acute and chronic wounds, ocular diseases and injuries and as a separating membrane to prevent adhesions in various surgical situations.

#### Challenges and perspectives:

In the recent few years a lot of research is going on in the use of HAM as a source of pluripotent stem cells, as a source of growth factors and cytokines for wound healing purposes, and as a carrier of in vitro cultured cells for clinical applications in both ophthalmology and plastic surgery.

#### Summary:

HAM have been used clinically for already more than one century in various clinical situations with good results. Their beneficial effects include barrier function, wound protection from outer environment and infection, wound preparation for optimal take of skin grafts, pain reduction, enhancement of angiogenesis, epithelialisation and reduction of scarring. Their advantages include availability, simple procurement, preparation and preservation methods, low antigenicity, and, last, but not least, relatively low costs.

## LEARNING FROM BIOVIGILANCE

### THE NOTIFY PROJECT

*Alessandro Nanni Costa, Italian National Transplant Centre*

From September 2010 to February 2011, the World Health Organization (WHO), the Italian National Transplant Centre (CNT) and the EU-funded Project 'Vigilance and Surveillance of Substances of Human Origin' (SOHO V&S) joined forces to organise a global initiative aimed at raising the profile of vigilance and surveillance of substances of human origin; the initiative was called Project NOTIFY. The scope of the project included organs, tissues and cells for transplantation and for assisted reproduction. Ten international expert groups were tasked by WHO with gathering documented cases of reactions and events across the scope of the substances under consideration, using published articles and vigilance system reports as their information sources. The work was conducted on a Google site where over 100 participants (regulators, clinicians, professional society representatives, scientific experts) inserted more than 1,500 published references and added comment regarding alerting signals and the methodology applied for confirmation of imputability. The cases were used as the basis for developing draft guidance on detection and confirmation of reactions and events, with an emphasis on the key role of the treating physician.

The NOTIFY project culminated in a meeting that took place in Bologna from February 7<sup>th</sup> to 9<sup>th</sup> 2011. The 113 invited experts from 36 countries represented regulatory and non-regulatory government agencies, professional societies and scientific and clinical specialities from all WHO regions. The meeting was made possible with funds raised by CNT together with those allocated within the SOHO V&S project for an international meeting on vigilance reporting and investigation. The meeting explored the work already carried out on-line and agreed on priorities for the future development of global V&S for organs, tissues and cells.

From the meeting, the Bologna Initiative for Global Vigilance and Surveillance (**BIG V&S**) was established and a number of outcomes will result. A detailed report of the meeting is in the process of publication, together with 5 didactic guidance documents, each one addressing one category of adverse reaction or event. The SOHO V&S project is using the data and commentary in the development of its guidance for tissue and cell V&S in the EU. WHO will publish a booklet for clinicians that will summarise the guidance on detection and investigation of adverse reactions and events that was developed by project Notify.

A new dedicated website is being developed by CNT, as part of a sustained collaboration with WHO, for the promotion of V&S. The site will host the Notify compendium of adverse incident cases in a database that will be publicly accessible. These cases, and new cases as they arise, will be posted on the site using key words and a minimum data set which will enable searching by, for instance, type of human substance, type of infectious disease transmission agent, type of logistical error. The tool will be a source of information for clinicians, potential donors and patients who wish to understand better the risks associated with particular types of donation or human application; for professionals who need information when deciding on the suitability of a potential donor and for regulators who need information on previous experiences of specific types of reported events and reactions.

An international Editorial Board with regulatory and professional representatives from the fields of organs, tissues and cells, has been established to oversee the work of the new website and to take forward the other outputs of the Bologna Initiative including the development of correspondence tables for terminology and agreement on common definitions, where possible.

It is intended that this initiative will facilitate global sharing of V&S information and guidance for the enhancement of donor and recipient safety and for greater public transparency in transplantation and assisted reproduction.

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## **VIGILANCE AND SURVEILLANCE OF SUBSTANCES OF HUMAN ORIGIN - THE SOHO V&S PROJECT**

*Deirdre Fehily PhD, Italian National Transplant Centre.*

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The project 'Vigilance and Surveillance of Substances of Human Origin' (SOHO V&S) was launched in March 2010 and will run for 3 years, co-funded by the European Union Public Health programme. The key objective is to develop a shared view of how serious adverse events and reactions associated with tissue and cell donation or human application should be reported, evaluated and investigated within the EU. A series of work packages address specific areas of concern: vigilance in assisted reproduction, vigilance for living donors, how investigation of adverse incidents should be performed, the promotion of vigilance and surveillance among clinicians and the detection and prevention of illegal and fraudulent activity. The project aims to facilitate harmonisation of terminology and documentation and consensus on how information should be exchanged between EU Member States, the European Commission and third countries.

The project is co-ordinated by the Italian National Transplant Centre (CNT). It has a Steering Committee and a large number of collaborating partner organisations, including all of the major European professional societies in the field and a number of professional. The involvement of the World Health Organisation and many collaborating partners, both professional societies and regulators, from outside the EU ensures that the guidance developed in this project reflects international needs and realities, in the context of global movement of human tissues and cells for human application.

Although the EU Directives include gametes and embryos in their scope, it emerged during the EUSTITE project that the Directive definitions and the EUSTITE vigilance tools were not fully adapted to the field of assisted reproduction and the reporting requirements are interpreted in different ways in Member States. A SOHO V&S work-package led by the French Biomedicine Agency has explored these issues with the active participation of the European Society for Human Reproduction and Embryology, ESHRE. The group has amended the EUSTITE vigilance tools to make them more relevant to the field and has developed specific guidance for EU vigilance and surveillance in assisted reproduction. The document has been submitted to the European Commission and the tissue and cell Competent Authorities of the EU.

Up to now, most EU Competent Authorities for tissues and cells have focused their efforts on putting in place systems and procedures to implement the regulatory functions that are required by the tissues and cells Directives, notably inspection, authorisation and vigilance. Many, however, lack experience and training, as well as procedures to be followed, for the investigation of cases where illegal or fraudulent activity is suspected. A SOHO V&S work-package led by the French Agency for the Safety of Health Products has gathered information from Member States and non-EU countries on cases that have been investigated and concluded, in some cases with enforcement action. The work-package has drawn on this experience to developed guidance, including tools and recommendations, to support EU Member States in this particular area of work.

Guidelines on the investigation of adverse reactions and events will be delivered by two work-packages in SOHO V&S. CNT is leading a work-package developing guidance for authorities and the Polish partner is leading a work-package developing guidance for hospitals and clinics where tissues and cells are applied to patients. Both groups will draw on the outcomes of the NOTIFY project, an initiative led by the World Health Organization in which this project participated, where experts from across the globe gathered information on documented cases of adverse reactions and events for organs, tissues, cells, gametes and embryos, drawing on the data to develop didactic guidance. The database created by the project will be publicly available as an interactive searchable tool on a dedicated website, hosted by CNT on behalf of WHO, and will provide data on how different types of reactions and events have been detected and confirmed. The new website with the database will be publicly available early in 2012 ([www.notifylibrary.org](http://www.notifylibrary.org)).

In the later stages of this three-year project, the Irish Medicines Board will lead a work-package delivering training for EU Vigilance officeres, based on the various principles of good practice identified and documented by all of the work-packages. The courses will be delivered following the successful model developed in the EUSTITE project, with a combination of e-learning followed by a residential module.

An effective vigilance and surveillance system plays a pivotal role in enhancing the safety of tissue and cells for human application. In some cases it facilitates rapid intervention by professionals or regulators to prevent further harm to patients. In general, it ensures the sharing of invaluable information to support improvements in systems and procedures for the benefit of donors and patients. The SOHO V&S project aims to maximise this learning opportunity through international collaboration between regulators and professionals.

*Further information and updates can be found at [www.sohovs.org](http://www.sohovs.org)*

*The SOHO V&S project is supported by co-funding from the Department of Health and Consumer Protection of the European Commission. Grant agreement n. 20091110*

## GLIMPSE INTO THE FUTURE; ADVANCED THERAPIES (I)

### TISSUES AND ATMPs; MARRIAGE OF CONVENIENCE. AIMING A UNIQUE QUALITY STANDARD

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#### Introduction:

The Transplant Services Foundation (TSF) is a good example of organization that develops different activities under different regulations frameworks. Donation, procurement, evaluation, processing, preservation, storing, distribution and biovigilance of tissues and cells are regulated by the 2004/23/EC, 2006/17/EC and 2006/86/EC Directives, with the objective of guarantying their quality and safety, whereas the production of ATMPs (Advanced Therapy Medicinal Products) must fulfil the EU Good Manufacturing Practices (GMP) and are regulated by the European legislation that covers the Advanced Therapies and by the European rules for medicinal products.

#### Aim:

The purpose of this challenge is to assess the benefits and inconveniences on the integration of the tissues quality standards and the ATMPs ones.

#### Methods:

The starting point of the GMP requirements implementation was an ISO 9001 certification and a TE authorisation. Such implementation was planned through a risk assessment basis in order to assess the positive, negative or neutral impact of the diverse adaptations on the quality system in place. The requirements related to the Quality Management (Product Quality Review, Risk Management), organizational requirements related to the personnel roles (independency between Quality Control and Production), requirements on the design and use of facilities (A/B grade for aseptic processes, clean rooms and equipment qualification, microbes and airborne particles monitoring), requirements related to the documentation used (production and packaging batch records, archive), requirements related to production (processes validation), requirements related to quality control activities (good laboratory practices, stability studies), requirements related to outsourced phases and products and services suppliers (suppliers management, quality agreements), requirements derived from out of specifications products (recall and return systematic) and requirements related to the self-inspections (integrated checklist), were all of them analysed to determine if the implementation could be performed immediately, with some adaptations or were not suitable to implement.

Hence, the study to assess the suitability of the Good Manufacturing Practices requirements implementation is based in a risk assessment methodology, assuming in all cases that the subsequent residual risk was acceptable. This analysis was combined with a change control exercise to assess the actions that should be taken and their impact.

#### Results:

In this practical approach, 23 requirements such as PQR, facilities monitoring during the process, media-fill validation, air quality, cleaning systematic, training requirements for the Responsible Person, relationship between production and quality control affairs, retention samples, on going stability studies, product specifications file/registration dossier, extensive clinical studies, batch definition, parametric release, investigational products, validation of processes, primary materials suppliers evaluation, archive retention period, traceability and biovigilance, have been assessed with the following summarized results:

- 7 requirements were implemented without adaptations
- 14 requirements were implemented with adaptations
- 2 requirements were not implemented

**Conclusions:**

The Transplant Services Foundation decided to extend the scope of the strict requirements to all the processes, understanding that it was the problem and the solution at the same time. Fulfill the GMP requirements in all the processes means a significant effort in terms of personnel and materials, but at the same time is understood as the solution to the coexistence of different quality standards.

The fact of providing the Hospital Clínic de Barcelona (HCB) with facilities, personnel and a whole quality system that respect the GMP requirements allowing to develop and obtain the Advanced Therapy Medicinal Products, supposes the mandatory satisfaction of the needs arising from the scientific advance that houses and promotes the HCB. Furthermore, it allows merging in an optimal manner the steps of detection of the therapeutic need, development of the solution and the assessment of its efficacy.

On the other hand, the bet to extend the scope of the certification, not only to the obtention of ATMPs but also to the tissue processing, answers to a realistic and regulatory need of the scenario where both therapeutic solutions do coexist.

The need of adapting and completing the current regulation, to make it cover the process running from the donation till the tissular and/or cellular products obtention, is been identified by the experience of the TSF. To have an unique process covered by different regulations, that at the same time do not respond to the reality of both of them, is an unresolved matter within the European Union that arises as a immediate challenge for the TSF in line with its compromise to stand as a reference in the tissular and cellular field.

The production of ATMPs is currently regulated within the European regulation of medicinal products and it is with no doubt and excellent reference for them to achieve an adequate grade of quality and safety. However, the coincidence on the initial steps of donation, with regard to the processing of tissues for transplant, the similarity between tissues and ATMPs concerning the specifications and quality controls needed, the unclear borders between the different types of tissues and ATMPs and the fact that both therapeutic solutions share actors and scenario in the moment of transplant and application, leads to believe in the necessity of a specific, adapted and integrated regulation to guarantee the c

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## ARTIFICIAL CORNEAL TISSUE: A NEW CHALLENGE

*Miguel González-Andrades. Spain.*

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Corneal diseases are one of the most important causes of visual impairment in the world. Thus, many patients affected by this pathology end up needing a corneal transplant. This treatment implies two major problems: the lack of donors and the possibility of graft rejection. Therefore, this necessity has encouraged the generation of a corneal substitute in the laboratory. In order to accomplish this challenge, tissue engineering appears as promising science, whose aim is to generate artificial tissues and organs that can replace the damaged one within the human body. Regarding tissue engineering of the cornea, different approaches have been attempted.

We present two models of artificial corneas based on fibrin-agarose scaffolds and acellular xenografts, developed by our research group. Both models are based on the combination of corneal cells and scaffolds. Corneal epithelial cells and keratocytes were obtained from sclerocorneal limbus of human cadavers. On the one hand, the fibrin-agarose model was developed seeding these cells into an artificial matrix, which was constructed with a mixture of agarose VII and fibrin from human donors. On the other hand, the acellular xenograft model was created applying a decellularization process to pig corneas, based on NaCl. After obtaining an acellular corneal stroma from pig corneas, the human keratocytes were seeded over it. Once we developed both artificial corneal models, histological, genetic expression profile and optical analyses were carried out.

Both models showed a well-developed stroma based on the presence of collagen and proteoglycans. The keratocytes proliferated and spread, migrating across the corneal matrix. Some immunohistochemical assays were performed, showing the differentiation and characteristic expression of corneal proteins. Optical analyses revealed the high transparency level that both models presented, observing that UV-light was mostly absorbed by the corneal substitutes. All these results suggest that corneal substitutes made by tissue engineering show similar characteristics to human corneas. Thus, artificial corneas could represent a promising treatment for many corneal diseases that do not currently have an adequate and established therapeutic procedure.

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## CORNEA (I)

### DEKARIS IVA

*European Eye Bank Association – EEBA*

The European Eye Bank Association (EEBA) is a technical-scientific organization comprising individual members from 84 eye-banks from 24 European countries and 4 international colleague eye-banks. EEBA is today the leading pan-national association in Europe dedicated to the advancement of eye-banking and an authoritative reference point for eye banks wishing to work according to quality standards.

#### **Aims:**

The Association is formed: to contribute to the development of standards for eye banking in Europe, to establish an agreed set of EEBA Standards, to promote data collection on graft outcome, to facilitate the information interchange between eye banks, to provide opportunities of eye banking practice, to encourage relevant research and development, to provide informed comment to external agencies, to foster education in eye banking, to maintain national and international links with corneal transplant communities, to make knowledge of eye banking available to any person for the general good of society.

#### **Activities:**

In the period from 2005-2009 the number of corneas received in EEBA member eye-banks was 31 587 ( $\pm 3034$ ) corneas. The overall percentage of corneas issued for transplantation in 2009 was 49% (lower as compared to previous 5 years); one hypothesis for this decrease may be the implementation of the „24h-regulation“ on taking blood samples in several EU countries, which causes a high loss of potential donor material. Most of the corneas were transplanted in the hospital housing the eye-bank or in the own country. According to their activities, expressed as number of corneas issued per year, 36 European eye banks are issuing 100-500 corneas, 7 are issuing 50-100 corneas and 7 eye banks >1000 corneas. Of the received corneas, 47 % was retrieved by enucleation and 53 % by corneoscleral disc excision. Organ-culture remains the preferred storage methods in EEBA (71% of preserved corneas). In recent years EEBA Technical guidelines have been developed and published describing selection criteria deemed acceptable for cornea evaluation. Of all corneas that were not selected for grafting, 16.2% were excluded because of abnormal morphology and other reasons were serology (7.9%), medical history (2.7%), microbiology (3.3%) and others. All banks test all donors for HIV Abs, Hepatitis B and C, either antigen, antibodies or both and most eye banks additionally test for syphilis. Mean frequency of positive serology ranges from 5.0-8.5% in the period from 2005-2009. In 2009, 16% of corneas was selected and issued for lamellar grafting, and sometimes lamellar grafts were pretreated in the bank. Only a small proportion of the transplanted corneas were tissue-typed; typing for HLA I was done for 10.4 % of all donors and for HLA II for 8.7 %. Adverse reactions (such as primary failures and endophthalmitis) occurred at 0.2 and 0.05 % risk. Of other tissues used for ocular surgery EEBA banks were issuing sclera, limbal graft, stem cells and amniotic membranes.

## EUROPEAN GOOD PRACTICE STANDARD

### EURO-GTP PROJECT

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*Transplant Services Foundation-Hospital Clinic. Barcelona, Spain*

#### Introduction

In Europe, there are currently neither common procedures for how the procurement, the processing and the preservation of tissues for transplant should be carried out, nor for how the donor selection and screening of tissue donors according to the type of tissue retrieved should be done.

It is important that the methodology of tissue banking activities is harmonized among all tissue establishments in Europe so that high quality and the safety of transplanted tissues can be guaranteed. There are regulations which provide the basis for performing tissue banking activities in a professional and coherent manner. However, this project aims to go one step further, and provide the specific tissue practices (GTPs) to be followed in European tissue banks, in order to harmonize these decisive procedures. This will also contribute to a higher confidence in the exchange of tissues for transplant among the Member States. To guarantee the highest level of quality and safety of the tissue grafts for recipients it is very important to carry out the tissue banking procedures in adequate environmental conditions and facilities. The GTPs will be very helpful tools for all kinds of TEs and TEs that are in different phases as concerns their development and evolution.

#### Objectives

The main objective of this project is to develop common Good Tissue Practices (GTPs) for European tissue establishments (TE), as well as a Training Model for TE personnel concerning the activities that are carried out in TE, including donor selection and recovery, and processing, preservation and storage of tissues for transplant. The aim is to apply these practices European-wide to increase the know-how and the level of performance of tissue banking staff, and to harmonize the techniques used in order to provide tissues for transplant of high quality and safety, hence reducing the risk of disease transmission to recipients.

#### Methods

- To develop European Good Tissue Practices (Euro-GTPs Guide) for Tissue Establishments activities (such as donor selection process, recovery, processing, preservation and storage of tissues).
- To develop a training model for TE personnel.
- The development of generic Euro-GTPs for TEs personnel
- The development of tissue-specific Euro-GTPs
- Approval of the Euro-GTPs
- Design of a Training Model for TE personnel

#### Results

Before starting the elaboration of the GTPs, the following preliminary activities help us to know the current situation of the TEs both at regulatory and practical level;

To have a better understating of the above mentioned issues, a questionnaire has been developed and addressed to TEs for recollecting data on the methods used for tissue banking processes. The objective of this questionnaire is to find out: (i) information on the quality processes that are being used and (ii) whether TEs carry out activities related to tissue recovery and processing.

The questionnaire has been distributed to 147 TEs and completed by 22 TEs from several Member States through the EU (collaboration asked from some national authorities in order to have a representative analysis of the EU). Additionally, it will be uploaded in the project website and TEs from the countries of the consortium that are not direct participants in the project

(and other EU countries if possible) will be invited to complete it so that the project can have a better global idea of the current situation in Europe.

The project coordinator elaborated a survey to be distributed among the Eustite Final Conference participants (25 EU inspectors). The current or future inspectors were asked to answer some questions regarding their audit findings, the difficulties they find to apply the current regulation and their opinion about the worth of a more detailed and realistic guidance such as GTPs at European Level.

The following documents and regulation have been analyzed:

- The different European and national existing legal regulations
- Quality systems applied in TEs
- Procedures followed by TEs
- Standards followed in TEs
- EQSTB project results
- Good Manufacturing Practices

As a result of the analysis of the above mentioned questionnaires and documents and more specifically the GMP, we have identified the processes to be included in the GTPs guide.

As regards the generic GTP, these include requirements related to:

- Personnel
- Facilities
- Documentation
- Donor selection and evaluation
- Recovery
- Processing
- Storing and distribution
- Quality management
- Risk assessment
- Validation methodologies
- Documentation

As regards the specific GTPs, they contain the following:

- Donor selection
- Recovery methods
- Minimum quality criteria
- Preservation methods
- Storage and distribution

The key issues and the issues not covered or covered in a weaker way by standards or regulation have been identified and highlighted as "HOT-TOPIC". The web-based HOT-TOPICS forum supplements the guidelines by highlights, practical examples and special tools for developing areas /conversation pieces of tissue banking practices. These HOT-TOPICS issues are under regular revision and tissue bankers are urged to participate in the HOT-TOPICS discussion on the web page.

This document covers the following:

- Donor selection
- Recovery and processing environments
- Tissue specific quality criteria
- Risk management
- Traceability and vigilance
- Validation
- Critical third party agreements

BARCELONA

2011 November, 9-10-11

# INVITED SHORT PAPERS

## **SPECIFIC CHALLENGES OF DONATION, PROCESSING AND CLINICAL APPLICATION IN PAEDIATRICS (II)**

### **vCJD RISK REDUCTION MEASURES FOR CHILDREN**

*Dr Akila Chandrasekar FRCPath., NHS Blood and Transplant, Liverpool, United Kingdom*

#### **Abstract:**

Variant Creutzfeldt-Jakob disease (vCJD) is a rare and fatal human neurodegenerative condition caused by abnormal prions. The incidence of vCJD in the UK is much higher than elsewhere in the world. The most likely route of transmission of vCJD is by exposure to prion from contaminated food infected with bovine spongiform encephalopathy (BSE). Various control measures were introduced in the UK to minimise the risk of passing BSE from cattle to humans.

Transmission of prions has occurred (iatrogenic CJD) during medical care through neurosurgical instruments, transplantation of corneal and dura mater grafts and administration of cadaveric-derived pituitary growth hormone and gonadotrophins. Transmission of vCJD by blood transfusions has been reported in the UK. The number of current or past blood or tissue donors incubating the disease at the time of donation is not certain.

To minimise the potential risk of secondary transmission from human to human by tissue transplantation, the tissue services associated with UK blood services have introduced a number of risk reduction measures. These include donor exclusion of transfusion and transplant recipients, additional vCJD testing for deceased tissue donors where possible and improved processing steps to remove as much blood and marrow content from tissue grafts as possible. In addition, for children under the age of sixteen, who would not have had exposure to vCJD through the dietary route, imported skin allografts from countries with low risk for vCJD were made available.

This presentation gives overview of challenges facing the tissue establishments due to emerging infections across the world.

- Import and export
- Continuity plans
- Tissue establishment dossier

### Conclusions

The European Good Tissue Practice (GTP) guidelines and the adjacent training model have been established as an outcome of the EU-funded project - *Euro-GTPs* - to provide a *complete and detailed tissue banking information package* for tissue bankers as well as for tissue establishment (TE) inspectors in Europe. These guidelines bring together the current minimum regulatory requirements of the European tissue and cells Directives and go one step further - incorporating useful good manufacturing practice (GMP) principles and utilizing the expertise of tissue bank experts to provide a set of practical recommendations for good practice in European TEs. The GTPs are developed to be a helpful tool for all kinds of TEs in different phases of their development and evolution as well as for competent authorities (CA) when performing TE inspections.

The web-based HOT TOPICS forum is an important supplement to the guidelines. It highlights areas where it is generally acknowledged that greater harmonization is needed, where consensus is lacking on the best practice to be applied, or where it is commonly thought that tools are needed to support improvements in practice. Practical examples and some particular tools for developing areas and where conversations can proceed on specific tissue banking topics are proposed. These HOT TOPICS issues are under regular revision and tissue bankers are urged to participate in the HOT TOPICS discussion on the web page. As consensus is reached and good practice on these topics is more clearly defined, these texts will move into the full guidance document. New hotspot topics will be

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## PRIMARY PEDIATRIC KERATOPLASTY: DONOR AGE AND GRAFT SURVIVAL

*Planas Domènech, N. Fernández Guardiola, A. Physician of the Department of Pediatric Ophthalmology. Hospital St. Joan de Déu. Barcelona. Spain.*

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### Background:

The ECD declines with age in the normal cornea. This process of cell loss is greatly accelerated after penetrating keratoplasty and persists for years after transplantation. Corneal clarity after penetrating keratoplasty can be affected by endothelial cell loss over time. The exact causa of postoperative cell loss is unknown but may be a result of donor or preservation factors, surgical stress, cellular interactions between the donor and recipient, immune reaction, normal or accelerated cellular aging, or glaucoma. Past studies evaluating endothelial cell loss after corneal transplantation have produced conflicting results with regard to the effect of donor age. Penetrating keratoplasty in children has been documented to have a higher rate of graft failure than adult keratoplasty.

### Purpose:

To report our experience of primary penetrating keratoplasty in children focusing on the donor age.

### Methods:

We undertook a retrospective review of penetrating keratoplasty performed in children 16 years and younger between 1995 and 2010 at the Department of Ophthalmology, Hospital Sant Joan de Déu. Barcelona, Spain.

### Results:

A total of 37 primary penetrating keratoplasties were performed in 34 patients during the study interval. The surgical indications were congenital opacities in 12 eyes (32,4%), acquired nontraumatic opacities in 21 eyes (56,8%), and acquired traumatic opacities in 4 eyes (10,8%). The mean recipient age was 7,8 years (range 5 months - 16 years). The mean donor age was 31,5 years (range 7 years - 68 years). The mean donor endothelial cell density was 2968 (range 2157 - 4700). Overall graft survival at 1 year was 70%. Graft survival at 1 year was different among the surgical indications categories (congenital opacities 42%, acquired nontraumatic opacities 86%, acquired traumatic opacities 75%). Graft survival at 1 year was different among the different age groups (50% for patients younger than 6 months, 44% for patients 6 months to 5 years, 83% for patients older than 5 years). Graft survival at 1 year was different among the different donor age groups (73% for donors younger than 16 years, 50% for donors 16 to 45 years, 88% for donors older than 45 years). Graft survival at 1 year was similar among the different methods of corneal preservation (Optisol 68%, tissue culture 67%).

### Conclusions:

Primary pediatric keratoplasty has an overall prognosis for graft survival of 70% at 1 year. Patients who received a cornea from a donor 16 to 45 years had worse 1-year graft survival compared with patients who received a cornea from a donor younger than 16 years or older than 45 years. However, it is difficult to compare graft survival among the different donor age groups because there is considerable variation in the indications for transplantation and in the recipient age, in each group.

## SKIN AND AMNIOTIC MEMBRANE (I)

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### ALLOGRAFTS AND CRITICAL BURNED PATIENTS: EXPERIENCE AND FUTURE

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Nowadays skin allografts or homografts represent a base of the huge surgical stock for treatment of critical burned patient. Cadaver skin, started to work as allograft at the beginning of 50's decade; allografts used as a temporary dressing have an important function in preventing infection and reducing fluid loss trough these burn wounds. Moreover, it carries out a basic function in order to prepare the surgical bed after debriding the burn wound, so autologous skin grafts could be inset, and as protection for this skin grafts when they are placed in the same surgical time, as it is described in the Alexander technique, also called Sandwich technique.

Extraction conditions must be the same than in other organ extracted before transplantation, but they are not used immediately so it is mandatory to process them in order to preserve and store the allograft.

There are 2 basic techniques in processing, cryopreservation and Glycerol preservation. Both have advantages and disadvantages. Because we have great experience in Glycerol preservation, and the bigger capacity in bacterial and viral inactivation of this technique is the reason we prefer this one.

In this short paper we present the full process, starting with the extraction in the donor cadaver, the preservation and the surgical utilization in the critical burned patient surgery, going on with the postoperative evolution up to the elimination of the graft.

We present too how must, or at least how we would like the future of allografts be in the wide context of critical burned surgical treatment.

## GLIMSE INTO THE FUTURE; ADVANCED THERAPIES (I)

### TOLEROGENIC DENDRITIC CELL THERAPY IN REFRACTORY CROHN'S DISEASE

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#### Introduction:

Crohn's disease (CD) is a chronic transmural inflammation that can involve any part of the intestinal tract, predominantly the small and/or large intestine. Since CD is a consequence of loss of tolerance against the normal gut flora, a novel approach based on the modulation of dendritic cell (DC) function is especially attractive. DCs are the most important of professional antigen presenting cells in the intestine. It is known that DCs are involved in inducing tolerance against self/harmless antigens. Despite the promise of cellular therapy with immunogenic DCs in cancer patients, no clinical studies have taken advantage of their specific immunosuppressive properties so far.

#### Methods:

Tolerogenic dendritic cells (Tol-DCs) were generated from isolated monocytes using clinical grade reagents (IL-4 and GM-CSF) and synthetic culture media. DCs were cultured for 6 days and the cells activated by adding a cocktail of cytokines (TNF- $\alpha$ , IL-6, IL-1 $\beta$  and PGE2). Dexamethasone ( $1 \times 10^{-6}$  M) and vitamin A ( $10^{-9}$  M) were added at day 3 of differentiation as immunosuppressive agents. The stability of DCs was evaluated by adding second stimuli after washing Dex away from the culture.

#### Results:

Tol-DCs had limited expression of costimulatory molecules and impaired alloresponse induction. Tol-DCs depicted a semi-mature phenotype. Production of the anti-inflammatory cytokine IL-10 was significantly increased on tol-DCs whereas IL-12 was completely absent. Furthermore, tol-DCs were poorer stimulators of antigen-specific T lymphocyte response (PBLs or naïve T cells) than control DCs, showing the role of tol-DCs in dampening the specific T lymphocyte activation. Moreover, tol-DCs were stable to second stimuli preserving the phenotype and cytokine production pattern; keeping high levels of IL-10 secretion without IL-12. Interestingly, we were able to generate tol-DCs from Crohn's disease patients with the same tolerogenic profile than can be applied in future clinical trials.

#### Conclusions:

Our findings highlight that the combination of dexamethasone together with a cytokine cocktail renders clinical grade tolerogenic DCs, displaying a semi-mature phenotype, a shifted balance towards anti-inflammatory cytokines, low T cell stimulatory properties. The ability of these cells to induce tolerance represents a potential and novel therapeutic approach to treat autoimmune diseases to silence unwanted immune reactions.

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