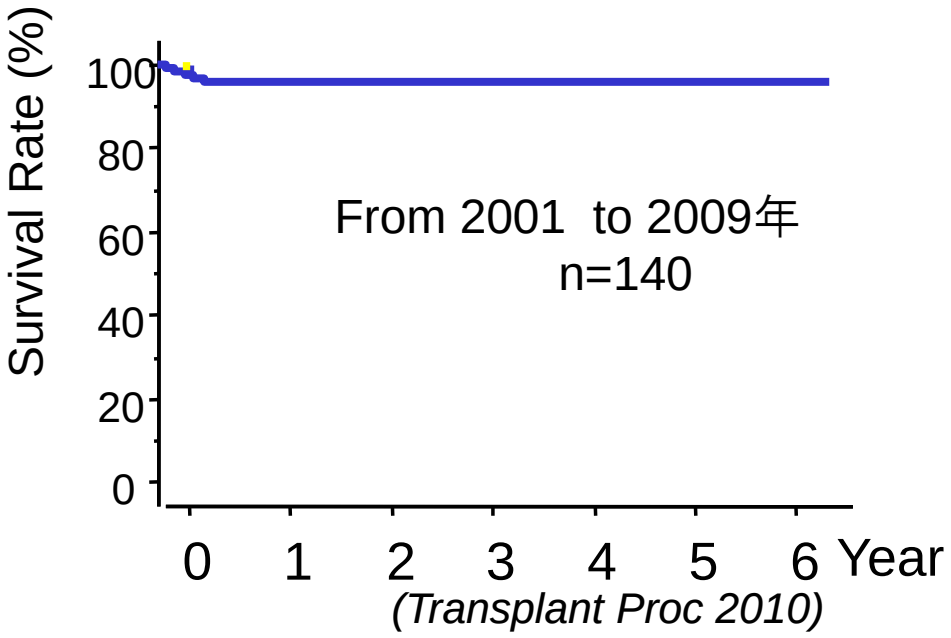
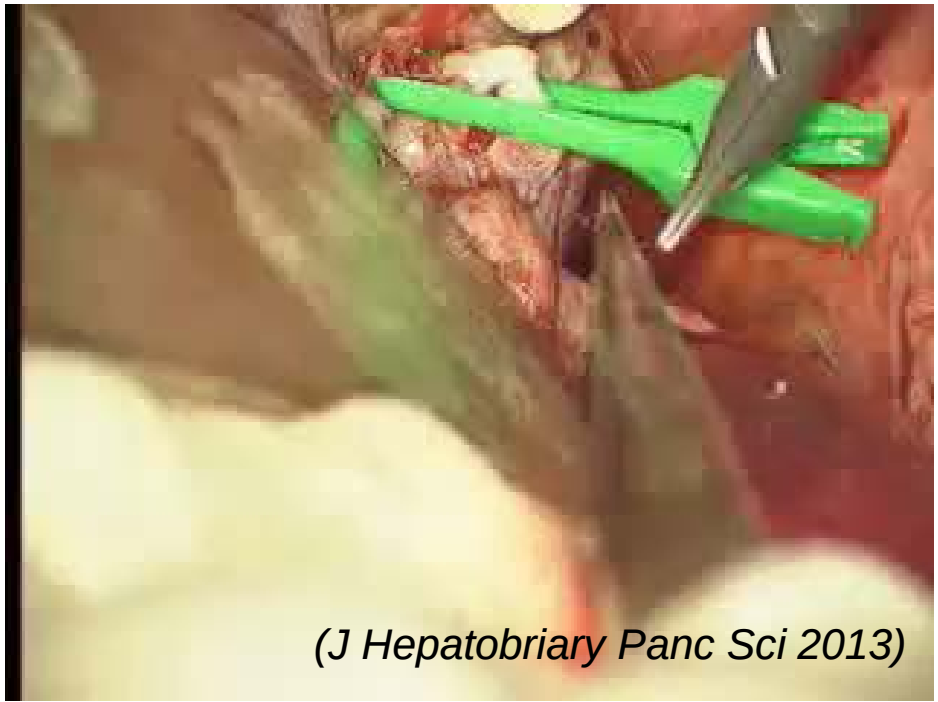
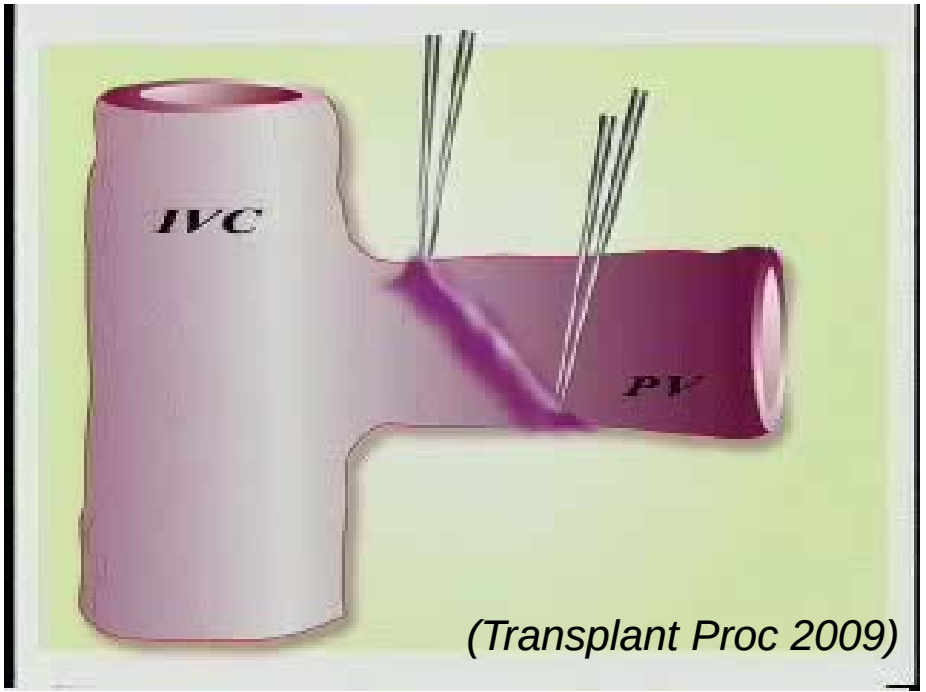
# Research Strategy for Human Organ fabrication-Pig as In vivo Bioreactor-

**Eiji Kobayashi, MD, PhD**
**Department of Organ Fabrication,**
**Keio University School of Medicine, Japan**


Survival Rate (%)
100
80
60
40
20
0
From 2001 to 2009年
n=140
0 1 2 3 4 5 6 Year
(Transplant Proc 2010)


(J Hepatobriary Panc Sci 2013)


IVC
PV
(Transplant Proc 2009)

# Declaration of Istanble

*(Istanbul, 30th April – 3rd May 2008)*


*152 professionals from 78 countries*

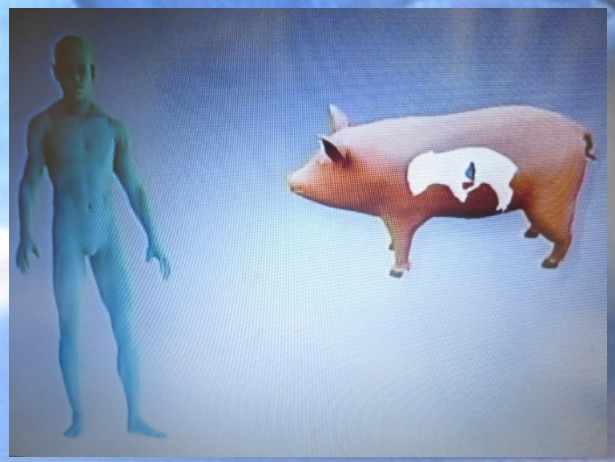
*(Lancet 2008)*

NHK Special 2009

# Human Body“Production”

## —The impact of Regenerative Medicine—

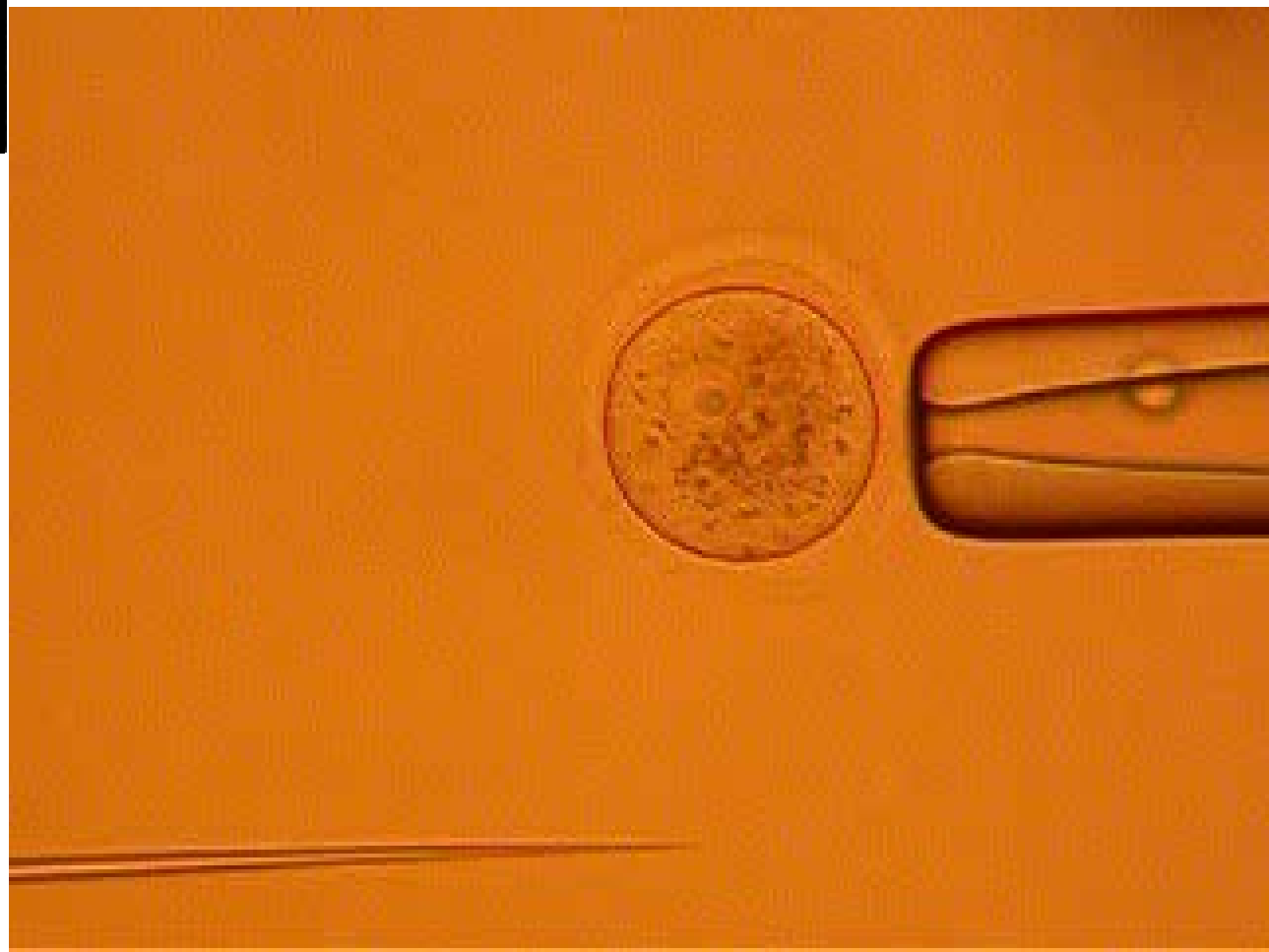
Eiji Kobayashi, MD
Jichi Medical university



# Green Fluorescence Protein

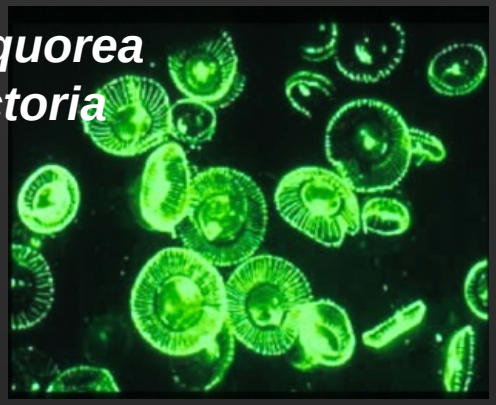
## Osamu Shimonura

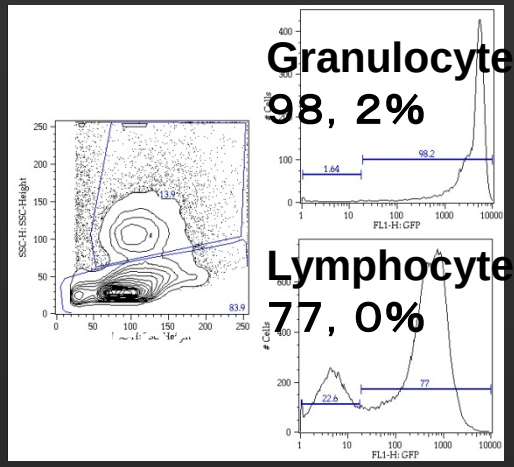
### Novel Prize in Chemistry 2008

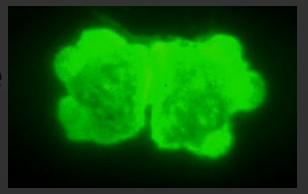


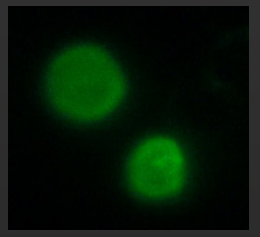
# Benefits in bio-imaging system using ‘fluorescence’

*Aequorea victoria*

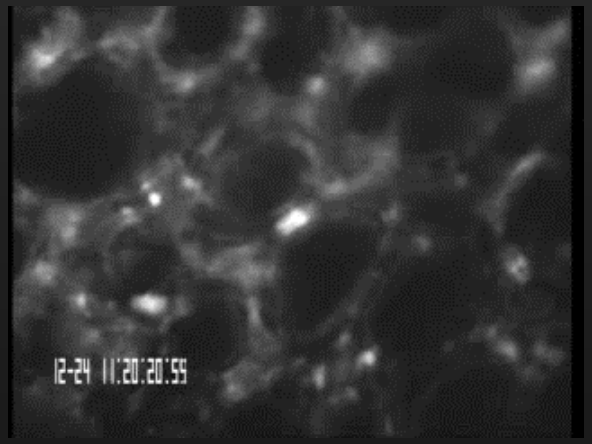






*(Sakuma Y, et al.Transplant Immunol 2004)*



Lung; 25 min after LPS injection

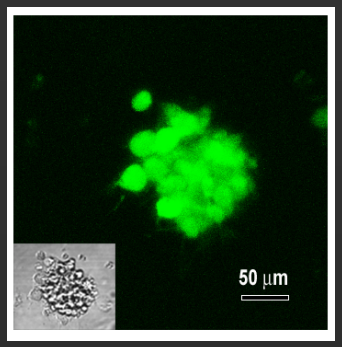
*(Sato A, et al. Anesth Analg 2005)*

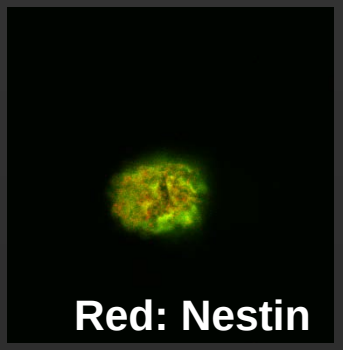
Transplanted lung immediately after re-perfusion

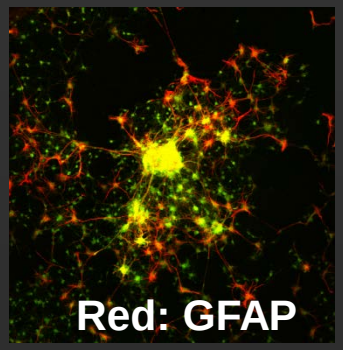
*(Enomoto A, et al. Microsurgery 2007)*

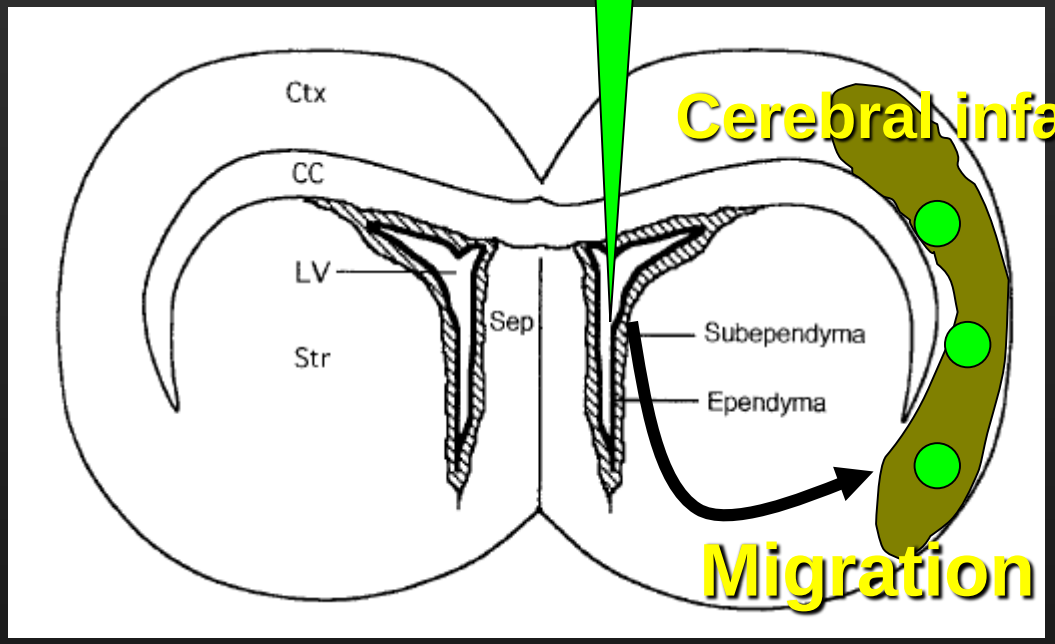
# Differentiation from the fetal tissue

Neurosphere




*(Inoue H, et al. BBRC 329:288,2005)*

**Promising future for the transgenic rat in transplantation research.**
Doorschodt BM, Teubner A, **Kobayashi E**, **Tolba RH**.
Transplant Rev (Orlando). 2014 Oct;28(4):155-62. doi: 10.1016/j.trre.2014.05.002. Epub 2014 May 27. Review

**The role of microstructured and interconnected pore channels in a collagen-based nerve guide on axonal regeneration in peripheral nerves**
Bozkurt A, Lassner F, O'Dey D, Deumens R, Böcker A, Schwendt T, Janzen C, Suschek CV, **Tolba R**, **Kobayashi E**, Sellhaus B, Tholl S, Eummelen L, Schügner F, Damink LO, Weis J, Brook GA, Pallua N.
Biomaterials. 2012 Feb;33(5):1363-75. doi: 10.1016/j.biomaterials.2011.10.069. Epub 2011 Nov 13

**The role of microstructured and interconnected pore channels in a collagen-based nerve guide on axonal regeneration in peripheral nerves**
Bozkurt A, Lassner F, O'Dey D, Deumens R, Böcker A, Schwendt T, Janzen C, Suschek CV, **Tolba R**, **Kobayashi E**, Sellhaus B, Tholl S, Eummelen L, Schügner F, Damink LO, Weis J, Brook GA, Pallua N.
Biomaterials. 2012 Feb;33(5):1363-75. doi: 10.1016/j.biomaterials.2011.10.069. Epub 2011 Nov 13

# International Collaboration using Photonics Rats






**Canada**
**A Keating (Toronto Univ)**

***U.S.A***
***John Critser (Missouri Univ)***
***SS Thorgeirsson (NCI)***
***P Leone(RWJ Med Sch)***

***Germany***
***EK Geissler(Regensburg)***
***G Nikkhah (Freiburg)***
***R Tolba (Bonn)***

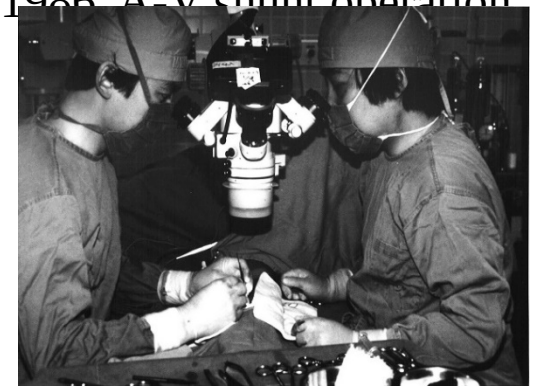
**Singapore**
**DW Hutmacher**
**S Cool (Bioporis)**



***Australia***
***GA Bishop (Sydney)***

# Clinical Microsurgery

1986 A-V shunt operation



2000 Hepatic artery reconstruction
in liver transplantation


# Experimental Microsurgery

Rome, Italy

K

W

L

C

2002 6th. ISEM San Diego, USA

2004 7th. ISEM Debrecen, Hungary

2006 8th. ISEM Montreal, Canada

2008 9th. ISEM Shanghai, People's Republic of China

2010 10th. ISEM Sao Paulo, Brazil

2012 11th. ISEM Timi

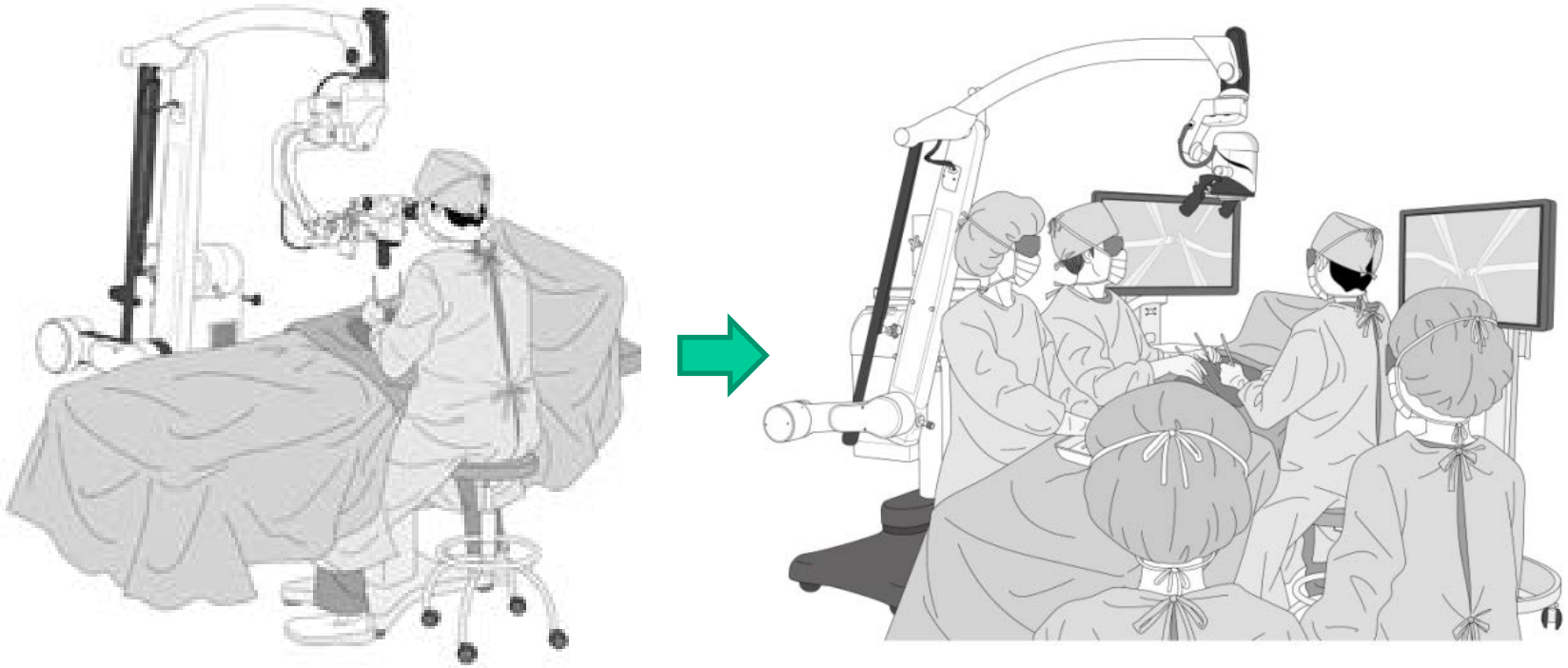
2014 12th. ISEM Kyot

2016 13th. ISEM Tianj

2018 14th. ISEM Debr

**Defining Standards in Experimental Microsurgical Training: Recommendations of the European Society for Surgical Research (ESSR) and the International Society for Experimental Microsurgery (ISEM)**

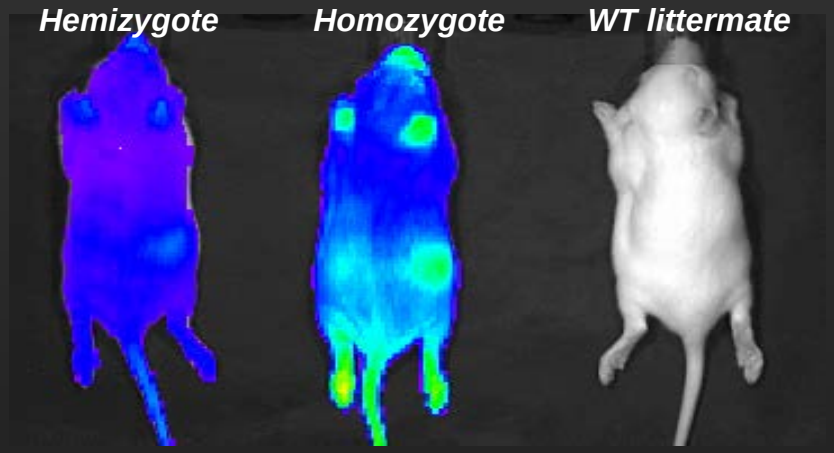
**Tolba RH**, Czigány Z, Osorio Lujan S, Oltean M, Axelsson M, Akelina Y, Di Cataldo A, Miko I, Furka I, Dahmen U, **Kobayashi E**, Ionac M, Nemeth N. Eur Surg Res. 2017;58(5-6):246-262. doi: 10.1159/000479005. Epub 2017 Jul 26.

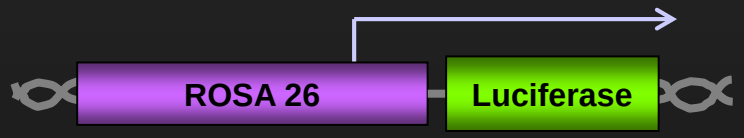


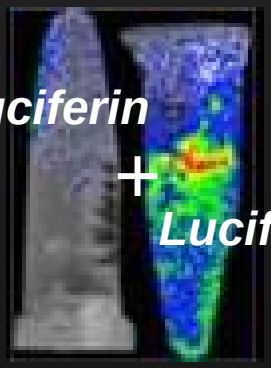
# Materials and Methods

## Photnus pyralis

## Firefly Tg Rat



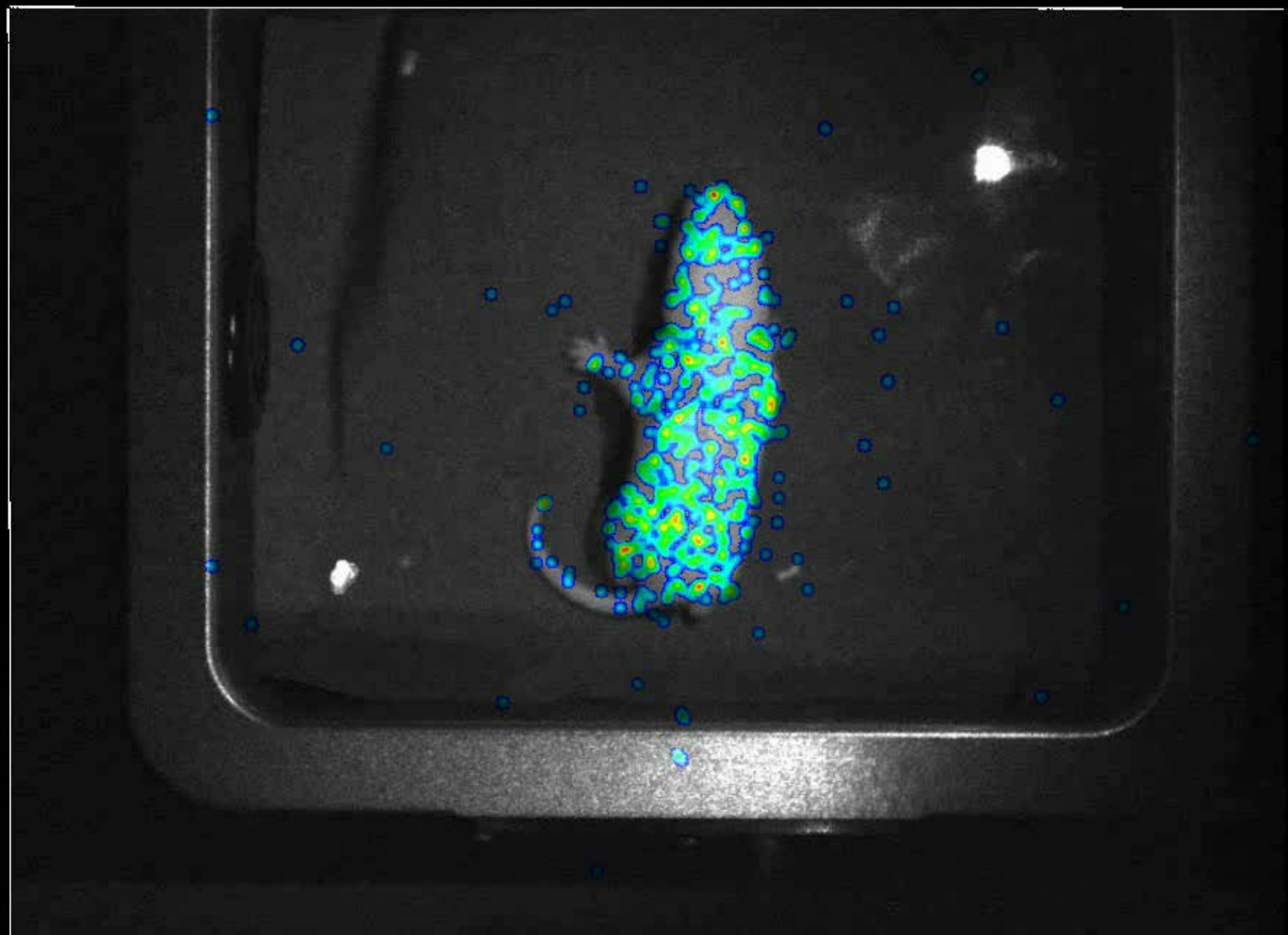



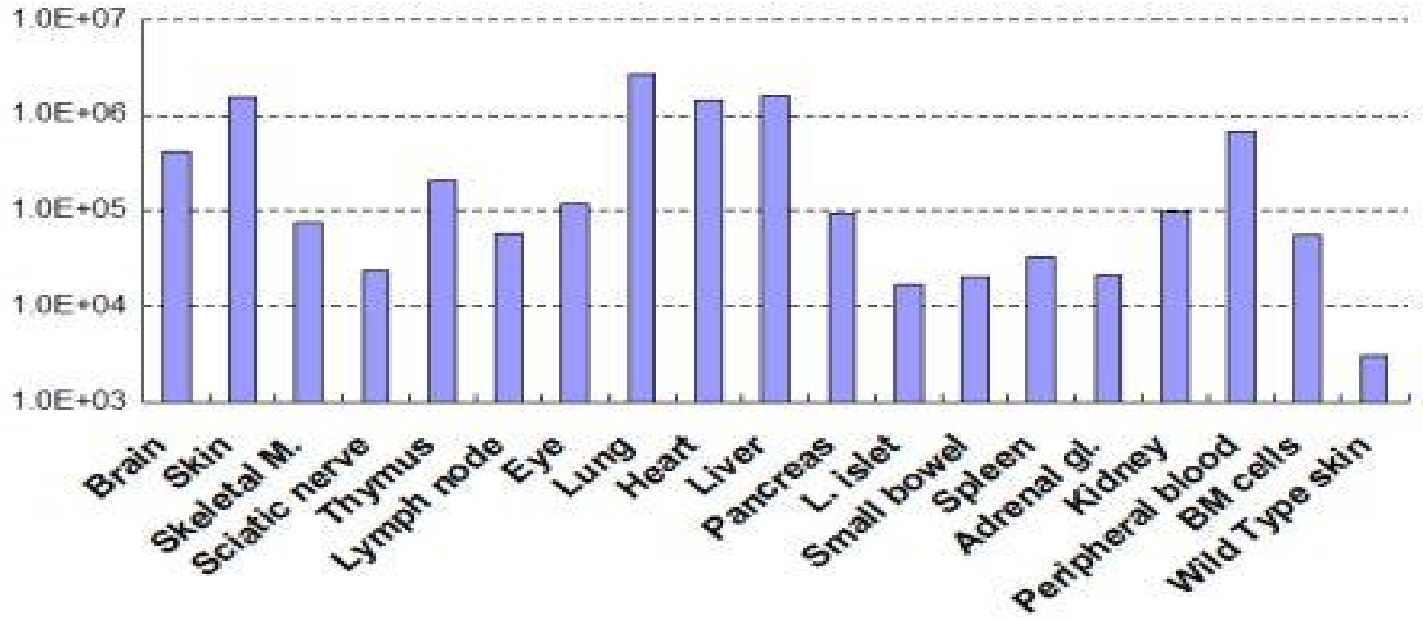
Luciferin + Luciferase → Luminescence

*(Hakamata Y, et al. Transplantation 2006)*

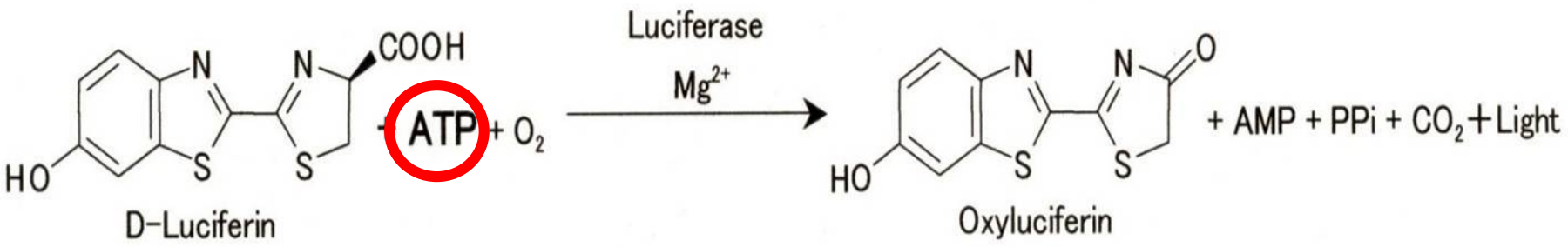
# 蛍ラット



# Light Emission of Luciferase Transgenic Rat



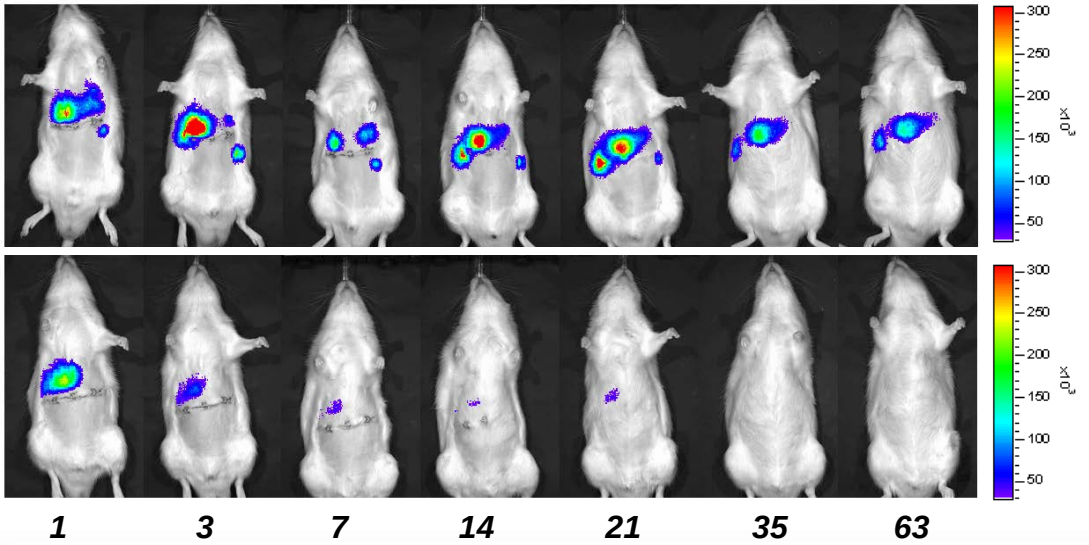

*(Hakamata Y, et al. Transplantation 2006)*

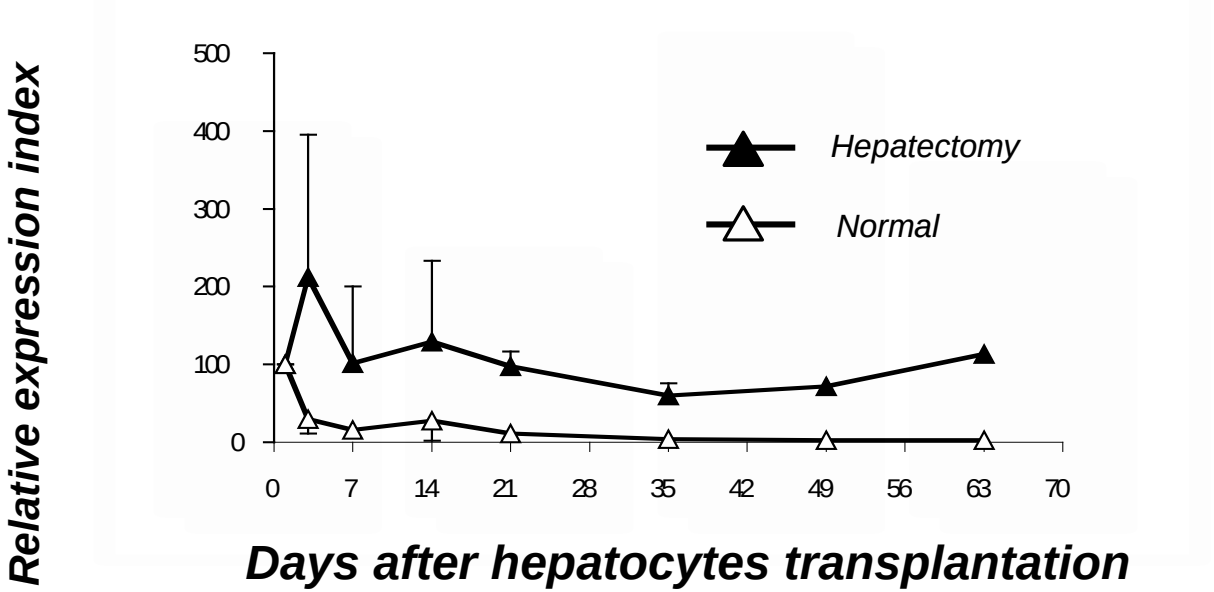


# *Chase the transplanted hepatocytes*

*Hepatectomized*

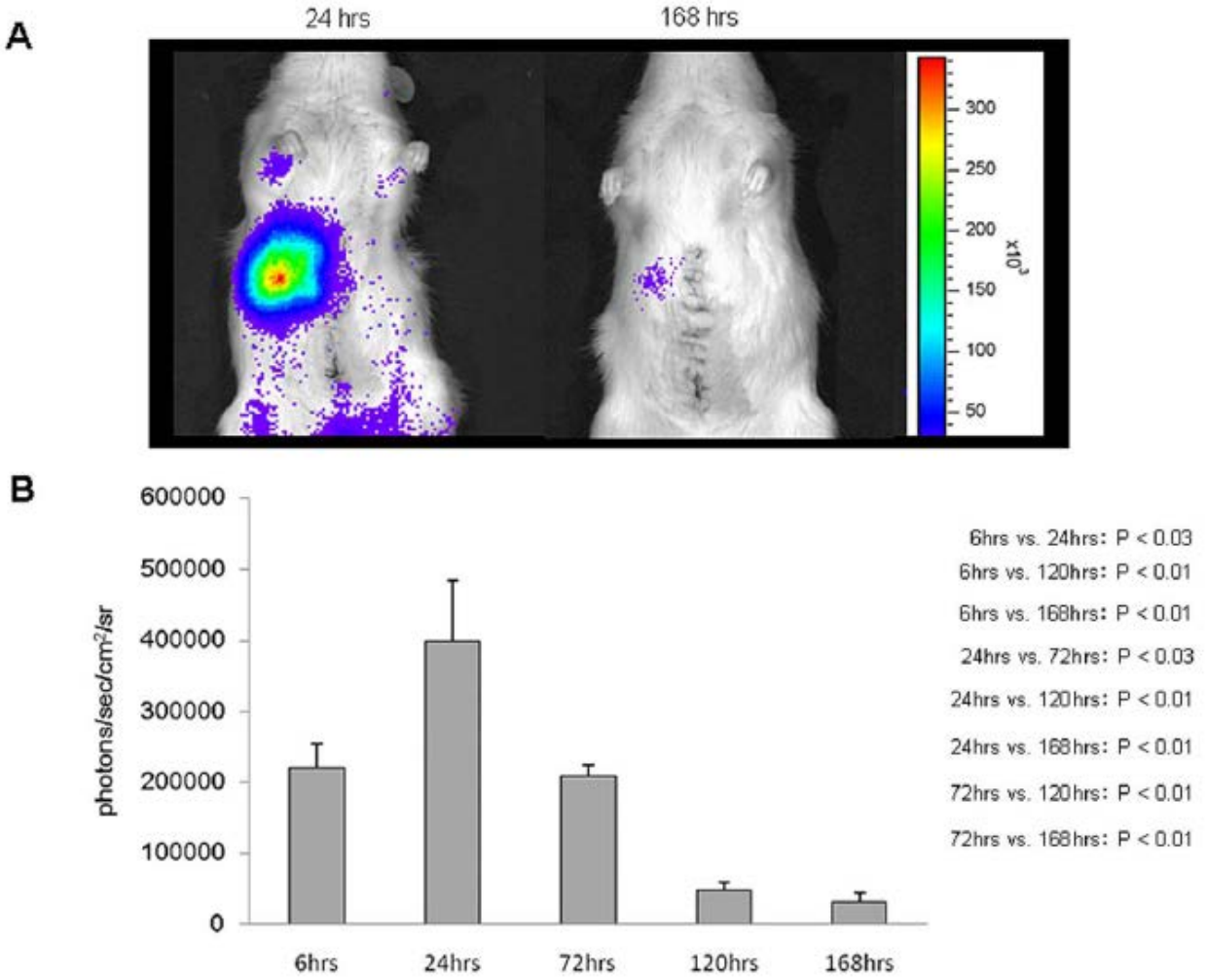
*Normal*



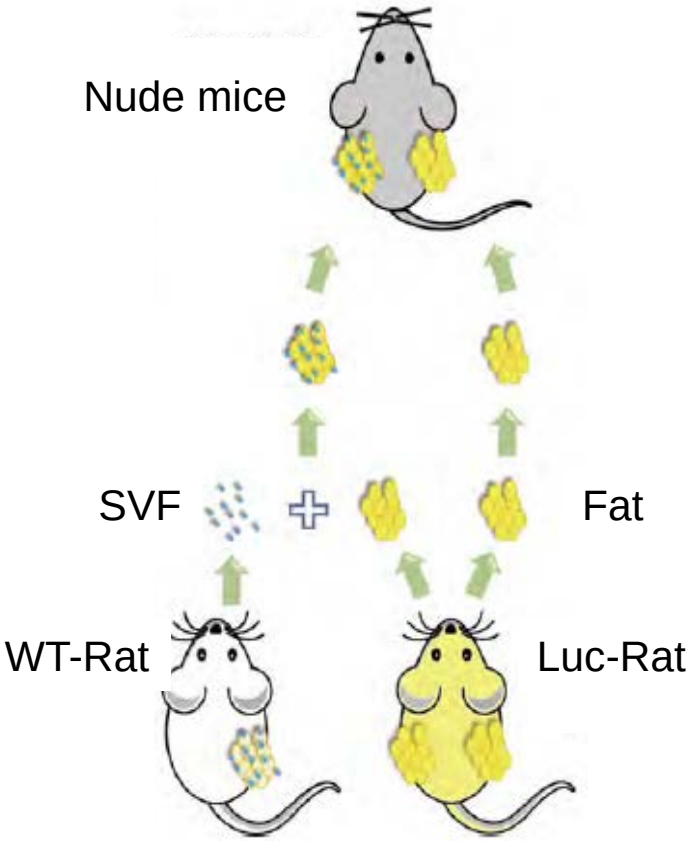

*(Hakamata Y, et al Transplantation 2006)*

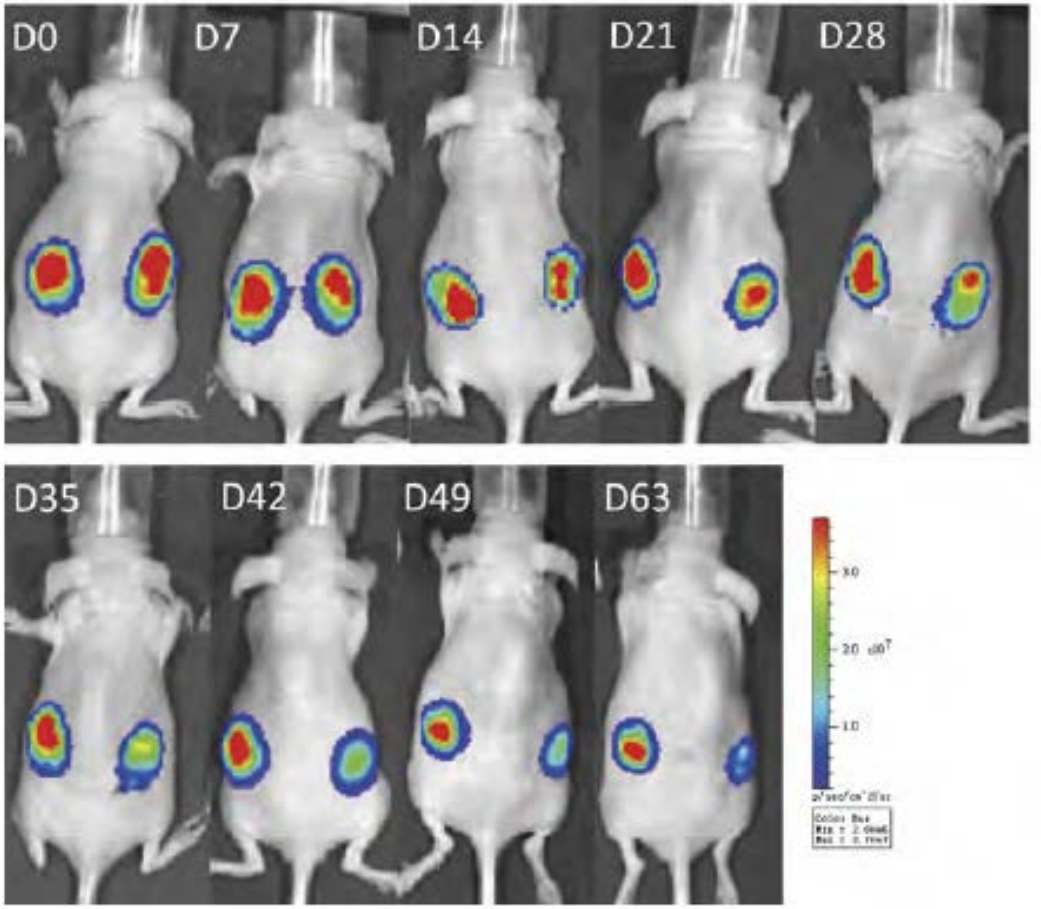
# *Bone Marrow-Derived Mesenchymal Stem Cells Ameliorate Hepatic Ischemia Reperfusion Injury in a Rat Model*




*(H Kanazawa et al. PLoS One 6(4):e19195 2011)*

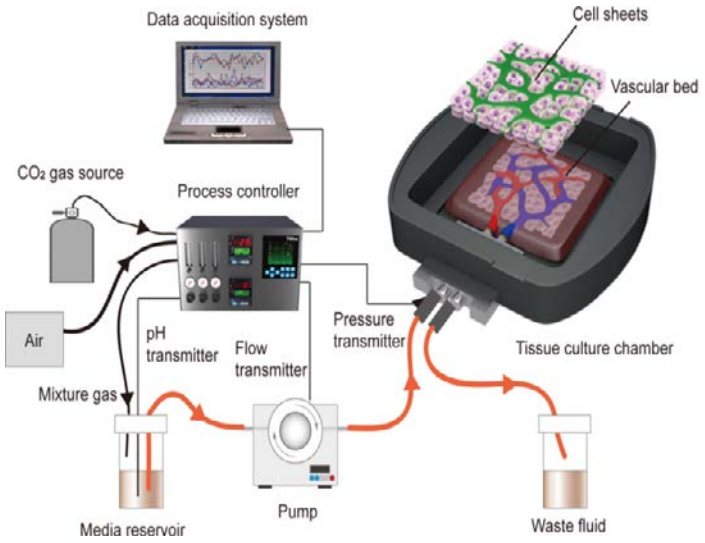
# In Vivo Bioimaging Analysis of Stromal Vascular Fraction-Assisted Fat Grafting: The Interaction and Mutualism and Cells and Grafted Fat

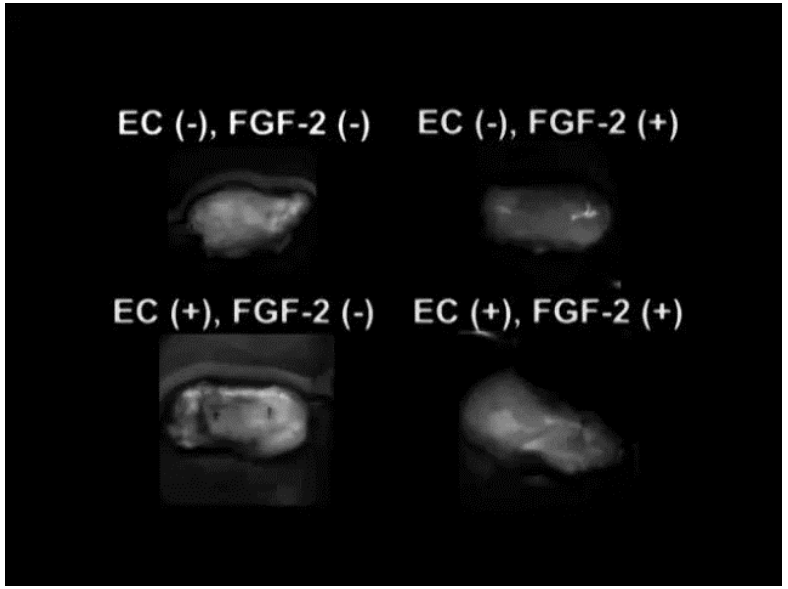


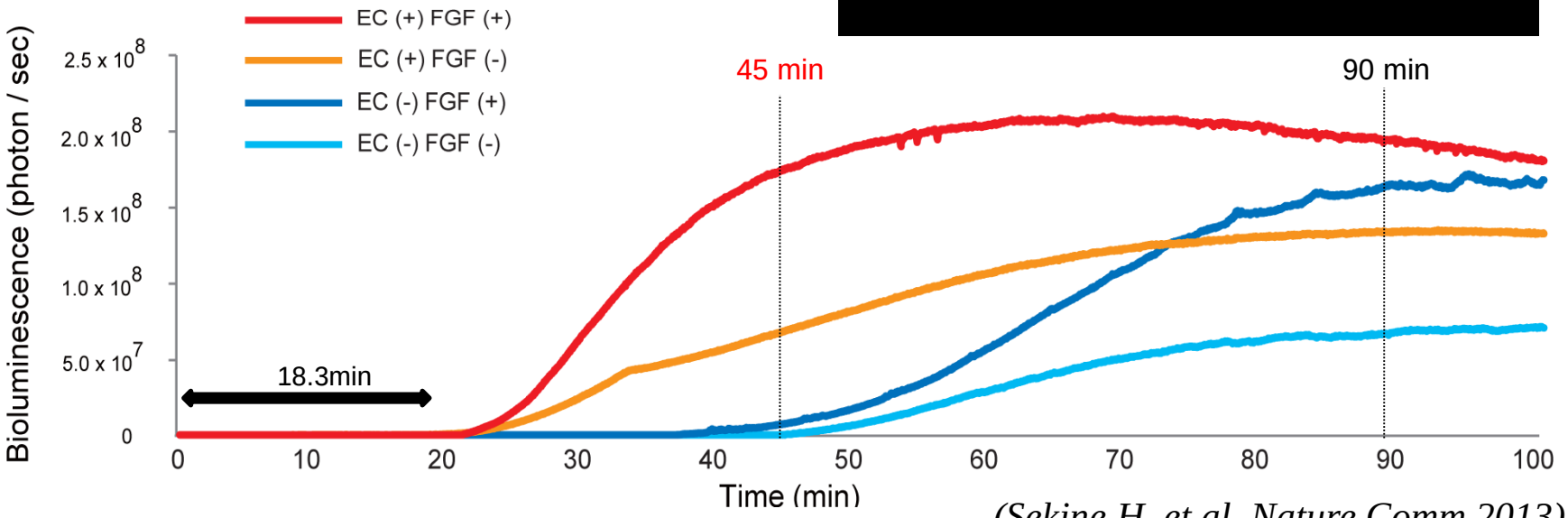




*(Zhou SB, et al. Transplantation 2014)*

# *In vitro* fabrication of functional three-dimensional tissues with perfusable blood vessels

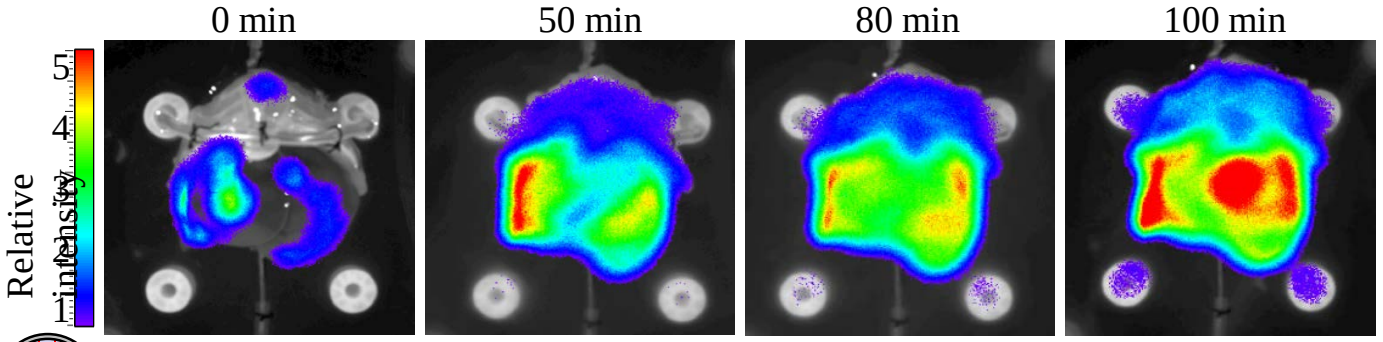


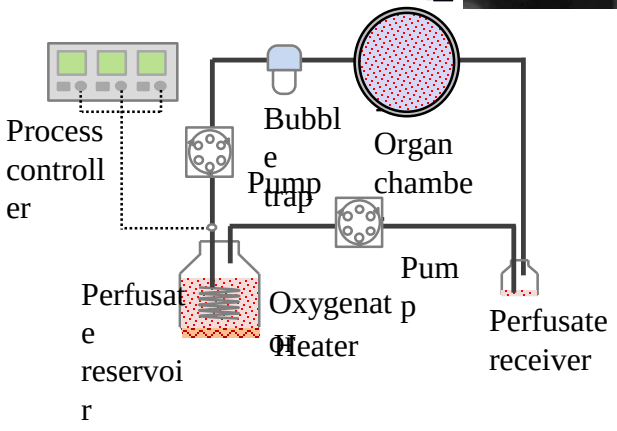



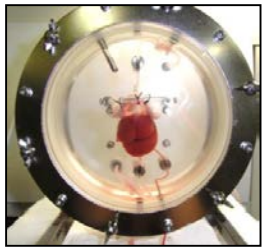



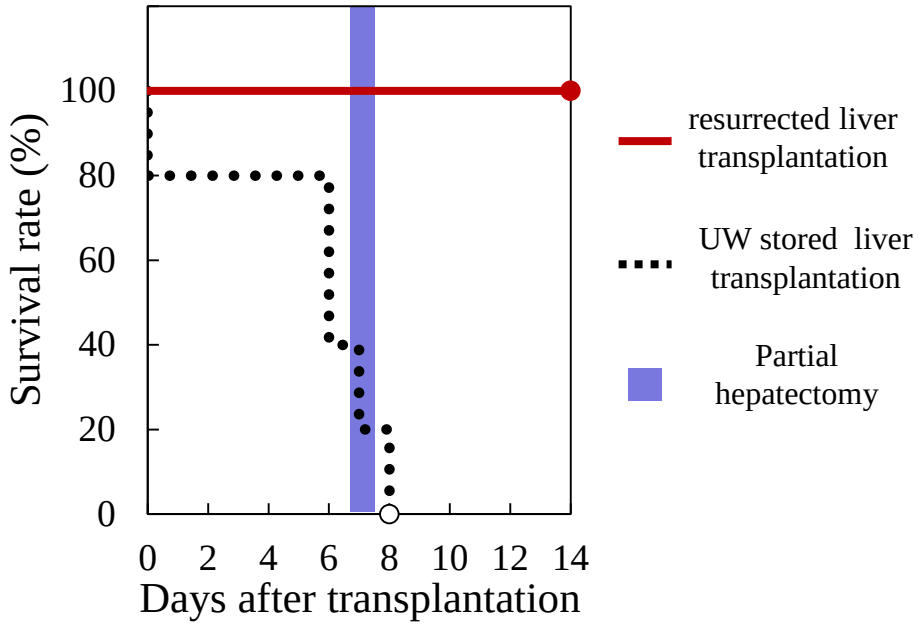

*(Sekine H, et al. Nature Comm 2013)*

# *Hypothermic temperature effects on organ survival and restoration*



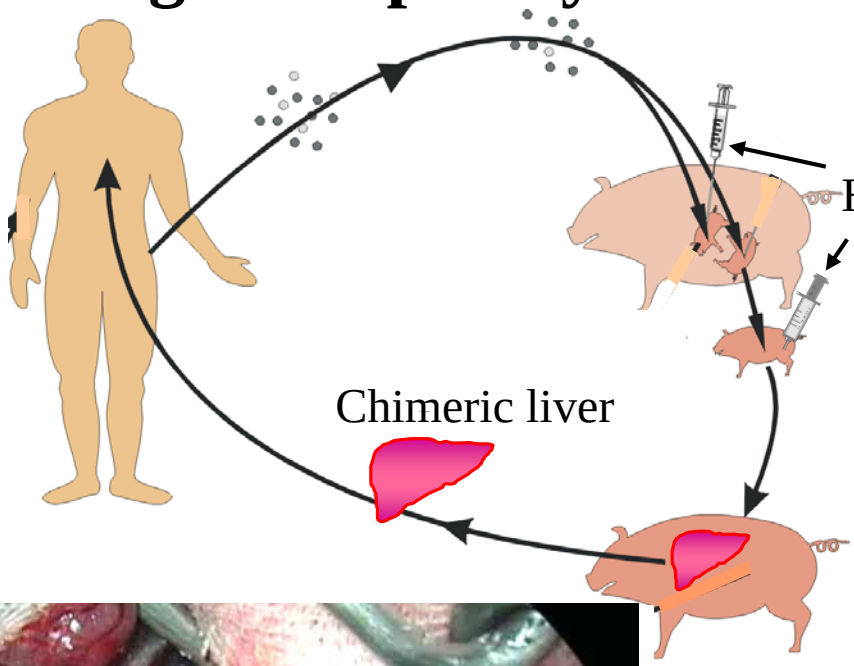



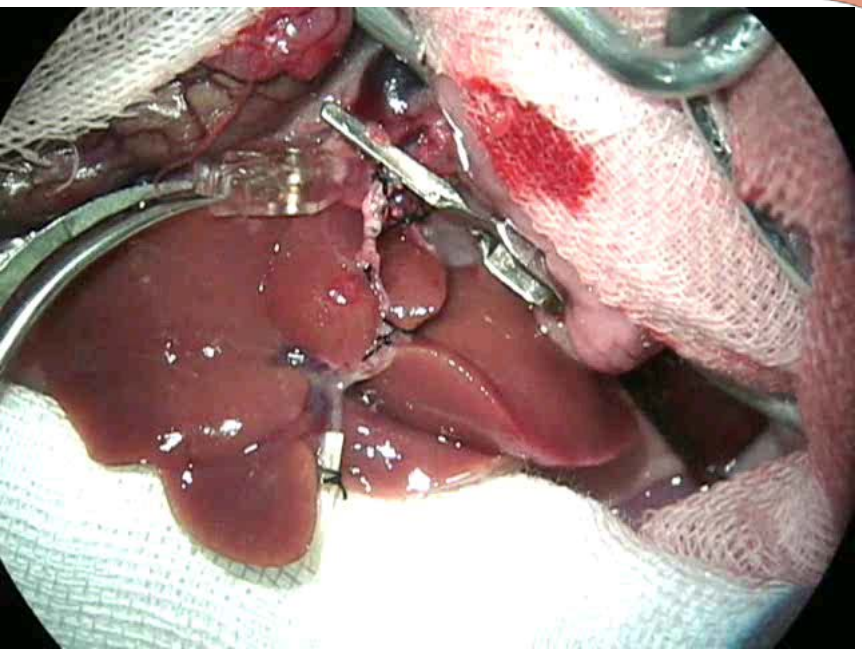



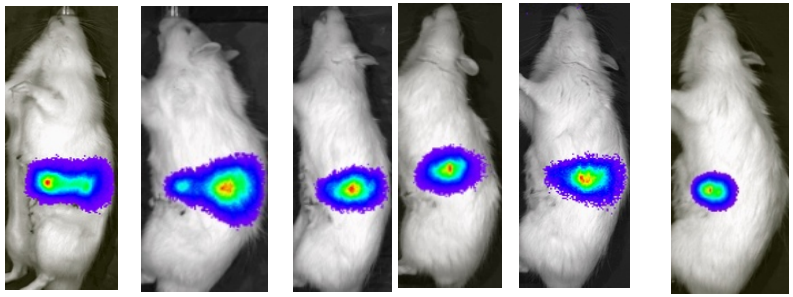


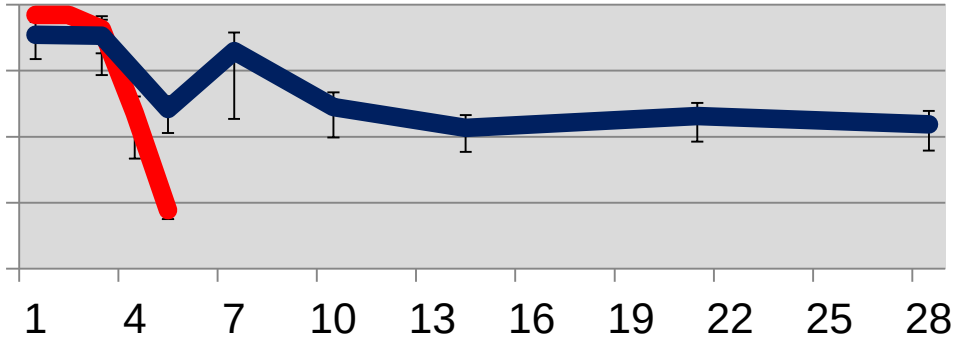

*(Ishikawa J, et al. Scientific Reports 2015)*

# Transplantation of engineered chimeric liver with autologous hepatocytes and xenobiotic scaffold



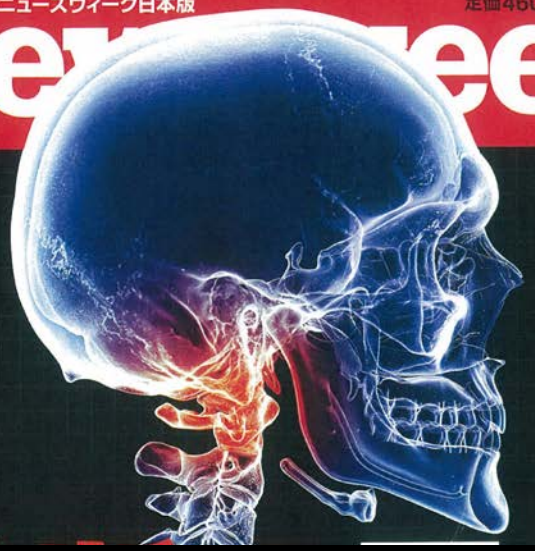




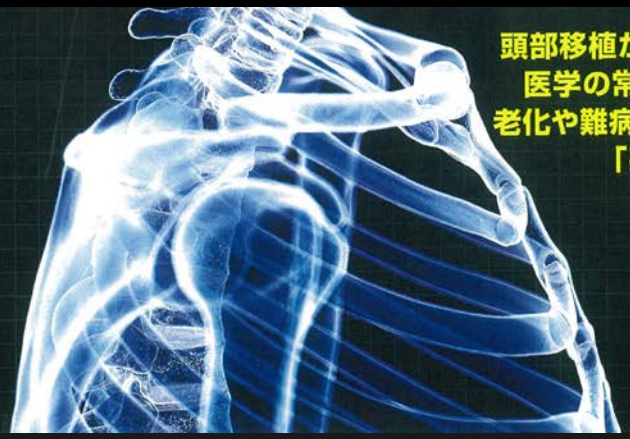

*(Hata T, et al. Ann Surg 2012)*

特集 頭部移植から人体冷凍保存、安楽死マシンまで
恐るべき「禁断の医療」
平昌オリンピック
アイスダンス
愛と成績の関係
米朝対立
「鼻血作戦」
未だ放棄せず

ニューズウィーク日本版
定価460円
Newsweek

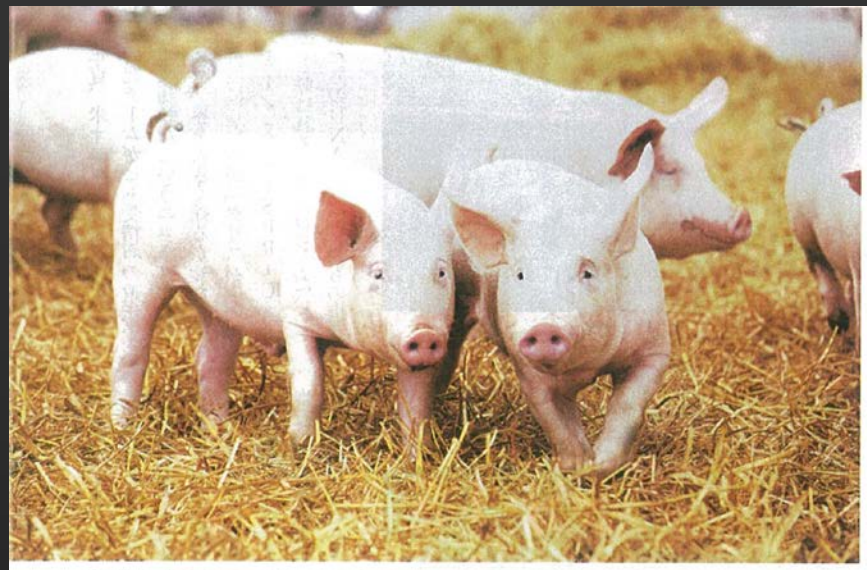


# Prohibited Medicine



頭部移植から人体冷凍まで
医学の常識を破る試みは
老化や難病克服の突破口か
「悪魔との取引」か

2018
3·6



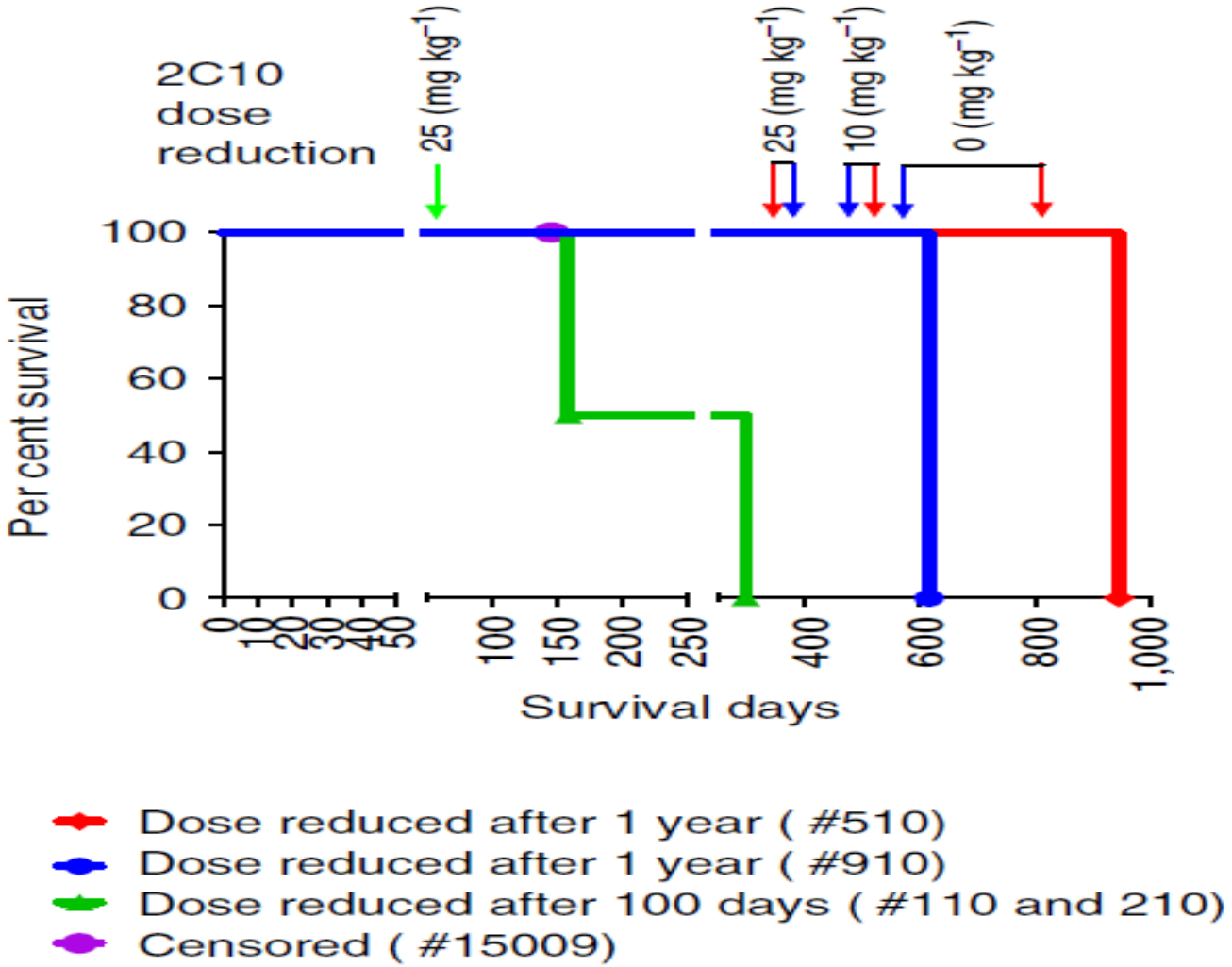
救世主？ 臓器の大きさも機能も人間に近いブタはドナーの最有力候補

MAKING PIG ORGANS SAFE

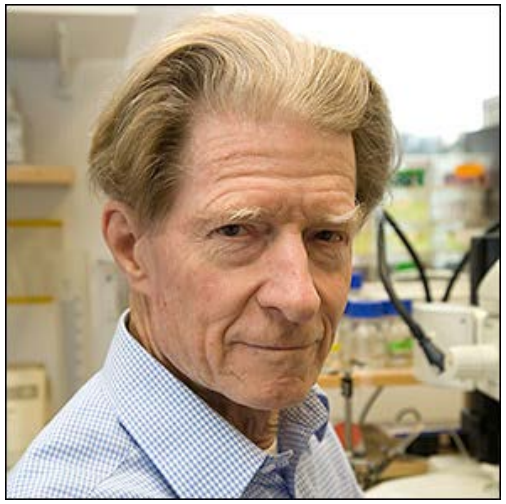
## 遺伝子改変ブタで ヒトの臓器を供給

異種移植 ゲノム編集で危険な遺伝子を除去
動物の内臓が人体に移植される日は近い

# Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft



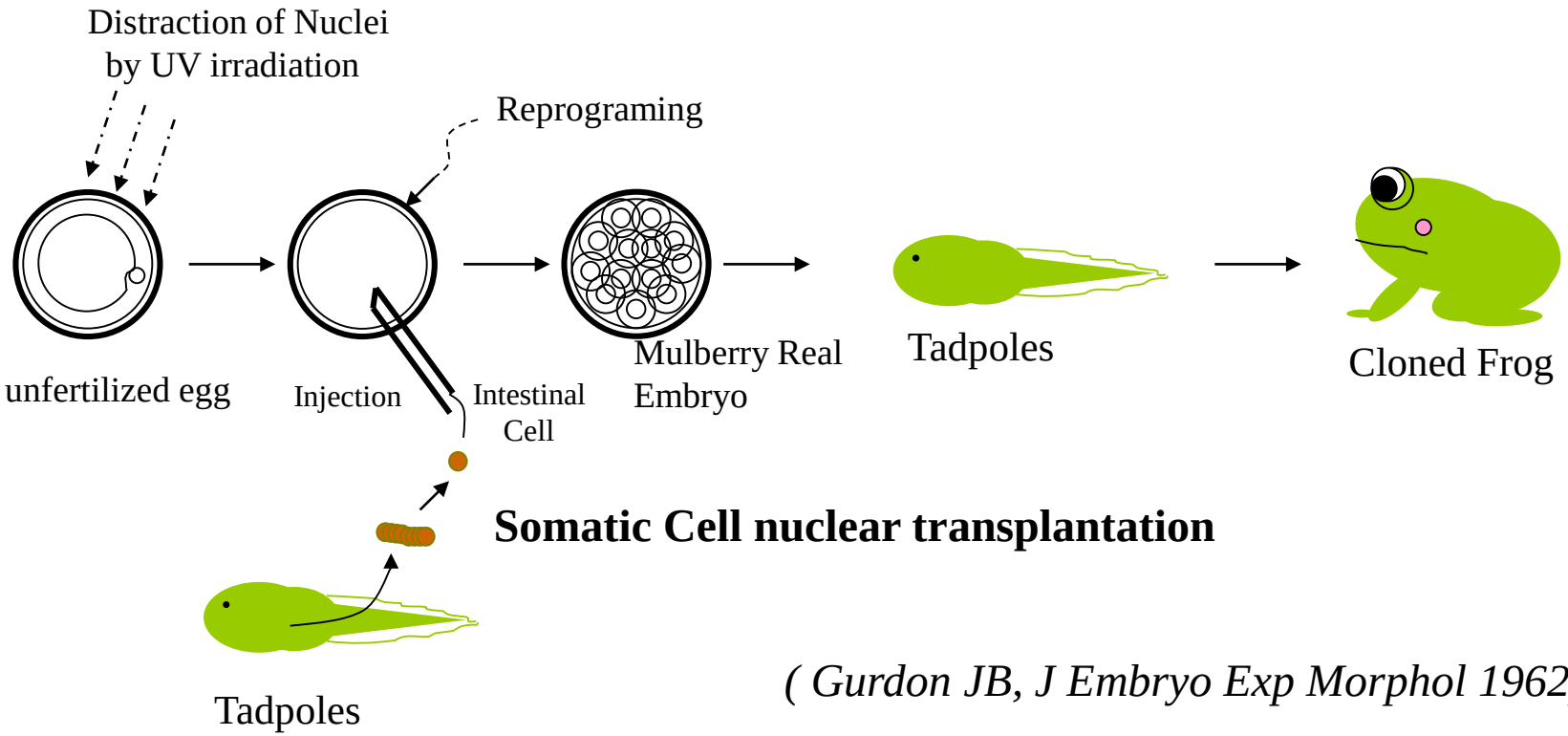

*(Muhammad M, et al. Nature Comm 2016)*



# Reprograming

**John Bertrand Gurdon**

**Novel Prize in Physiology or Medicine 2012**




*( Gurdon JB, J Embryo Exp Morphol 1962)*

# Cloning of Macaque Monkeys by Somatic Cell Nuclear Transfer

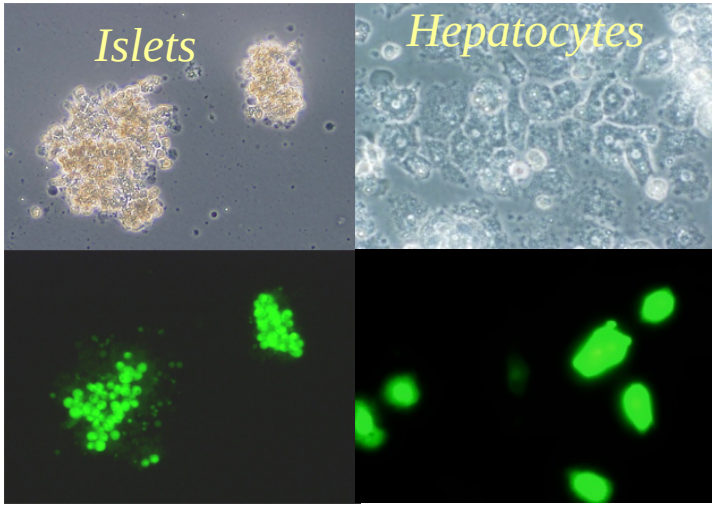



*(Liu Z, et al. Cell* *https://doi.org/10.1016/j.cell.2018.01.020)*

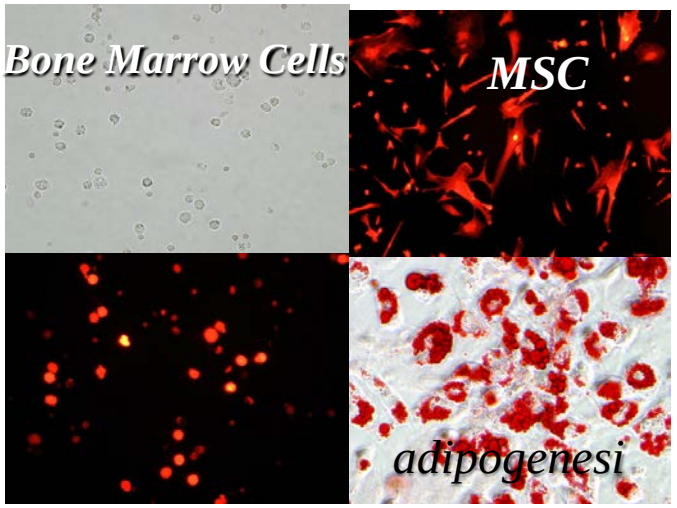
# *Genetically technology  for pigs*

# Production of `Colored` Pigs



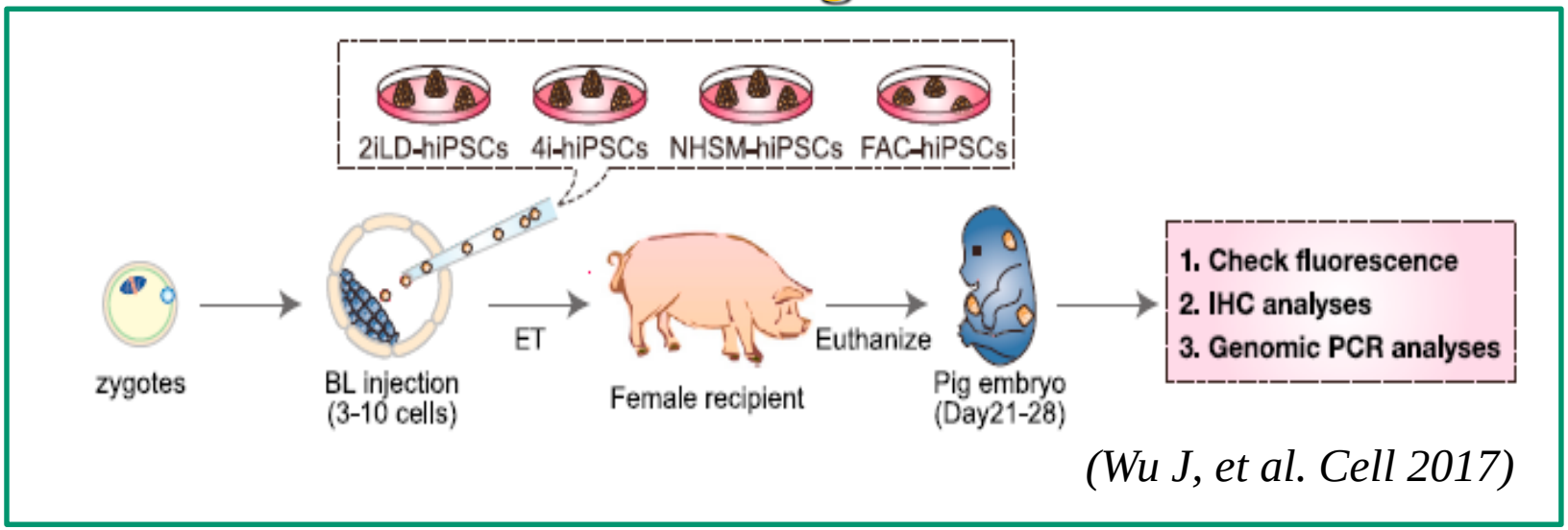



*(Kawarasaki T, et al. 2009)*

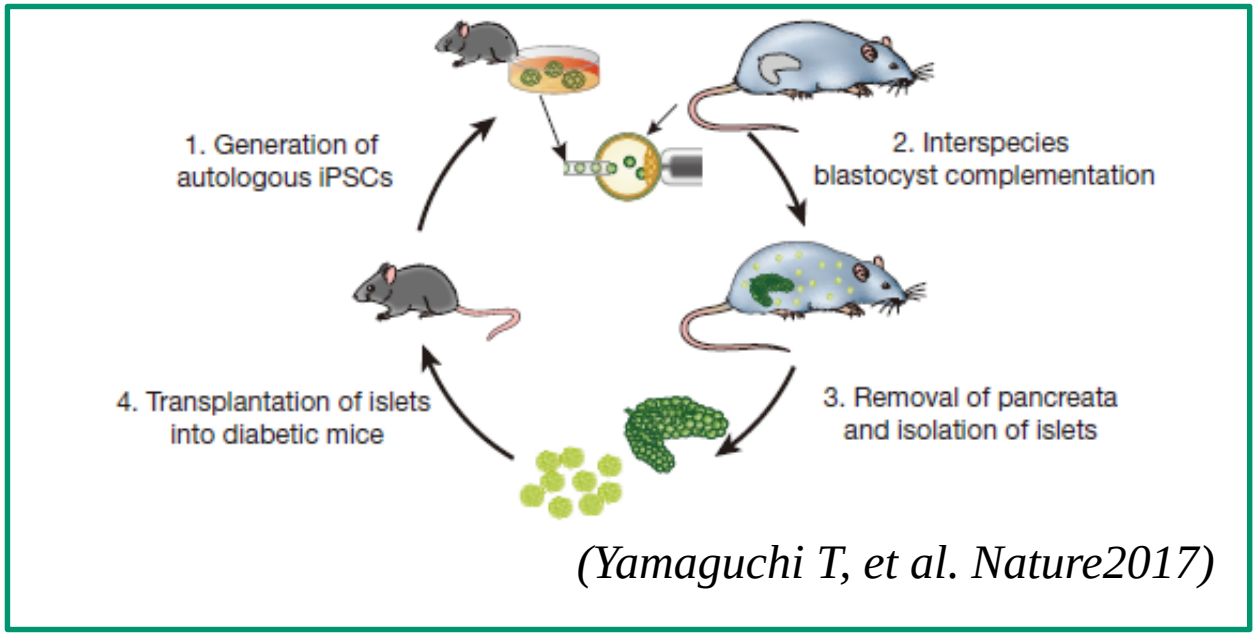



*(Matsunari H, et al. 2009)*

# In the heat of argument, Pig as In vivo bioreactor for human organs

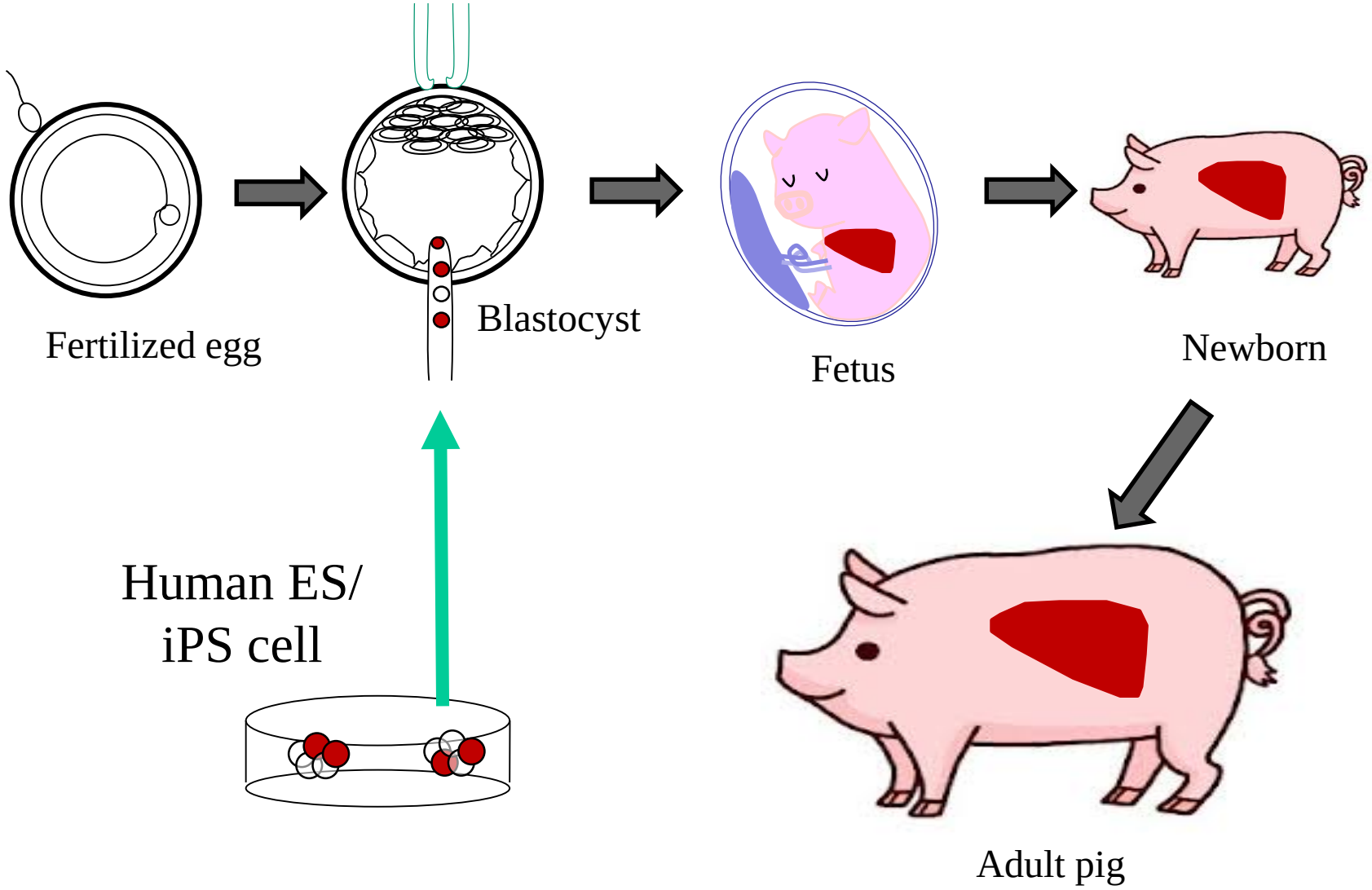


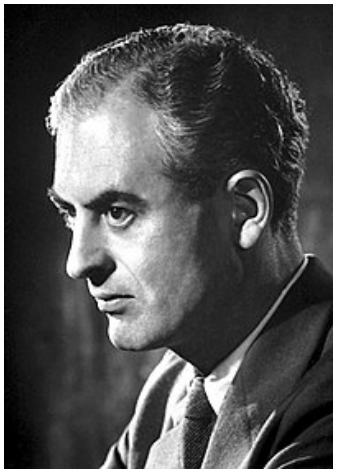

*(Wu J, et al. Cell 2017)*




*(Yamaguchi T, et al. Nature2017)*

# A theory of blastcyst complementation generating human organ in vivo in lacking organ cloned pig



| | **Experimental Medicine** | **Transplantation Immunology** |
|---|---|---|
| |  |  |
| | Dr. **Claude Bernard** | Sir **Peter Medawer** |
| Birth (Country) | 1813 (France) | 1915 (Brazil) |
| University | Universite de Paris | Oxford University |
| Professor(Age) | Sorbonne (41) | Birmingham (32)<br>Novel Award (45) |
| Death(Age) | 1878 (65) | 1987 (72) |

# IMMUNOGENETIC CONSEQUENCES OF VASCULAR ANASTOMOSES BETWEEN BOVINE TWINS[1]

Almost thirty years have passed since Lillie[2] used the demonstrated union of the circulatory systems of twin bovine embryos of opposite sex to explain, on an endocrine basis, the frequent reproductive abnormalities of the female twin. Since the appearance of Lillie's paper, the freemartin, as the modified female is called, has become an important example of the effects of hormones on sex-differentiation and sexual development in mammals.[3] Consequences other than endocrinological of nature's experiment in parabiosis have, however, received little attention.

Estimates of the frequency of identical as compared with fraternal twinning indicate that the former is relatively rare in cattle.[4] Tests for inherited cellular antigens in the bloods of more than eighty pairs of bovine twins show, however, that in the majority of these pairs the twins have identical blood types. Identity of blood types between full sibs not twins is infrequent, as might be expected from the large number of different, genetically controlled antigens[5, 6] (now approximately 40) identified in the tests. If, therefore, the frequent identity of blood types in twin pairs can be explained neither as the result of monozygotic twinning nor as chance identity between fraternal twins, nor as the sum of these two factors, it is evident that some mechanism is operating to produce frequent phenotypic identity of blood types in genetically dissimilar twins. The vascular anastomosis between bovine twins, known to be a common occurrence,[2] provides an explanation.

## Immunological Effects of Experimental Embryonal Parabiosis

According to Burnet and Fenner[1] and also Lopashov and Stroyeva[2], the inability to react against autologous antigens by the formation of antibodies develops during fœtal life (when the embryo is not yet able to produce antibodies), by the action of the antigens of the embryo's own tissues on the reticulo-endothelial system. According to Burnet and Fenner, a similar inability to form antibodies can be provoked by even a foreign antigen entering the reticulo-endothelial system during this stage. The experiments of Burnet, Stone and Edney[3], in which living influenza virus *A*, bacterial virus *C* 16 and human erythrocytes were introduced into chick embryos, did not confirm their hypothesis. In agreement with the theory are, however, the findings of Owen[4] in respect of bovine twins. In the case of twins this phenomenon is due to placental anastomosis, that is, to natural embryonal parabiosis.

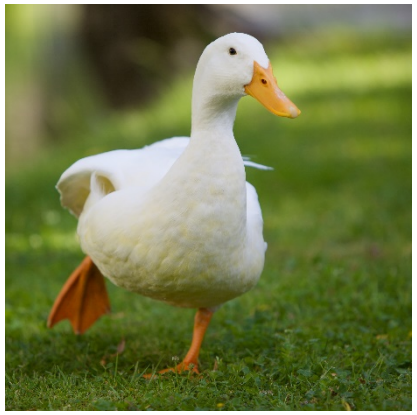


Table 1. Titres of Immune Agglutinins against Chick Erythrocytes in Ducks

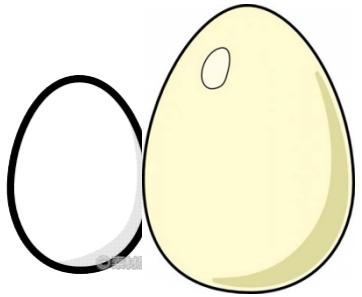
| Parabionts | Controls |
|---|---|
| 2* | 32† 64, 32 |
| 4* | 32, 64 |
| 8* | 258 |
| 4, 4 | 128, 128 |
| 4 | 32, 8 |
| 64‡ | 64 |
| | 32, 64 |

* Animal immunized by erythrocytes of embryonal parabiotic partner.
† Parabiont in which the exchange of blood was excluded by agglutination test.
‡ Exchange of blood not unequivocally demonstrated.
All parabionts or their partners were tested by agglutination immediately after hatching for the exchange of erythrocytes.
For earlier material, in which embryonic erythrocyte exchange was not tested, see Frenzl *et al.* (ref. 8).

Table 2. Titres of Natural (in parentheses) and Immune Agglutinins against Duck Erythrocytes in Chicks

| Parabionts | Controls |
|---|---|
| 64 (16), 256 (32) | 64 (4), 64 (8) |
| 128 (2) | 128 (2) |
| 256 (8)*, 256 (1) | 256 (8) |
| 512 (4)*† | 128 (16), 1,024 (2) |
| 128 (2) | 128 (2) |

* Represents an animal immunized by the erythrocytes of its embryonal partner.
† The skin of the duck partner was transplanted on the fifth day after hatching, the transplant surviving sixteen days.
All parabionts have been tested by agglutination immediately after hatching with respect to the exchange of erythrocytes.



# 'ACTIVELY ACQUIRED TOLERANCE' OF FOREIGN CELLS

By Dr. R. E. BILLINGHAM*, L. BRENT and Prof. P. B. MEDAWAR, F.R.S.

Department of Zoology, University College, University of London

## Experiments with Mice

A single experiment will be described in moderate detail: the recipients were mice of *CBA* strain, the donors of *A* strain. The data for transplantations between normal mice of these strains are as follows. The median survival time of *A*-line skin grafts transplanted to normal *CBA* adults (regardless of differences of sex, or of age within the interval 6 weeks–6 months) is $11{\cdot}0 \pm 0{\cdot}3$ days[7]. In reacting against such a graft, the host enters a state of heightened resistance; a second graft transplanted up to sixty days after the transplantation of the first survives for less than six days, and immunity is still strong, though it has weakened perceptibly, after four months. Heightened resistance may be passively transferred to a normal *CBA* adult by the intraperitoneal implantation of pieces of lymph node excised from a *CBA* adult which has been actively immunized against *A*-line skin[8].

In the experiment to be described (Exp. 73), a *CBA* female in the 15–16th day of pregnancy by a *CBA* male was anæsthetized with 'Nembutal', and its body wall exposed by a median ventral incision of the skin. The skin was mobilized but not reflected, and particular care was taken not to damage the mammary vessels. By manipulation of the abdomen with damped gauzes, six fœtuses were brought into view through the body wall. Each was injected intra-embryonically with 0·01 ml. of a suspension of adult tissue cells through a very fine hypodermic needle passing successively through the body wall, uterine wall, and fœtal membranes. (The inoculum itself, consisting of a suspension in Ringer's solution of small organized tissue clumps, isolated cells, and cell debris, had been prepared by the prolonged chopping with scissors of testis, kidney and splenic tissue from an adult male *A*-line mouse.) After injection of the fœtuses, the skin was closed with interrupted sutures.

## Preliminary Experiments with Chickens

Donors and recipients in these experiments were of Rhode Island Red and White Leghorn breeds, respectively. Skin transplanted from two weeks old Rhode Island Red chicks to White Leghorn recipients of the same age, using Cannon and Longmire's methods[8], is completely destroyed within ten days of grafting, to the accompaniment of an inflammatory reaction of conspicuous violence.

The embryonic chick is particularly well suited to experiments which make use of cellular inoculation, because the intravenous route is so easily accessible. Using methods demonstrated to us by Dr. C. Kaplan, whose help has been of the greatest value, we have obtained successful results by transfusing 0·2 ml. unmodified whole blood from an 11–12 day old embryonic Rhode Island Red donor into a chorio-allantoic vein of a White Leghorn embryo of the same age. Fourteen days after hatching, a test-graft of skin was transplanted to the recipient from its original donor. In seven such trials, five grafts showed prolongation of survival; of these, three succumbed within fifty days to the accompaniment of very much subdued inflammatory changes, and two still survive, with normal growth of red feathers, to the present time (125 days).

# Bone Marrow and Lymphoid Cell Injection of the Pig Foetus resulting in Transplantation Tolerance or Immunity, and Immunoglobulin Production

IT is known that the pig is capable at birth of immune responses to some antigens (phage[1,2]; animal viruses[3-5]; toxoids[6]; and homografts[7,8]). The finding that thymectomy in neonatal pigs is without effect on homograft rejection[8] also suggests that the pig, unlike many laboratory rodents[9,10] acquires the faculty of graft rejection before birth. Information on immune responses of pig foetuses to injected antigens has not been found in the literature. This communication outlines such research.

Large white pig foetuses at 60, 80 or 104 days of gestation were injected intraperitoneally through the uterine wall at laparotomy of the dam with an allogeneic white cell suspension taken either from blood and biopsied lymph nodes ($6 \times 10^8$ nucleated cells/kg of body weight) or from tibial bone marrow ($13{\cdot}5 \times 10^8$ nucleated cells/kg of body weight). Pig lymph contains very few lymphocytes[11], and so this rich source of lymphocytes in other species could
for postnatal
of a carbon–E
also for anti
litter were l
with killed
'Ampicillin' a
birth to the
cell suspensio

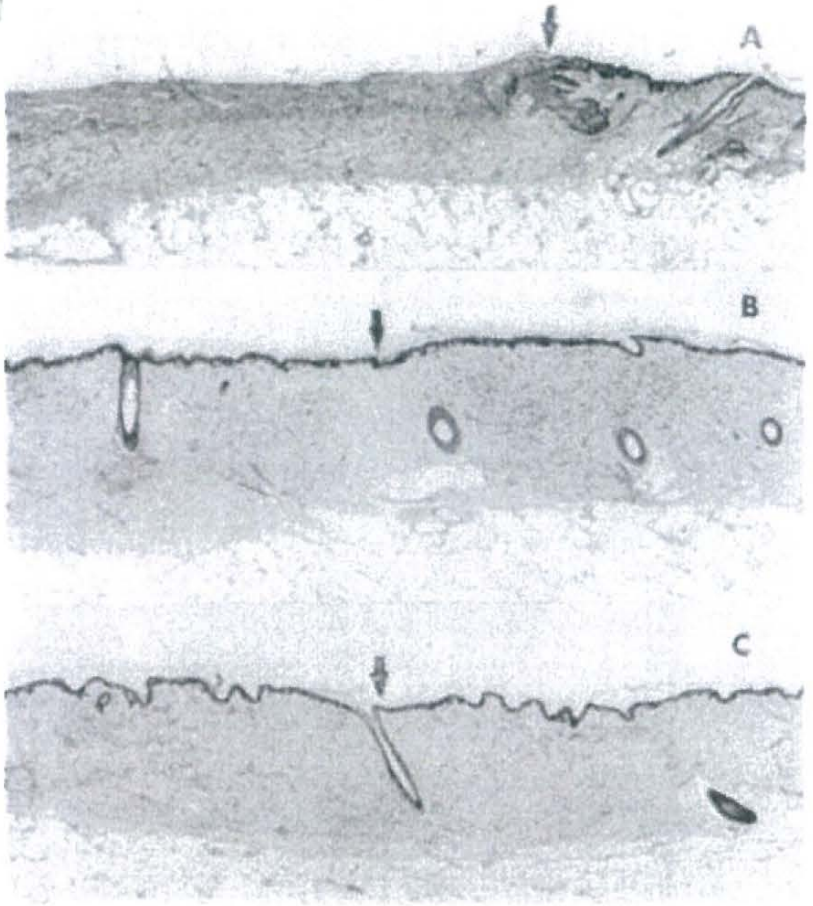
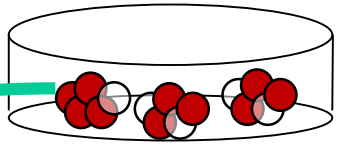
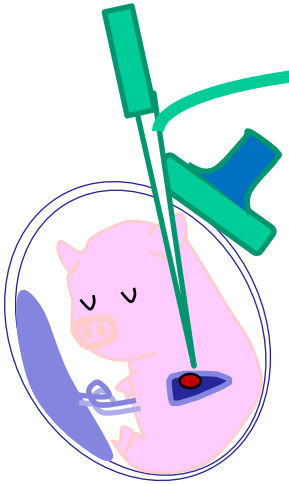


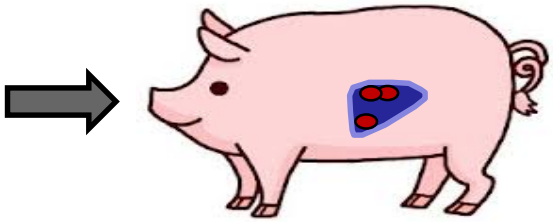

Fig. 1. Photomicrographs of biopsies from homografts on piglets treated at 60 days gestation with: *A*, control conventional antigens; *B* and *C*, bone marrow cell suspension from the skin graft donor (×7·5) (arrow denotes junction of graft on left and normal skin on right). *A*, Conventional antigen treated piglet 8 days after grafting. Primary homograft rejection. *B*, Bone marrow cell treated male piglet 50 days after grafting. Tolerant. *C*, Bone marrow cell treated female piglet 80 days after grafting. Tolerant.

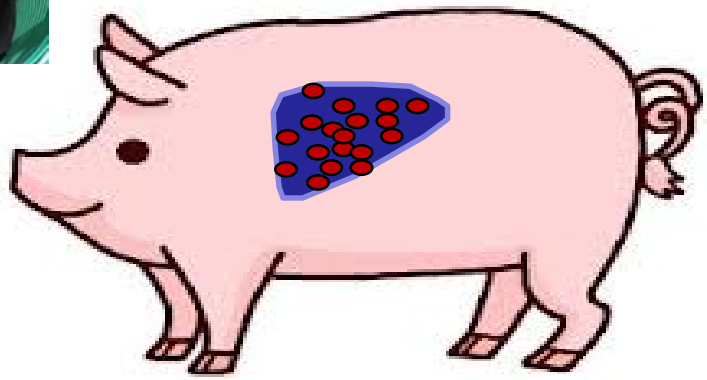
# Project of Production of Pigs with Human Organ by Actively Acquired Tolerance

Human Stem/
Progenitor Cells









fetus

neonate

Mature pig

Drug inducing
Apoptosis of Target Pig Organ


*(E Kobayashi )*


Thank you for ‘your attention’
for ‘Experimental Annimals’