第27回発達腎研究会

会長：琉球大学医学部小児科　中西浩一教授

(2018年9月15日、名古屋)

特別講演

ヒト由来幹細胞から作った‘腎芽‘を育てる戦略

Keio University

Eiji Kobayashi, MD,PhD

Keio University, School of Medicine

COI: EK is a medical adviser for Sysmex Ltd. and Screen Ltd.

腎臓オルガノイドと医療応用への道のり
The application potential of kidney organoids

表１　ヒトiPS細胞由来腎臓オルガノイドの比較

著者	雑誌	発表年	含まれる腎臓組織					
			糸球体	近位尿細管	遠位尿細管	集合管	血管内皮	間質細胞
Taguchi et al.	Cell Stem Cell 14(1): 53-67	2014	✓	✓	✓			
Takasato et al.	Nature Cell Biology 16: 118-126	2014		✓	✓	✓		
Morizane et al.	Nature Biotechnology 12(1): 195-207	2015	✓	✓	✓			
Freedman et al.	Nature Communications 6:8715	2015	✓	✓	✓		✓	
Takasato et al.	Nature 526(7574): 564-568	2015	✓	✓	✓	✓	✓	✓
Taguchi et al.	Cell Stem Cell 21(6): 730-746	2017	✓	✓	✓	✓		

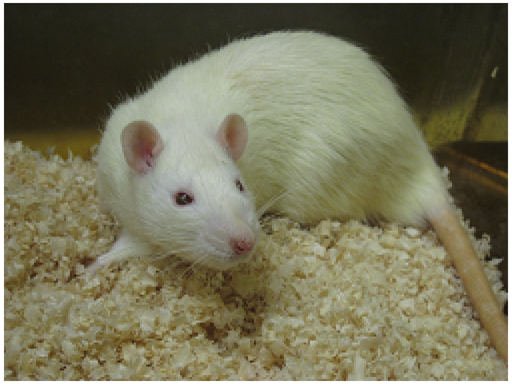
(高里　実、発達腎研究会誌 Vol. 26 No.1 p2-7, 2018)

腎臓オルガノイドの課題

項目	人の腎臓	腎臓オルガノイド
サイズ	握りこぶし程度	直径5mm 程度
成熟性	成人	妊娠15週目程度
形	尿の排出口である尿管がある	尿管がない
ネフロン数	100-200万個	300-500個程度
尿細管	各種輸送体を発現する	未発現の輸送体もある
糸球体	糸球体毛細血管がある	糸球体毛細血管が未形成

(高里　実、発達腎研究会誌 Vol. 26 No.1 p2-7, 2018)

再生医療におけるR＆Dで大動物を用いる意義

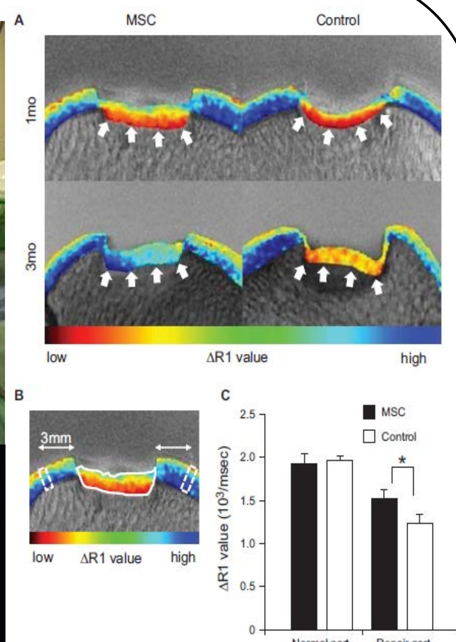
	小動物	大動物	臨床
			
細胞数	1.0〜3.0 x 10^6 cells	1.0〜3.0 x 10^8 cells	5.0〜8.0 x 10^8 cells
100mm dish	1〜2枚	100〜200枚	350〜550枚
移植時間	1〜10秒	10〜30分	15〜60分
移植法	シリンジによる 局注または静注	シリンジによる 局注または静注 点滴バックによる 静注	シリンジによる 局注または静注 点滴バックによる 静注

(寺谷、小林. 再生医療学会誌2013年)

非臨床・臨床一体型評価法の導入でいち早く臨床応用へ

（MSCによる軟骨再生療法）

豚での関節鏡



(Nakamura T, et al. Cytotherapy 2012)

患者での関節鏡

滑膜由来MSC

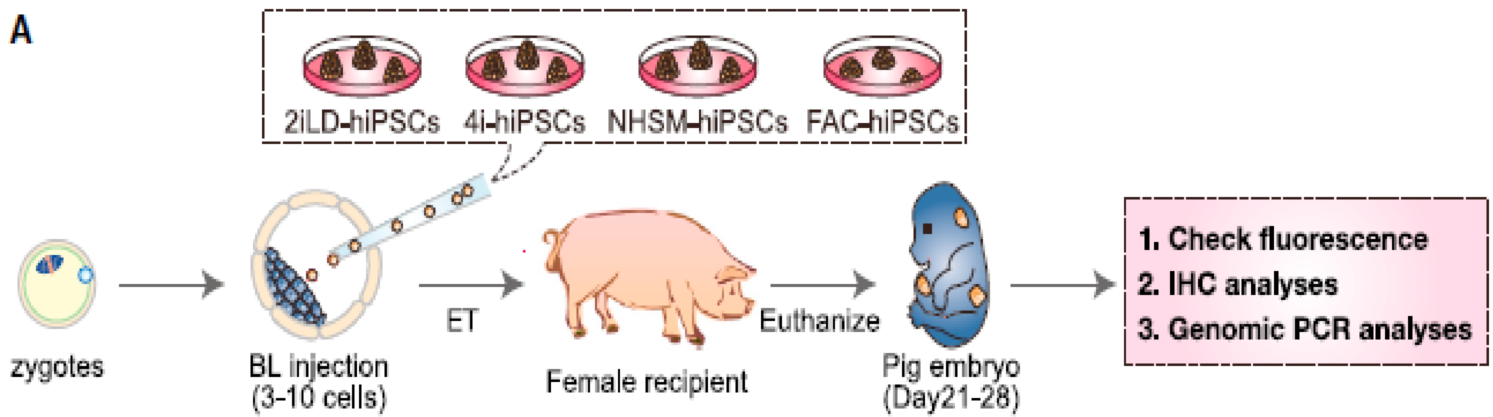
関矢教授、宗田教授らとの
（東京医科歯科大学との共同研究）

Strategies for Human Organ Fabrication

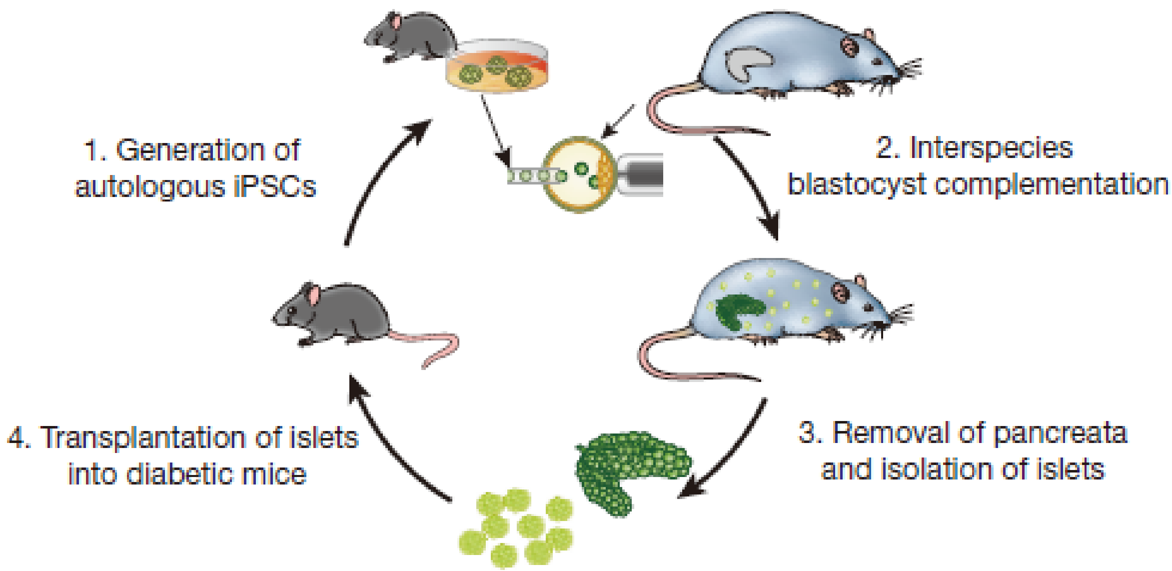
Methods How to Use Pigs	Experiments for Proof of Concept by Small Animals	In Pursuit of Fabricating Humanized Pigs
1) Whole Body (Animal Factory)	Pancreas（*Yamaguchi T, et al. Nature 2017*） Kidney（*Usui J, et al. Am J Path 2012*） Liver（*Hata T, et al. Ann Surg 2012*）	Pancreas（*Matsunari H, et al. PNAS 2013*） Liver（*Fisher JE, et al. Liver Transplant 2013*） Liver (*Hsu HC, et al. Transplant Proc 2017*) Pig/Human Chinera (*Wu J, et al Cell 2017*)
2) Use by Processing (Decellularized Graft)	Heart（*Ott HC, et al. Nature Med 2008*） Kidney（*Ross EA, et al. JASN 2009*） Liver（*Uygun BE, et al. Nature Med 2010*） Lung（*Ott HC, et al. Nature Med 2010*）	Heart *(Kitahara H, et al.Cardio Thrac Surg 2016)* Kidney（*Orland G, et al. Ann Surg 2012*） Liver（*Yagi H, et al. Cell Transplant 2012*） Lung *(Nichols JE et al, Science Trans Med 2018)*
3) Genesis Organs (Fetus Genesis Grafts)	Liver（*Takebe T, et al. Nature 2013*） Kidney *(Mae S, et al Nature Communi 2013*) Kidney（Takasato M, et al *Nature 2015*） Kidney（Yamanaka S, et al Nature Communi 2017）	Kidney（*Hammerman M, et al. Organogenesis 2012*） Kidney（*Yokote S, et al. PNAS 2013*）

（Modified Document for Bioethics Committee, Cabinet Office in 2013 Eiji Kobayashi）

白熱する「動物の体内でヒトの臓器を作る」 第３次ブタブーム

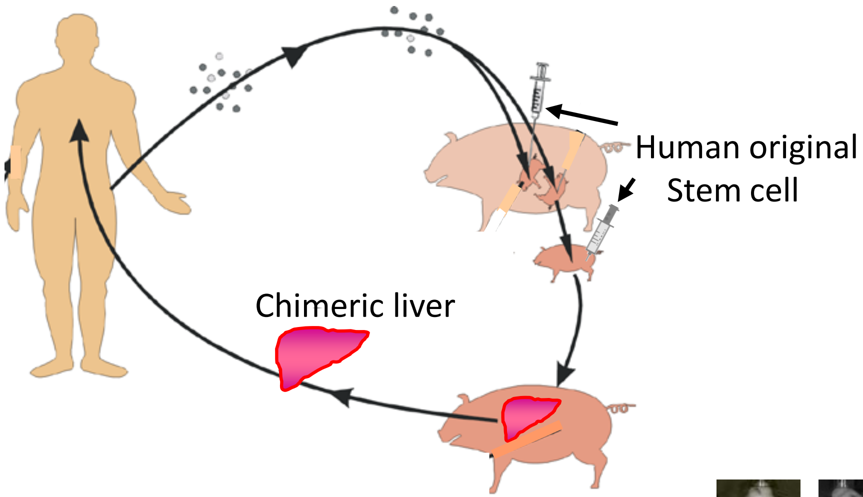


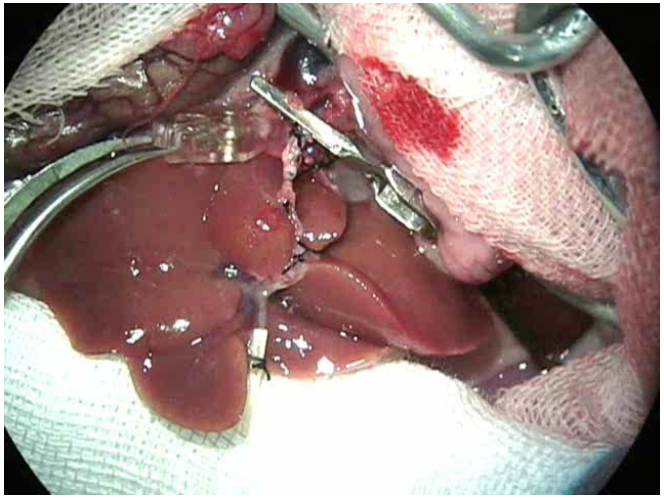
(Wu J, et al. Cell 2017)

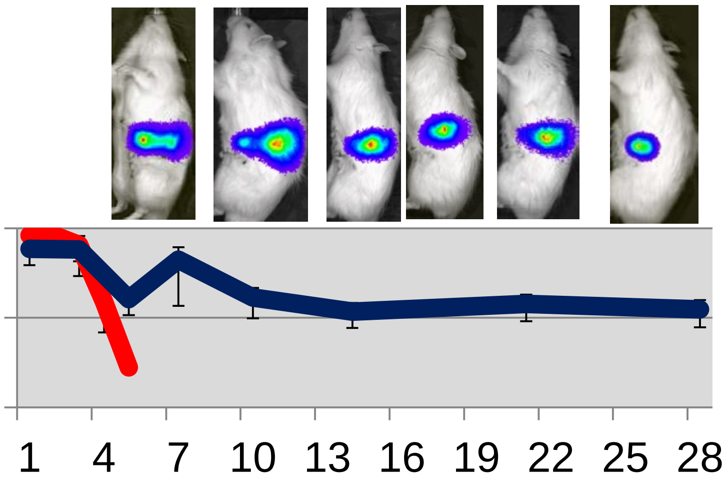


(Yamaguchi T, et al. Nature2017)

Transplantation of engineered chimeric liver with autologous hepatocytes and xenobiotic scaffold

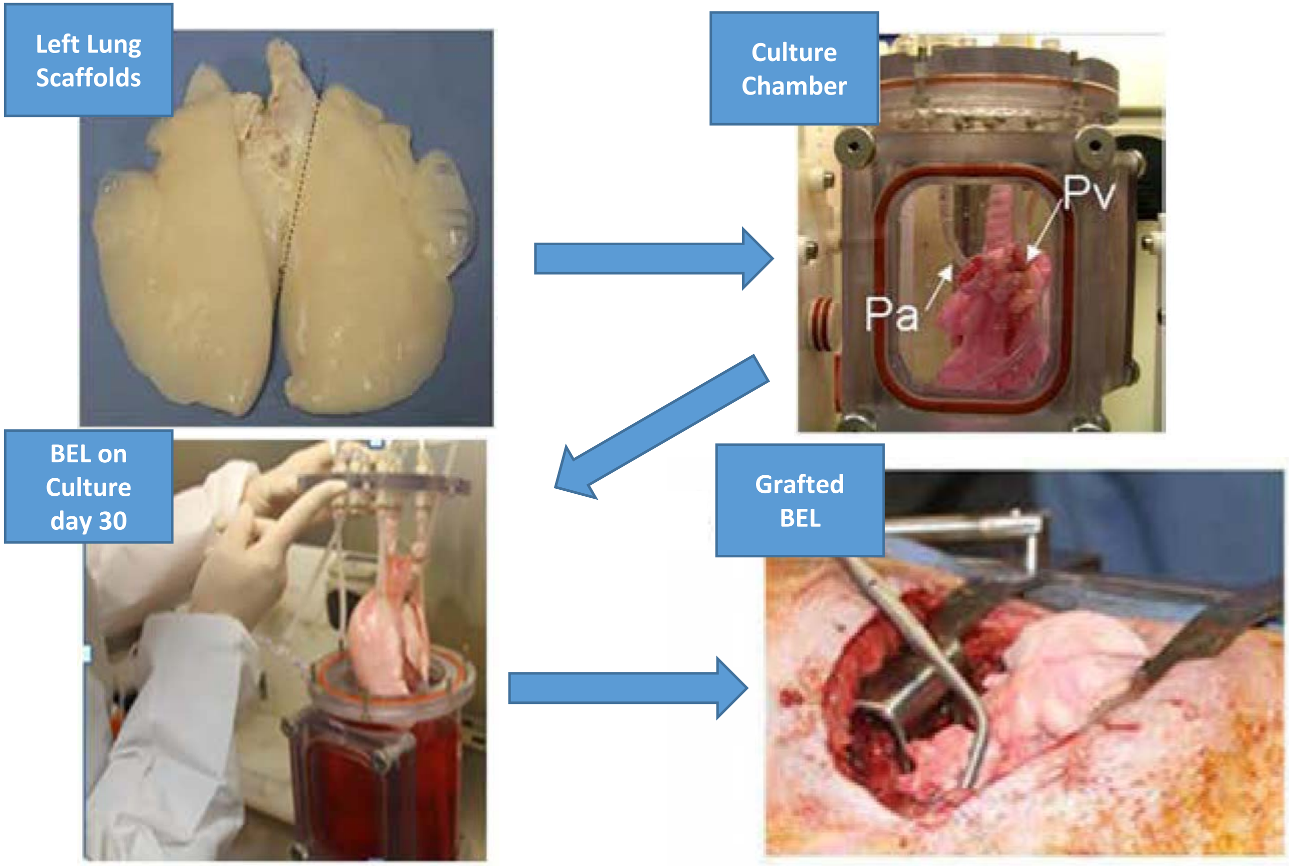






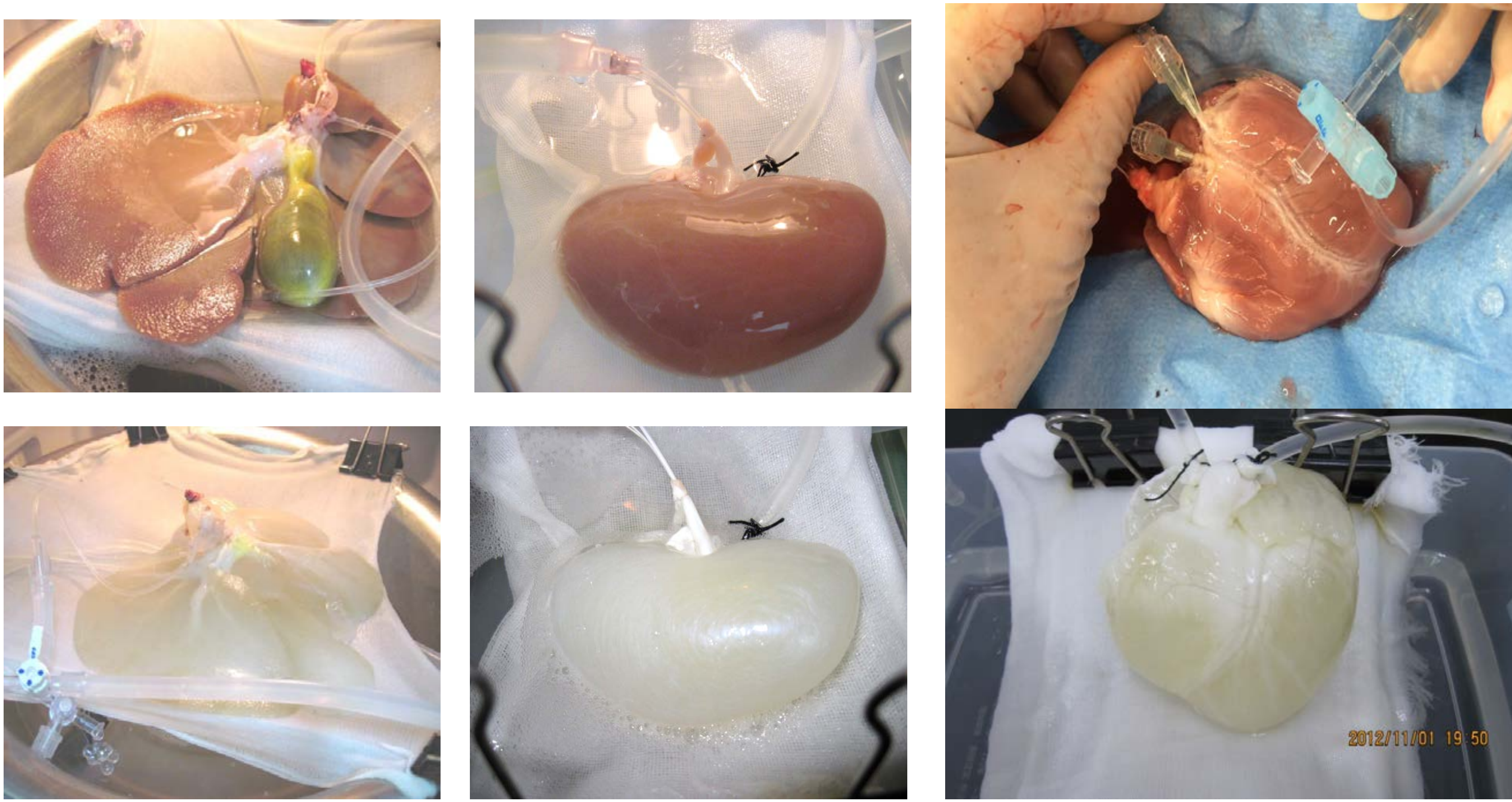
(Hata T, et al. Ann Surg 2012)

Production and transplantation of bioengineered lung into a large-animal model



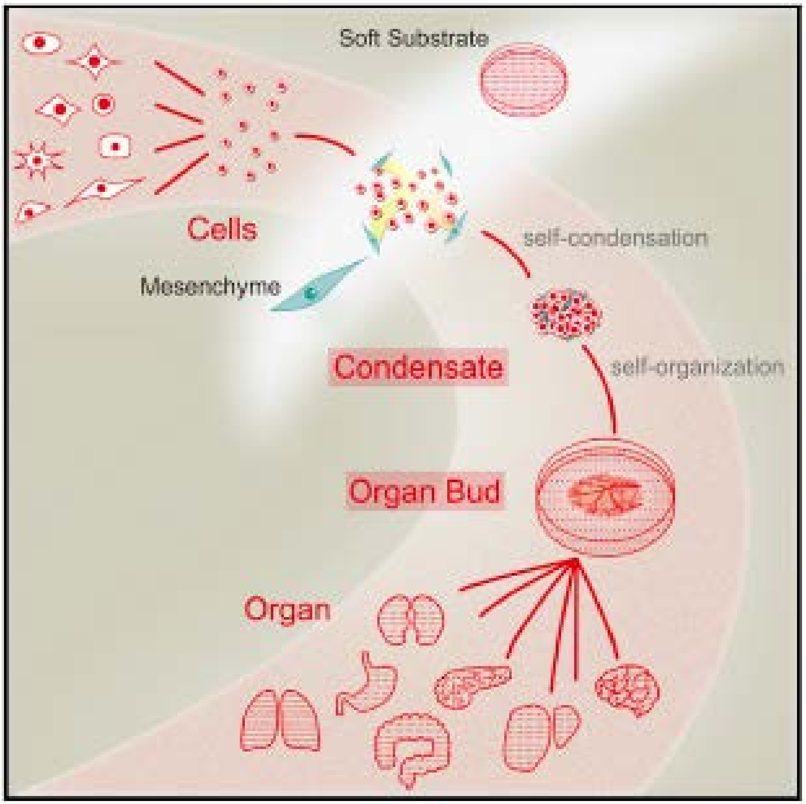
(*Nichols JE, et al. Science Transl Med 2018*)

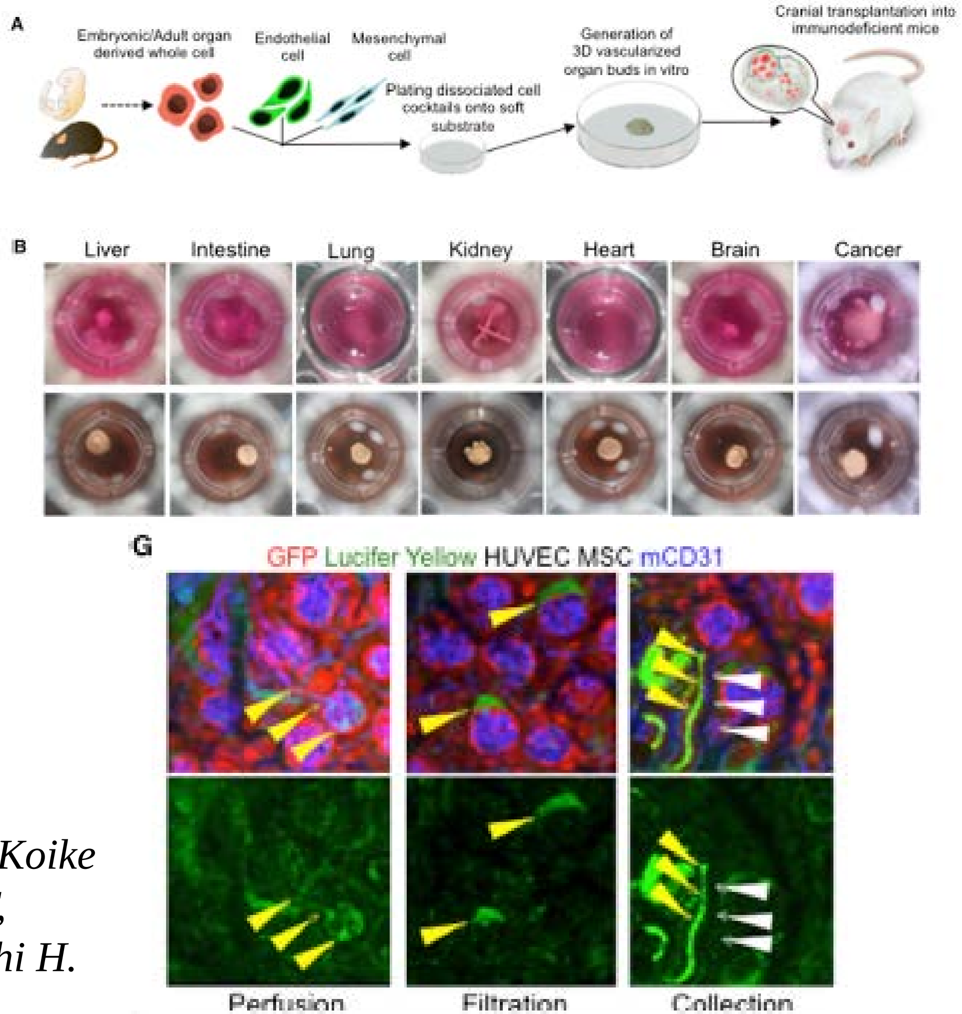
Perfusion based Decellularized Technique for Pig Organs



(Modified from Kobayashi E. Cell Medicine 2016)

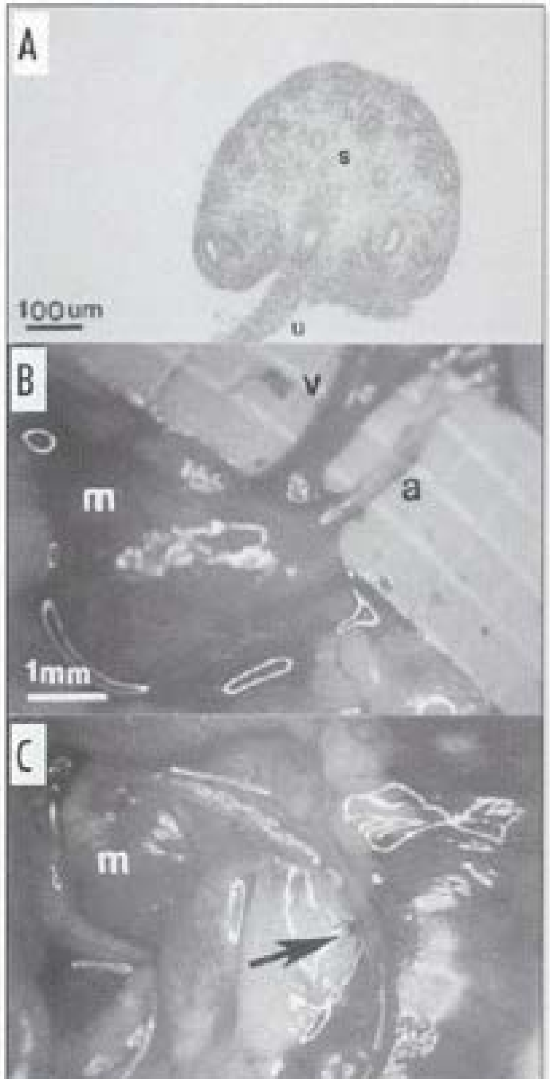
Vascularized and Complex Organ Buds from Diverse Tissues via Mesenchymal Cell-Driven Condensation

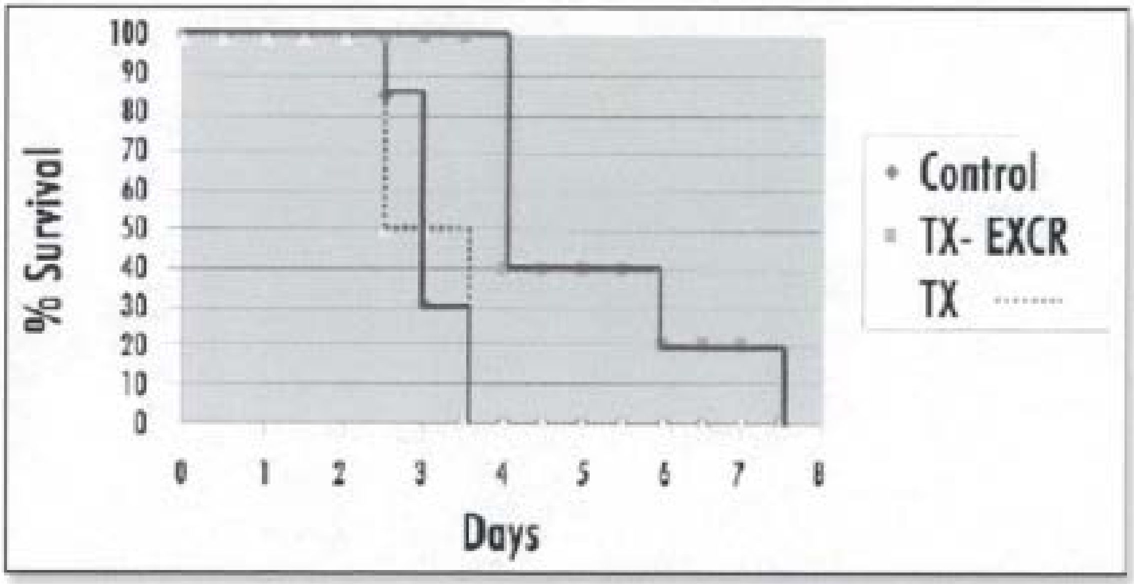




Takebe T, Enomura M, Yoshizawa E, Kimura M, Koike H, Ueno Y, Matsuzaki T, Yamazaki T, Toyohara T, Osafune K, Nakauchi H, Yoshikawa HY, Taniguchi H. Cell Stem Cell. 2015

Prolongation of life in anephric rats following de novo renal organogenesis.

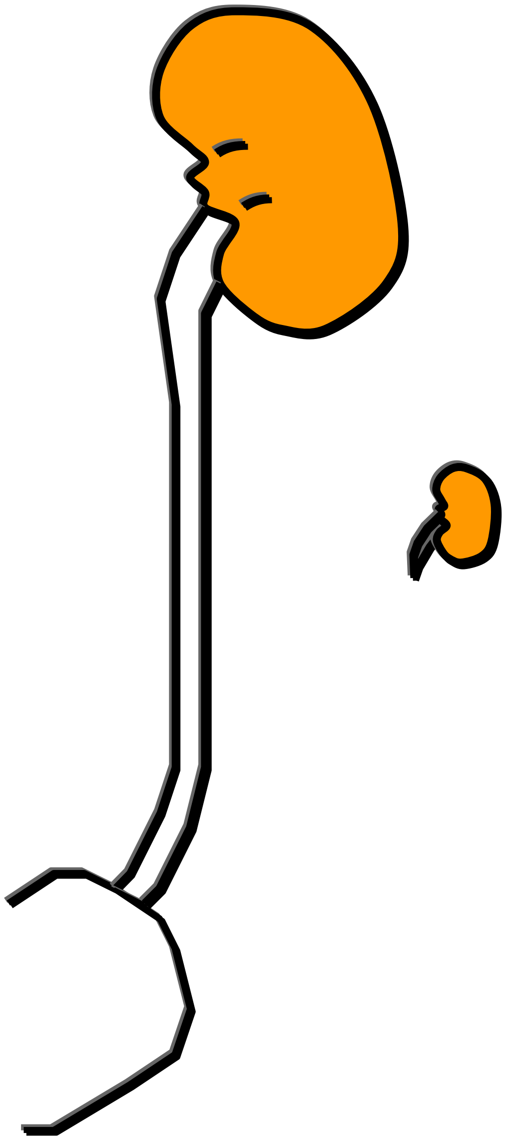




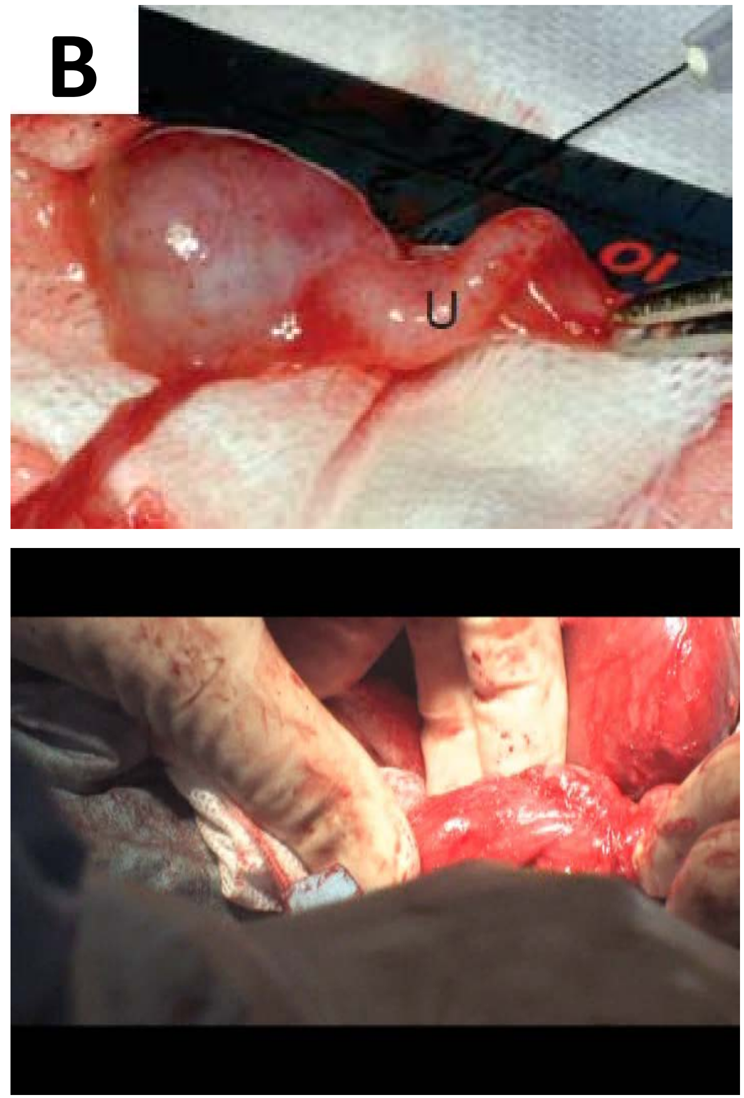
(Rogers SA, Hammerman MR. Organogenesis. 2004)

臨床に使える尿路作成への挑戦

A



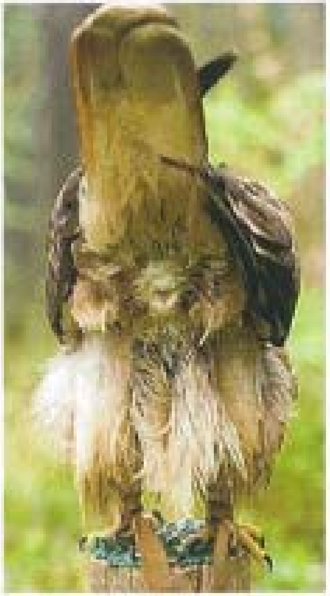
B



クローン豚由来メタネフロスの尿管吻合

「クロアカ・グラフト」開発（2012年）

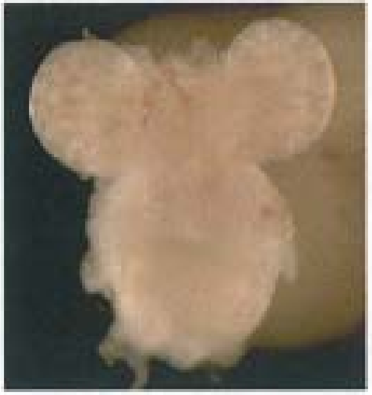
「クロアカ」とは



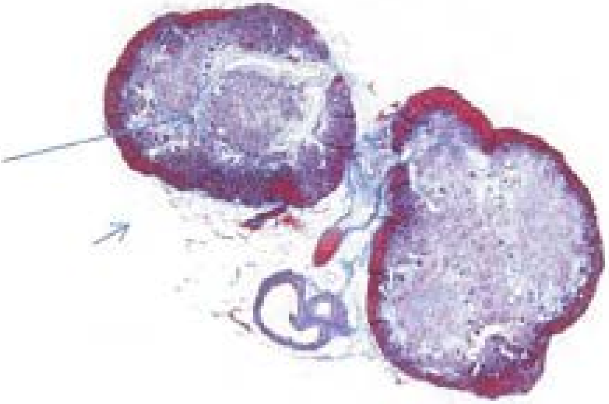
In zoological anatomy, a **cloaca** (/kloʊˈeɪkə/) is the posterior opening that serves as the only such opening for the intestinal, reproductive, and urinary tracts of certain animal species.

クロアカ・マキシマ（Cloaca Maxima）は、古代ローマの下水システム。

「クロアカ・グラフト」の移植とその発育

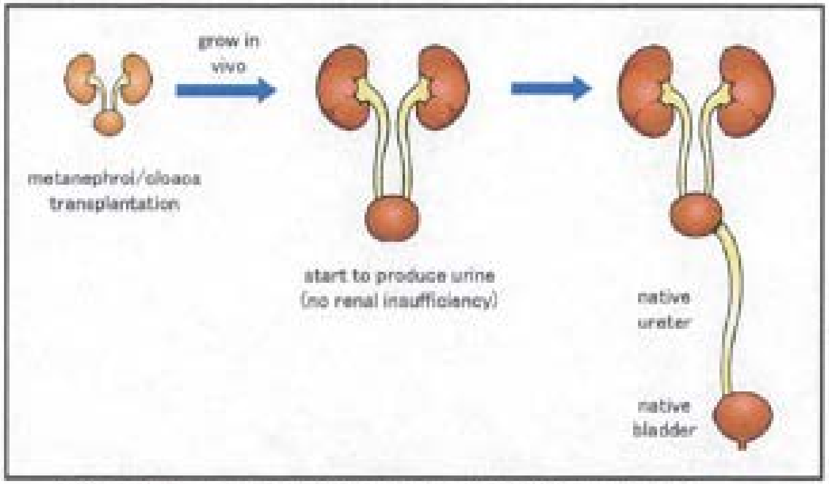


尿路と膀胱も育つ

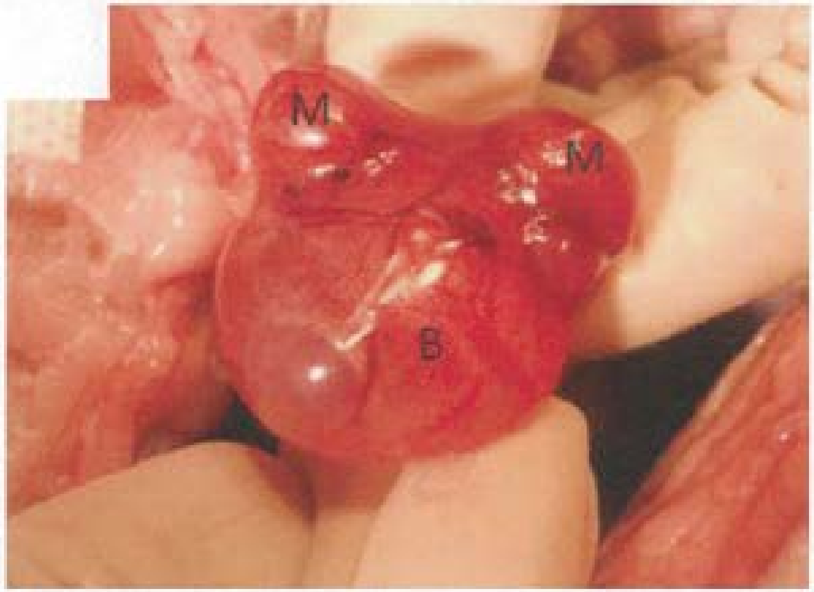


ブタクロアカの臨床応用に向けて

A. クローン豚間



Step-wise peristaltic ureter (SWPU) system

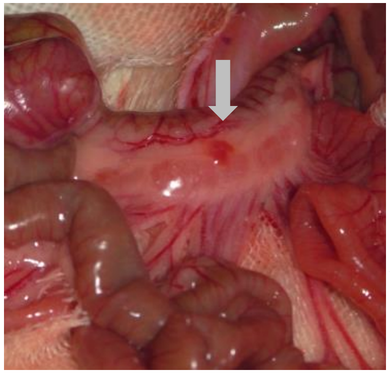


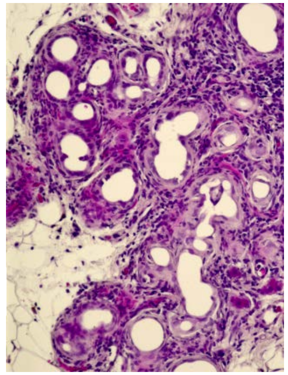
B. ブタ一猫間

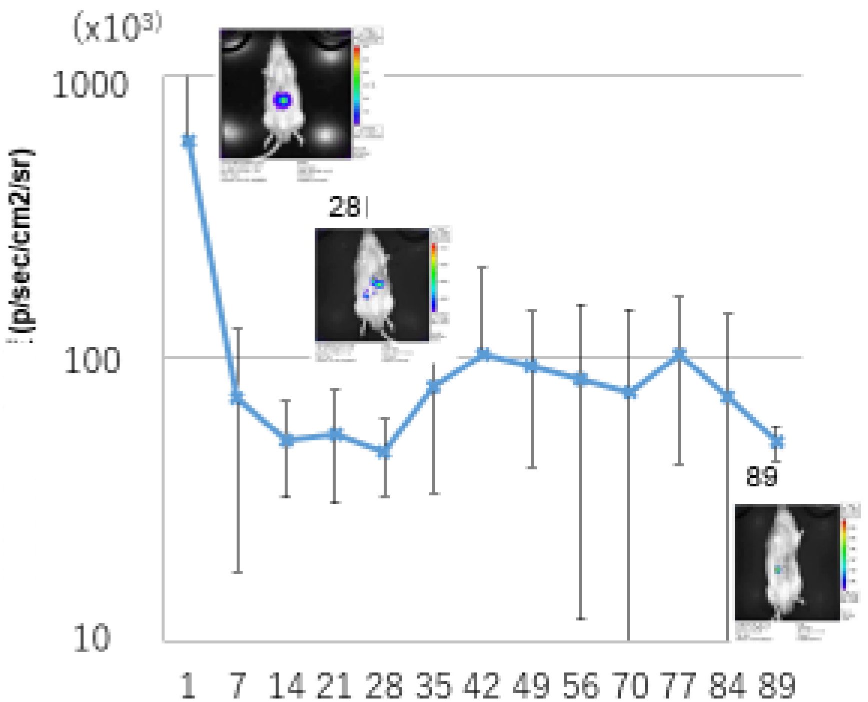
(*Yokote T, et al. PNAS 2015*)

Liver bud can not grow at the heterotopic site

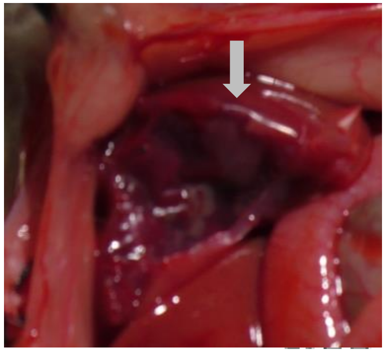
Heterotopic Transplantation

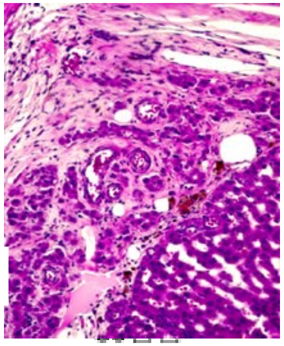


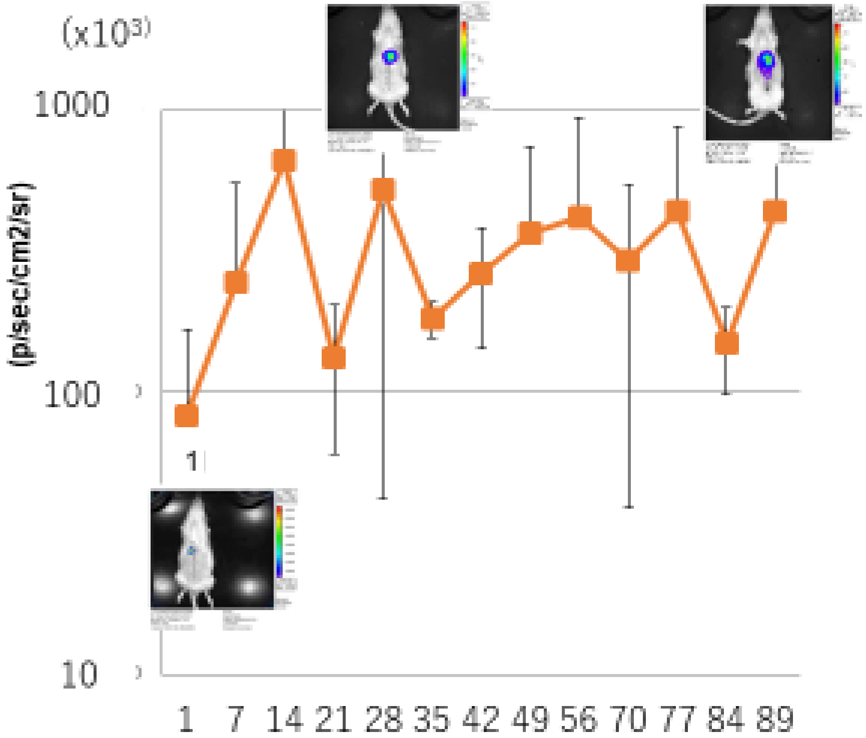




Orthotopic Transplantation



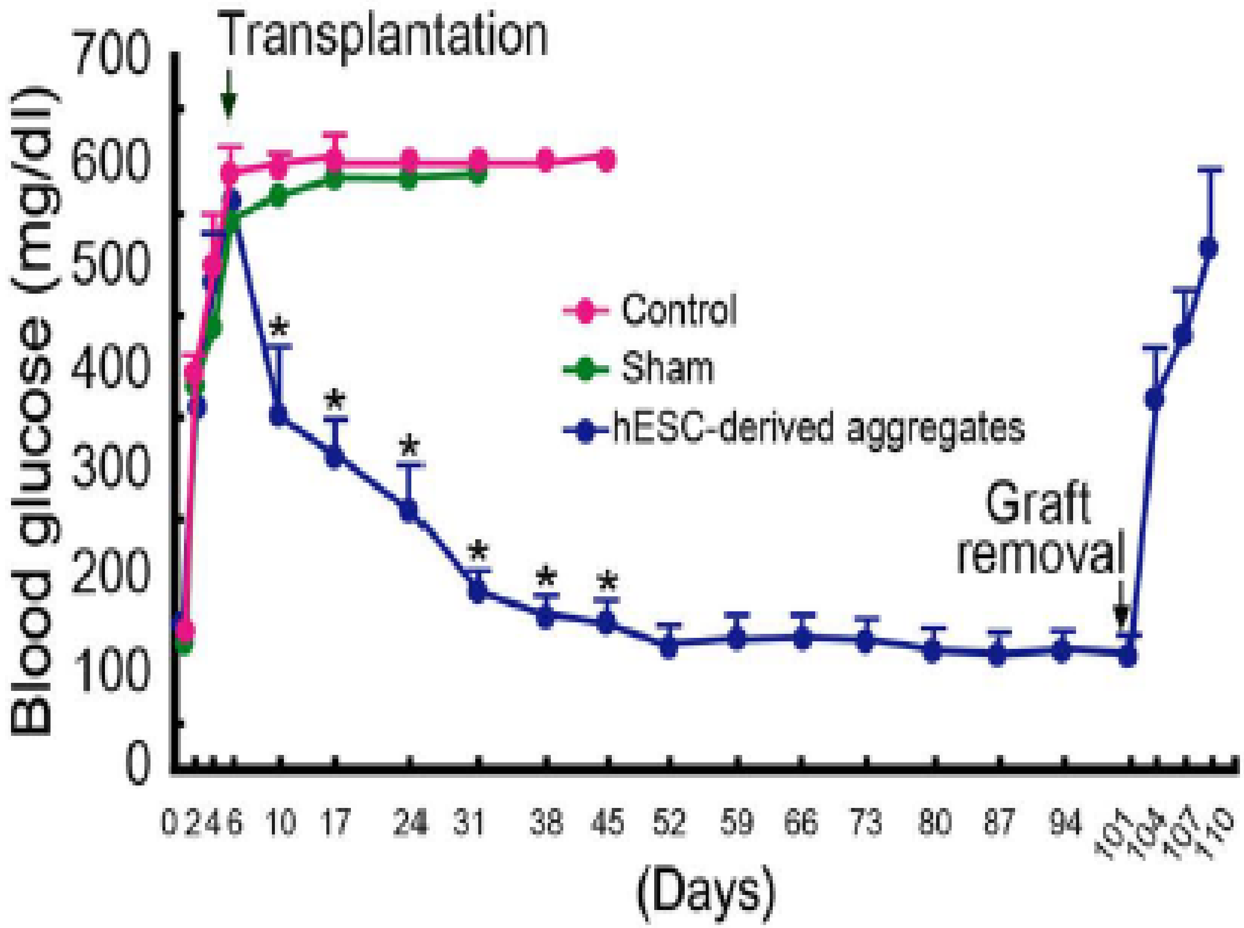




(Yanagi Y, et al. Scientific Reports 2017.

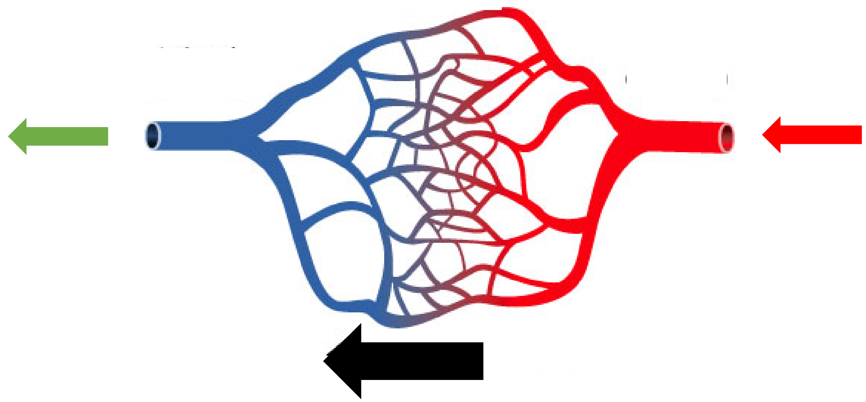
Organ budの機能発現には時間がかかる

Pancreatic Islet-Like Three-Dimensional Aggregates Derived From Human Embryonic Stem Cells Ameliorate Hyperglycemia in Streptozotocin-Induced Diabetic Mice

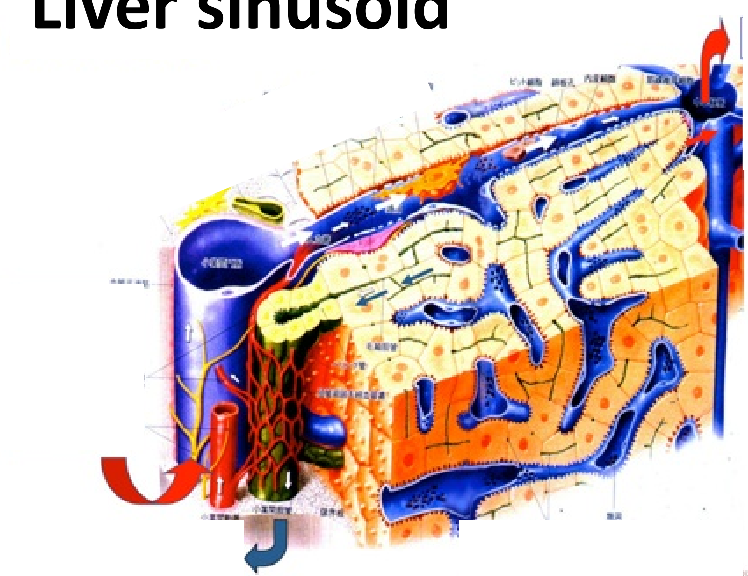


(Shim JH, et al. Cell Transplant 2015)

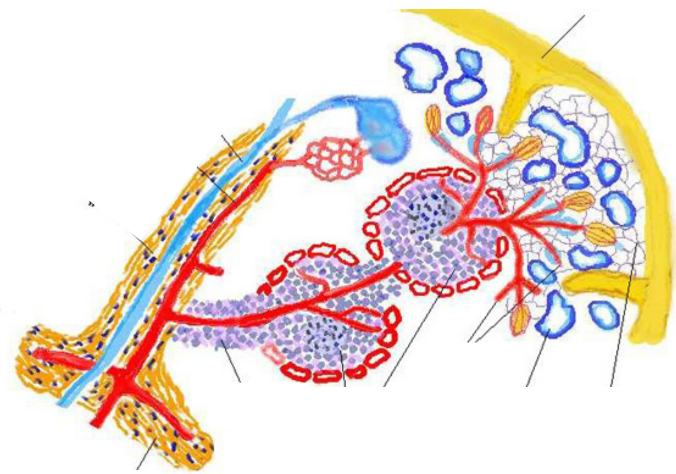
Anatomical Similarity of Sinus between Liver and Spleen



Liver sinusoid

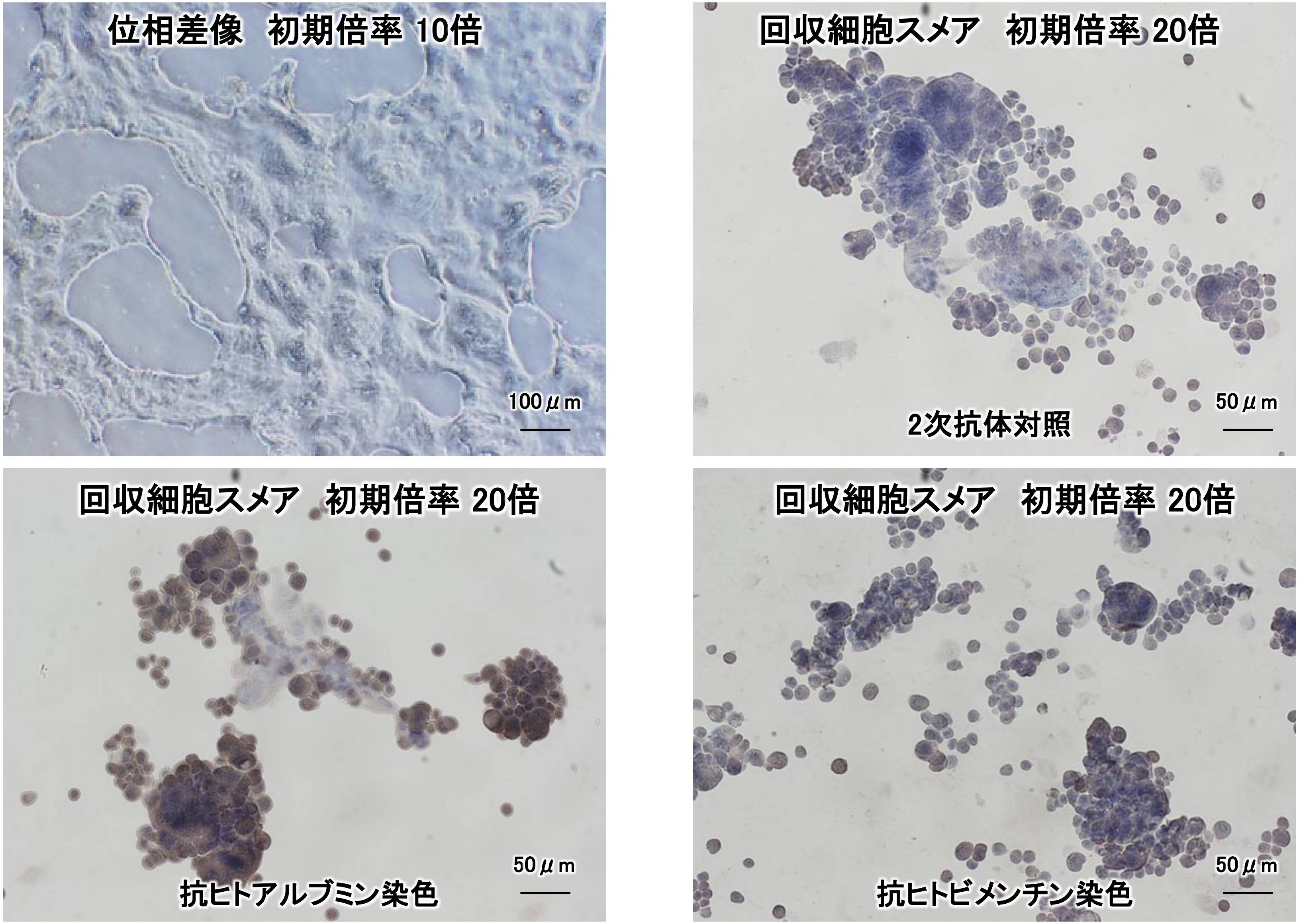


Sinus of the spleen



In Situでブタ脾臓内でヒト細胞を入れる技術

ヒトiPS細胞由来肝細胞様細胞（移植時）



（成育医療センターとの共同研究）

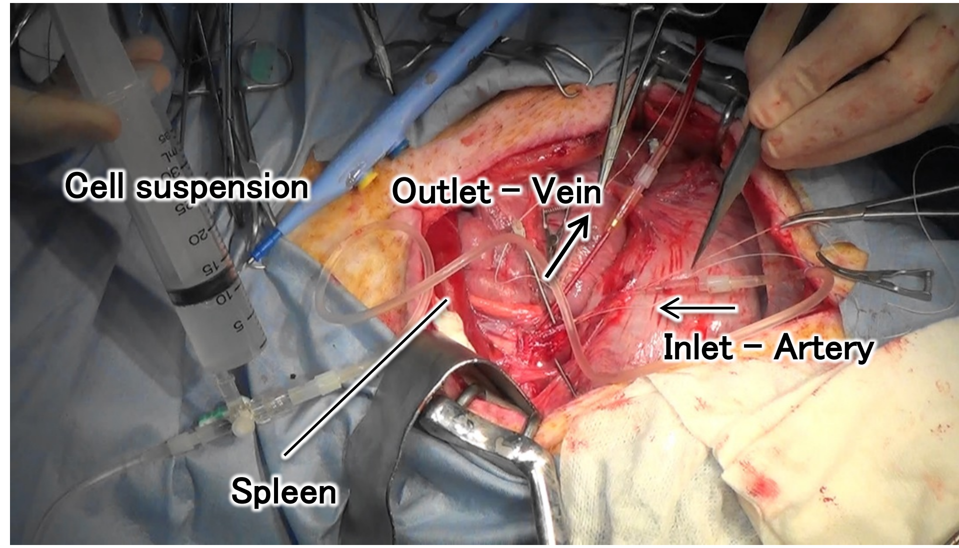
ヒトiPS細胞由来肝細胞様細胞移植

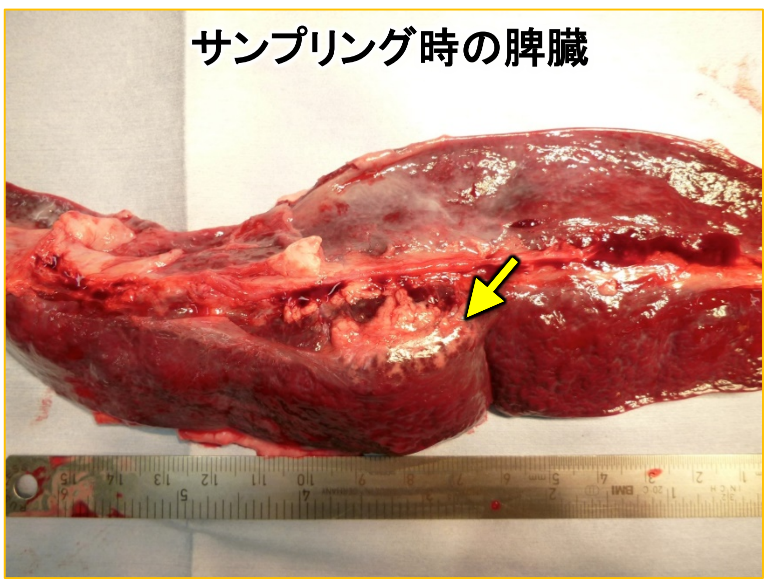
(Pretreatment)
Immunosuppression
Tacrolimus
MMF
Day −5 or −3

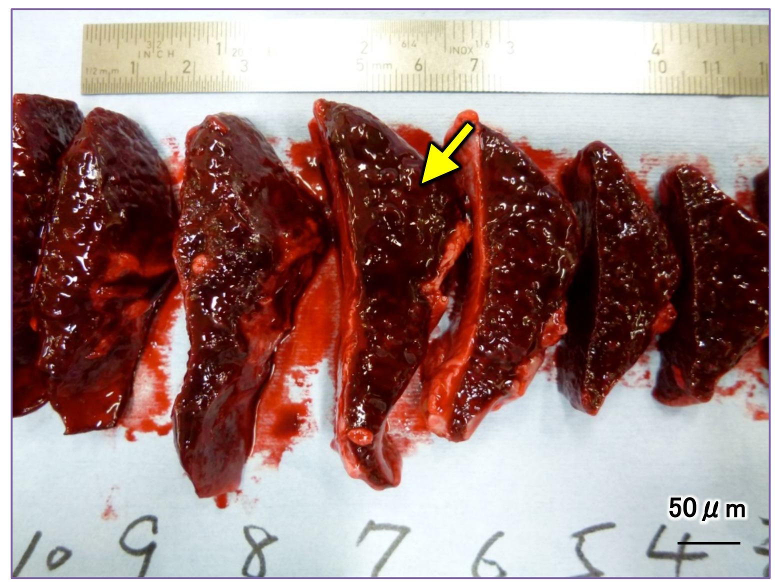
→ Cell Tx
Day 0
Pretreated with
Mitomicin C

Immunosuppression
Tacrolimus
MMF

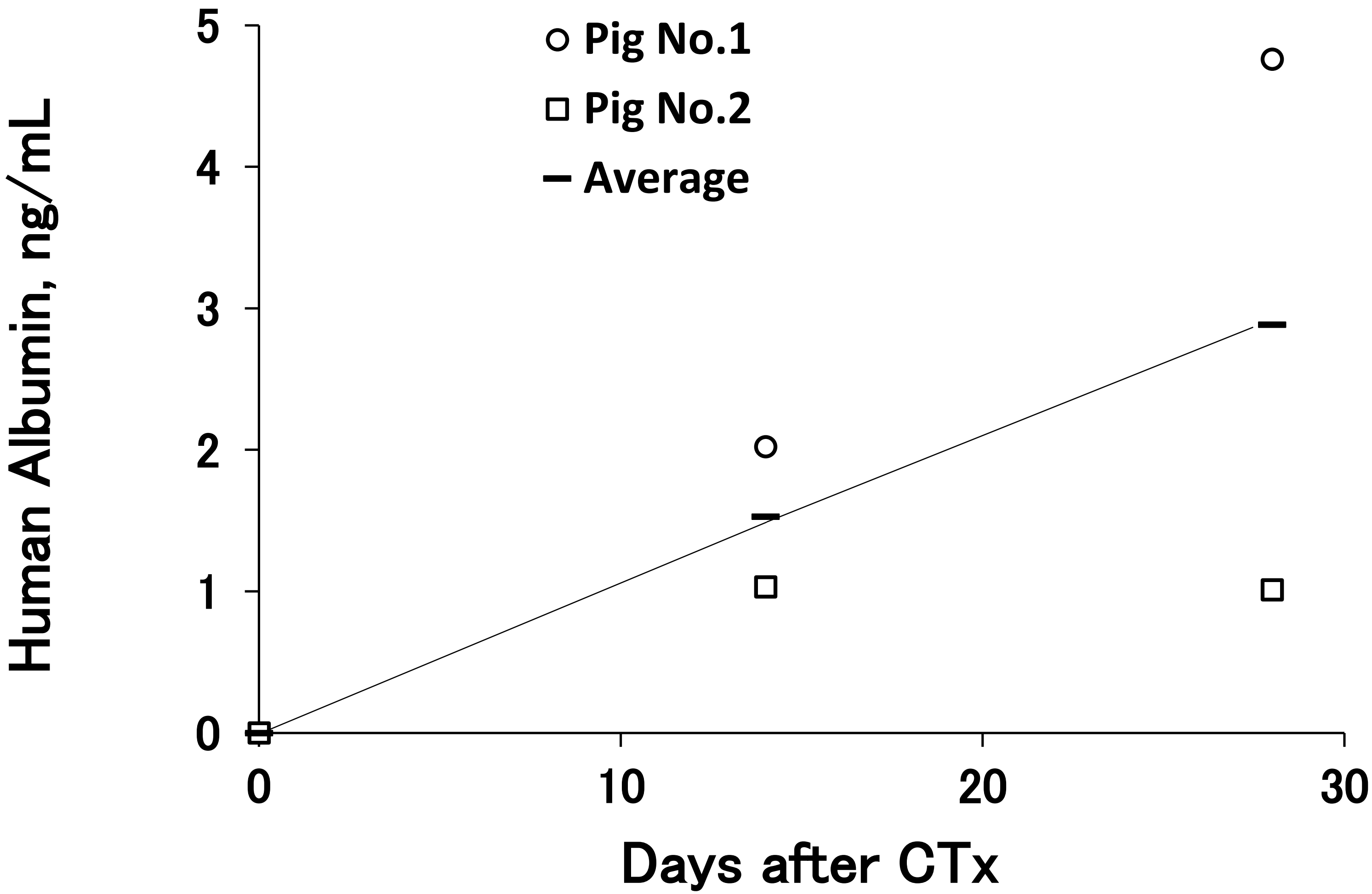
→ Sampling
Day28



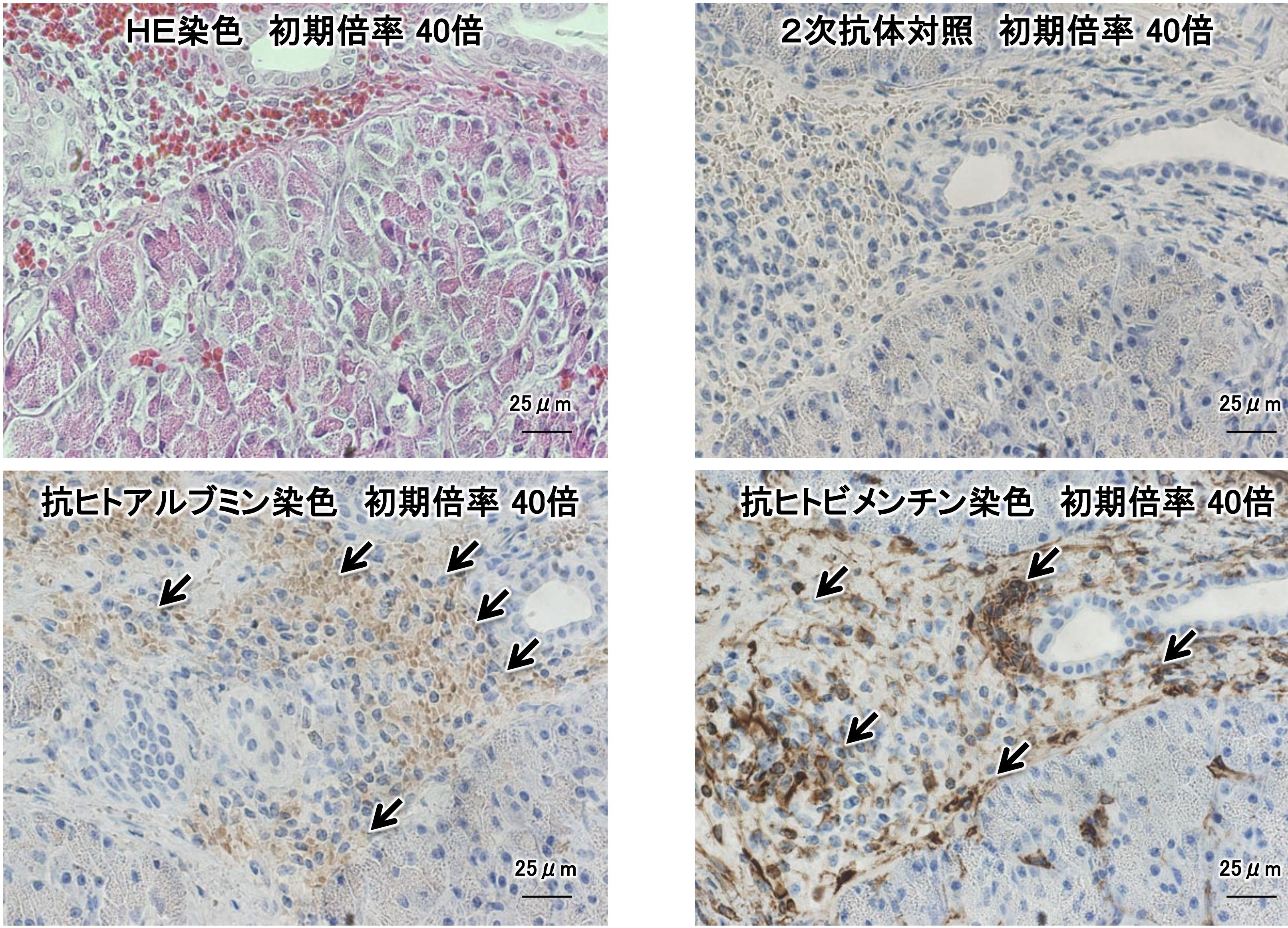




Human Albumin in the Pig Serum after Transplantation of iPS-Derived Hepatic Cells

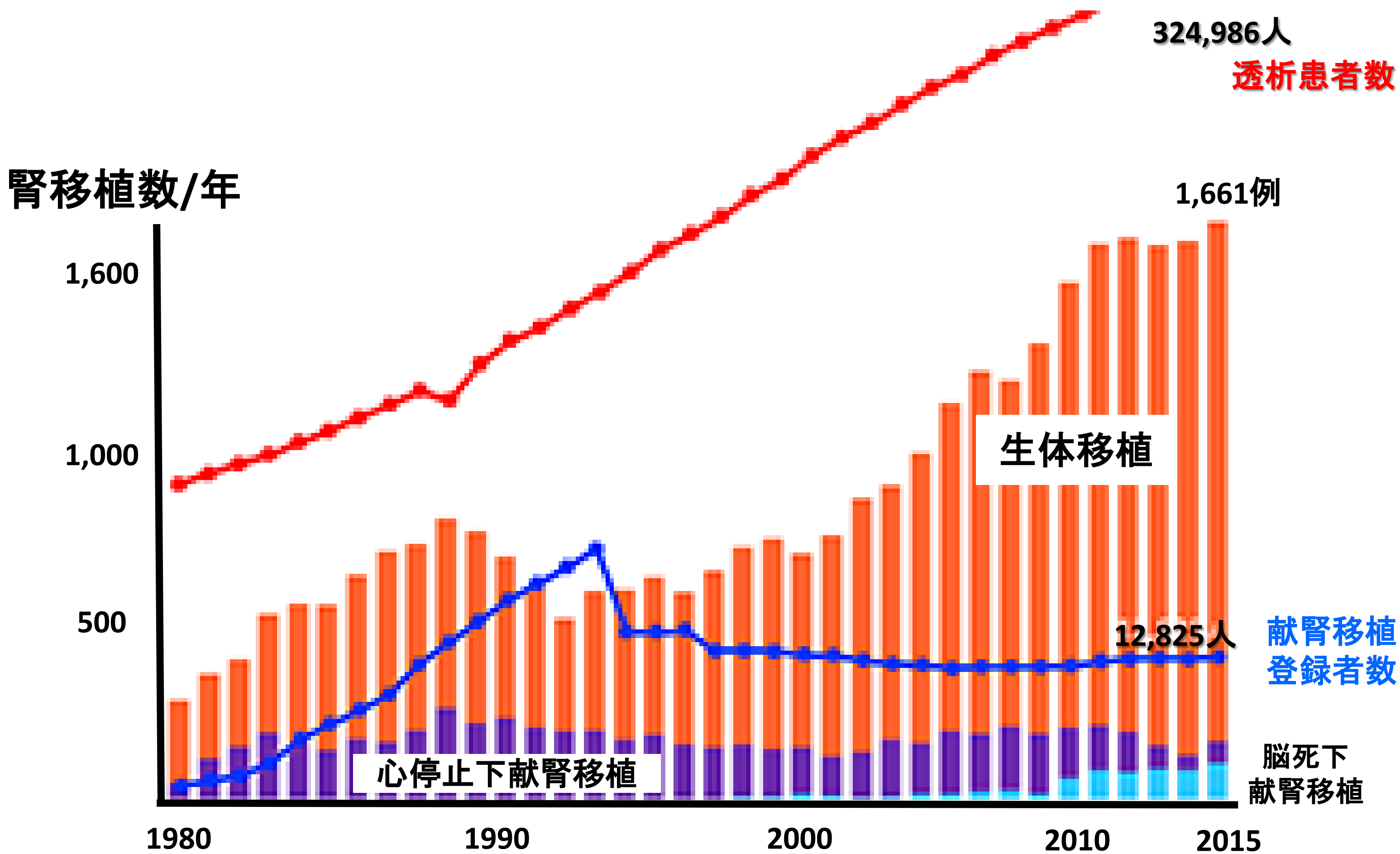


ヒトiPS細胞由来肝細胞様細胞移植後4週間の脾臓

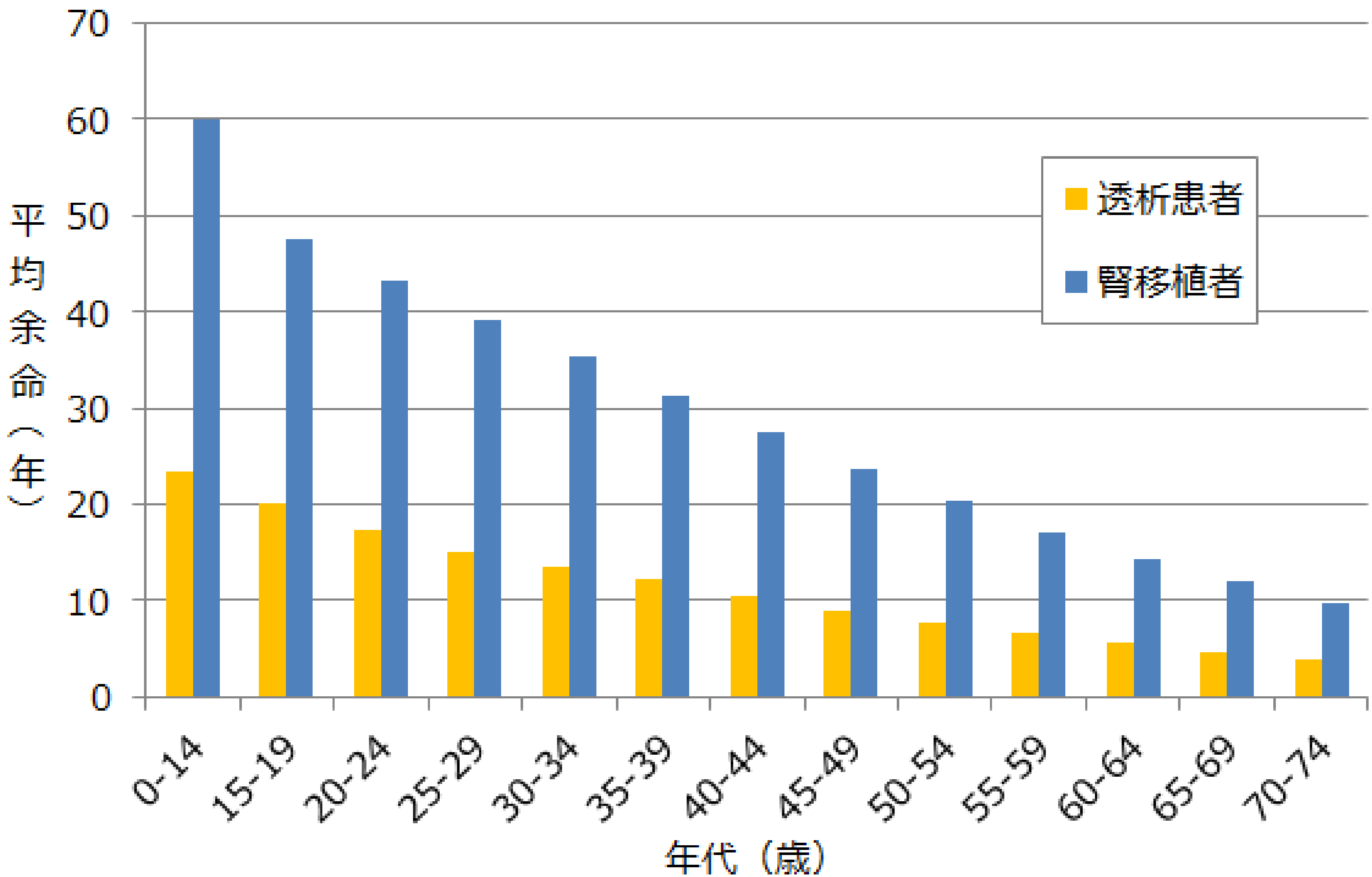


ファクトブック2016

Fact book 2016 of Organ Transplantation in Japan より

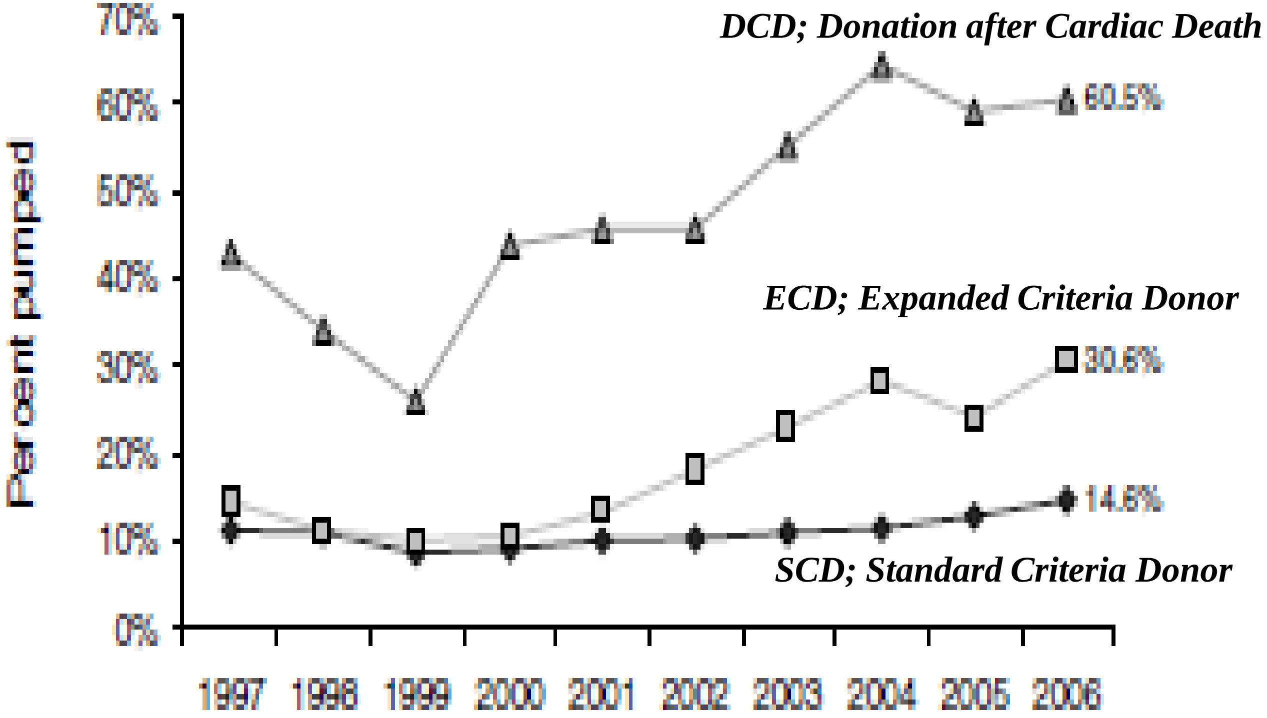


米国の透析患者・腎移植者の年代別平均余命



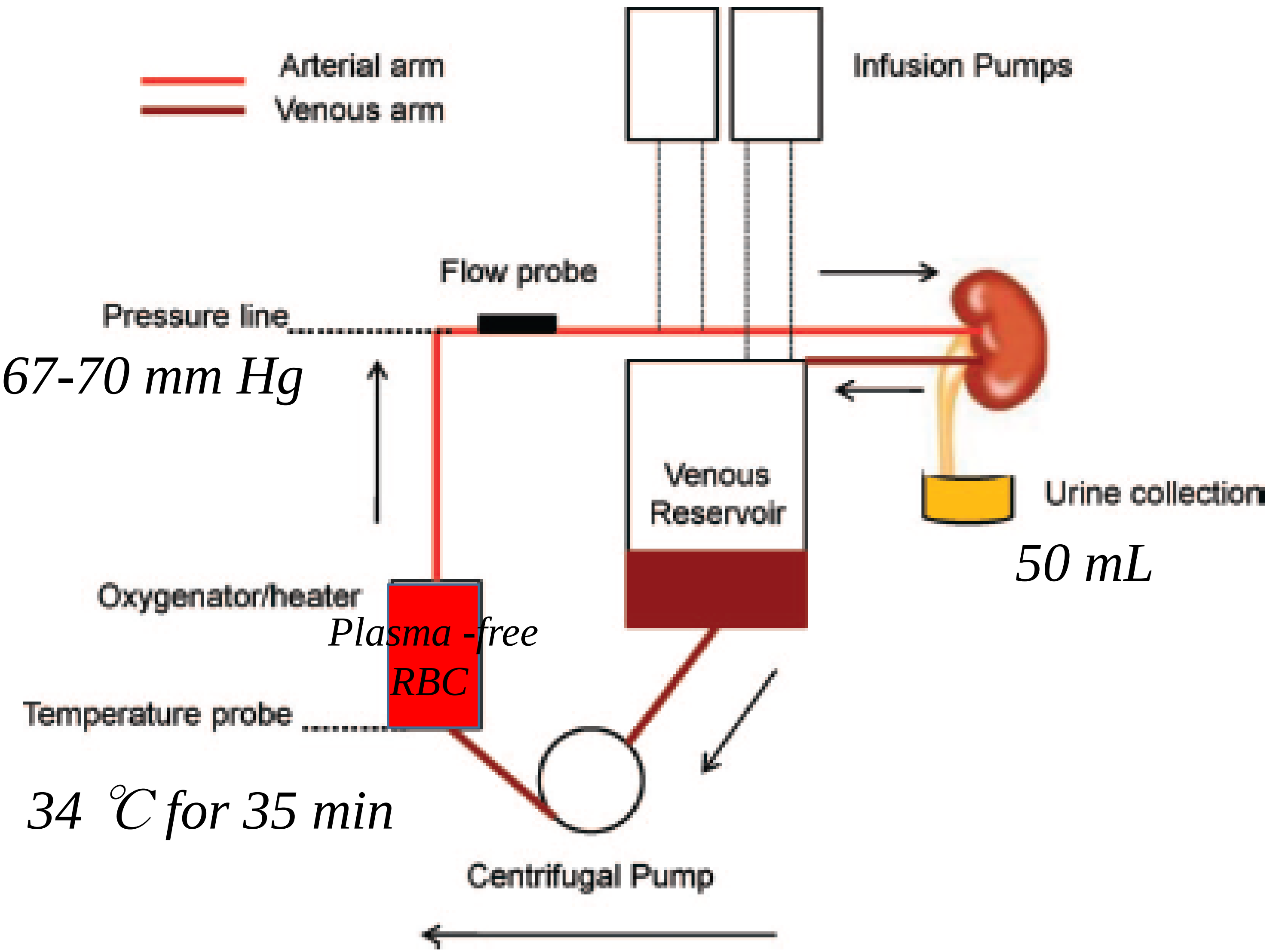
USRDS Annual Data Report 2015より作成

Organ Donation and Utilization in US (1997-2006)



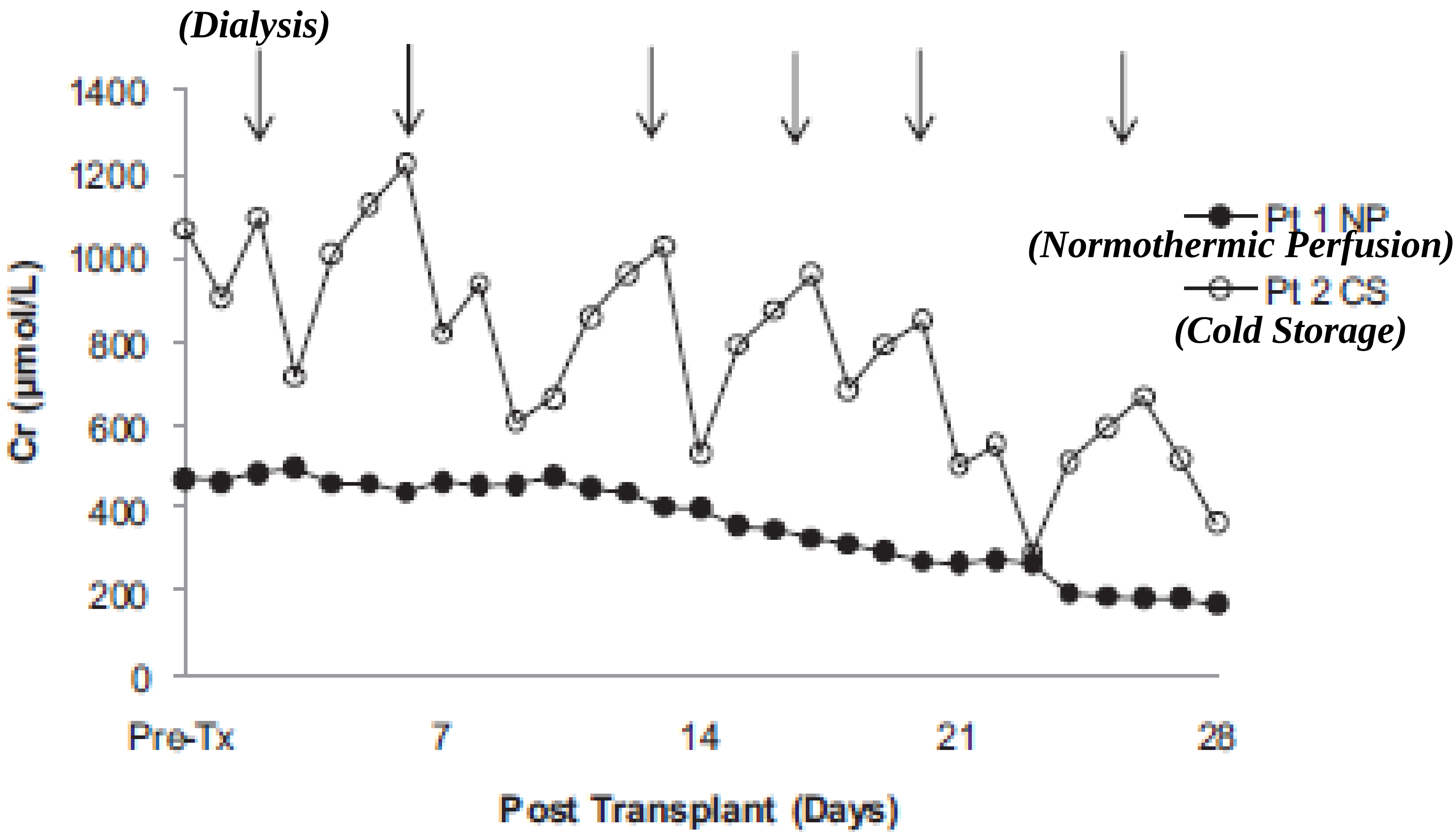
(R.S.Sung, et al. Am J Trasuplntation 8(2); 922, 2008)

Schematic diagram of ex vivo normothermic perfusion system



(Hosgood SA & Nicholson ML. Transplantation 2011)

First in Man Renal Transplantation
After Ex vivo Normothermic Perfusion



(Hosgood SA & Nicholson ML. Transplantation 2011)

A randomized trial of normothermic preservation in liver transplantation

a

NMP device and circuit.
OrganOx metra (generation 1)

Image of liver during
normothermic machine perfusion

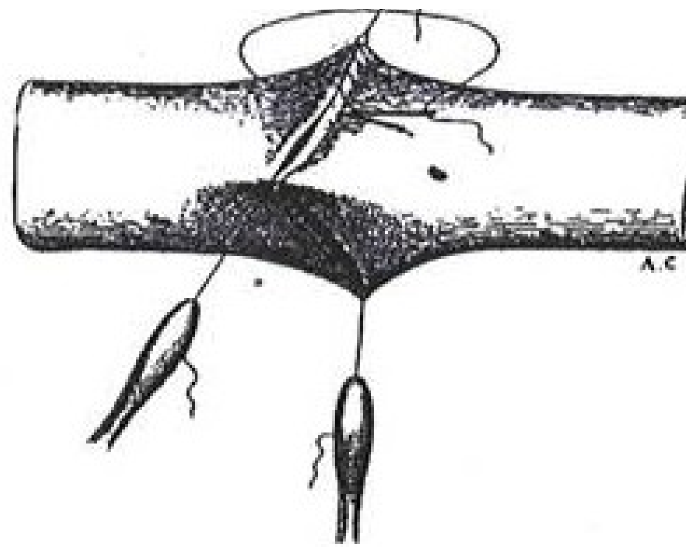
50 | NATURE | VOl 557 | 3 MAY 2018

Don Quixote Project for Organ Resurrection

(Kobayashi E with Screen Ltd. from 2015)

In 1912, **Dr. Alexis Carrel** was awarded **the Nobel Prize** in Physiology or Medicine for pioneering vascular suturing techniques

(1873-1944)



A New techniques in vascular sutures in 1902

The Culture of Whole Organs

Author(s): Alexis Carrel and Charles A. Lindbergh
Source: *Science*, New Series, Vol. 81, No. 2112 (Jun. 21, 1935), pp. 621-623
Published by: American Association for the Advancement of Science
Stable URL: http://www.jstor.org/stable/1660192
Accessed: 13-11-2017 02:50 UTC

Background

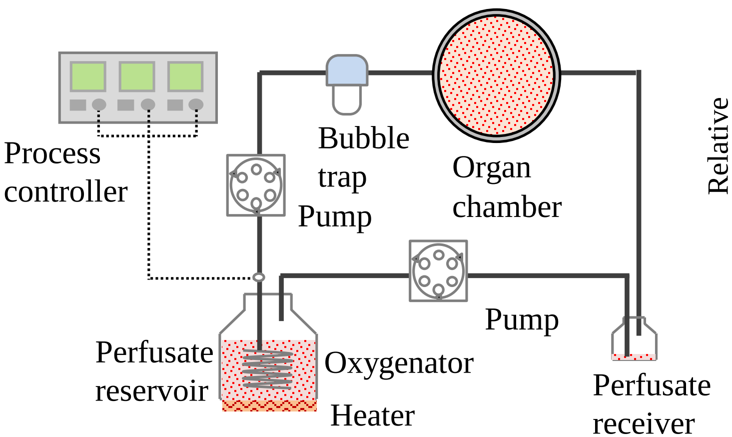
1812	
Le Gallois	Proposal for idea
1866	
de Cyron	Frog heart Beating for 48 hours Liver Urea production
Brown-Sequard	Brain circulation

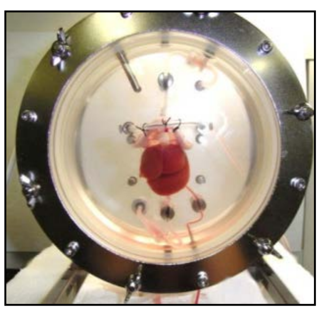
A cat's thyroid gland, varying in weight from 85 to 110 mgs, demands about 230 cc of nutrient fluid.

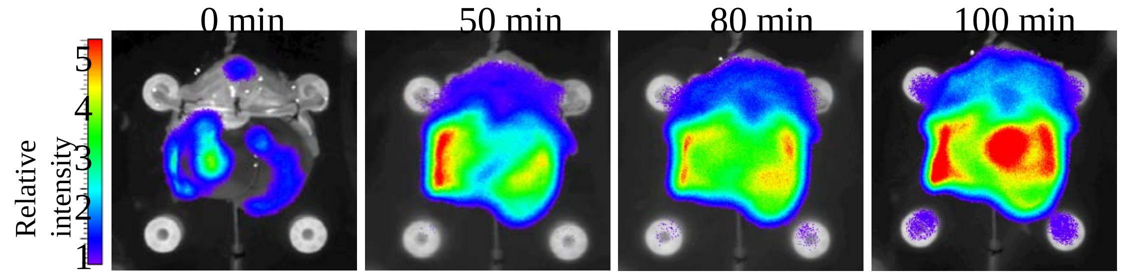
The solutions contain protein split-products, hemin, cysteine, insulin, thyroxine, glutathione, vitamin A, ascorbic acid, blood serum, et. The apparatus is kept in an incubator at a temperature of 37-38C.

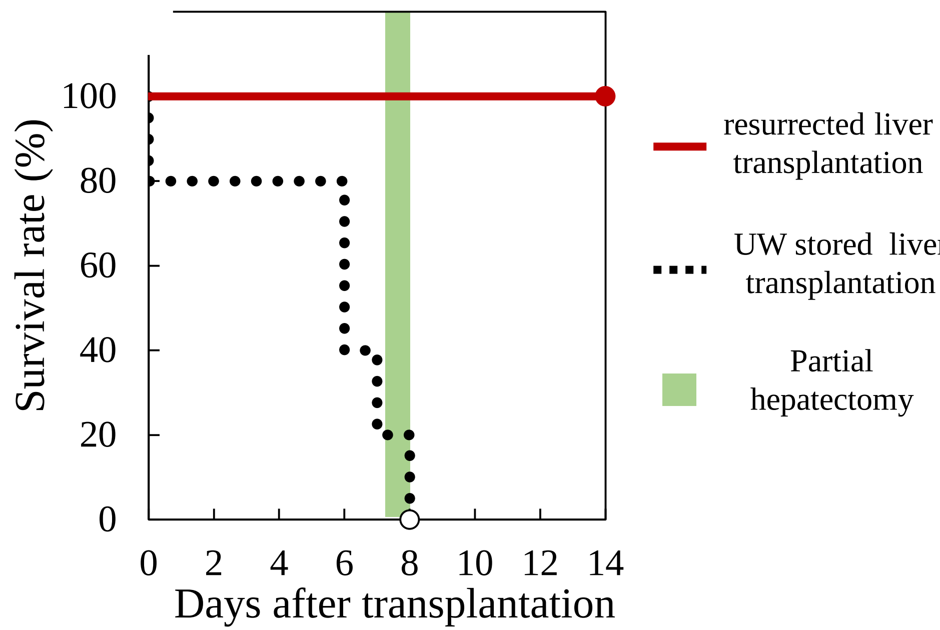
Thyroid glands were kept more than 20 days with pulsating arteries and active circulation.

Hypothermic temperature effects on organ survival and restoration





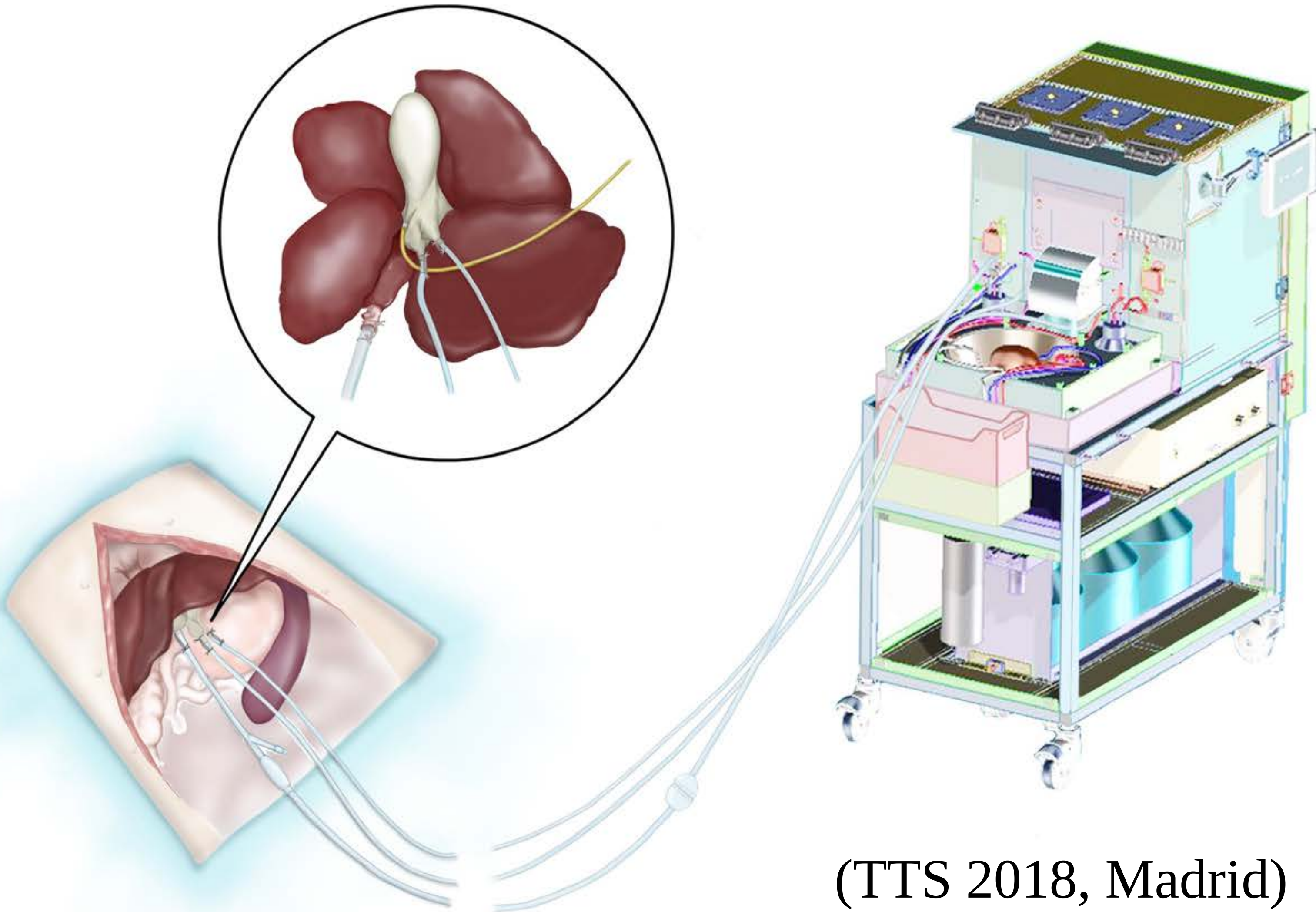




(Ishikawa J, et al. Scientific Reports 2015)

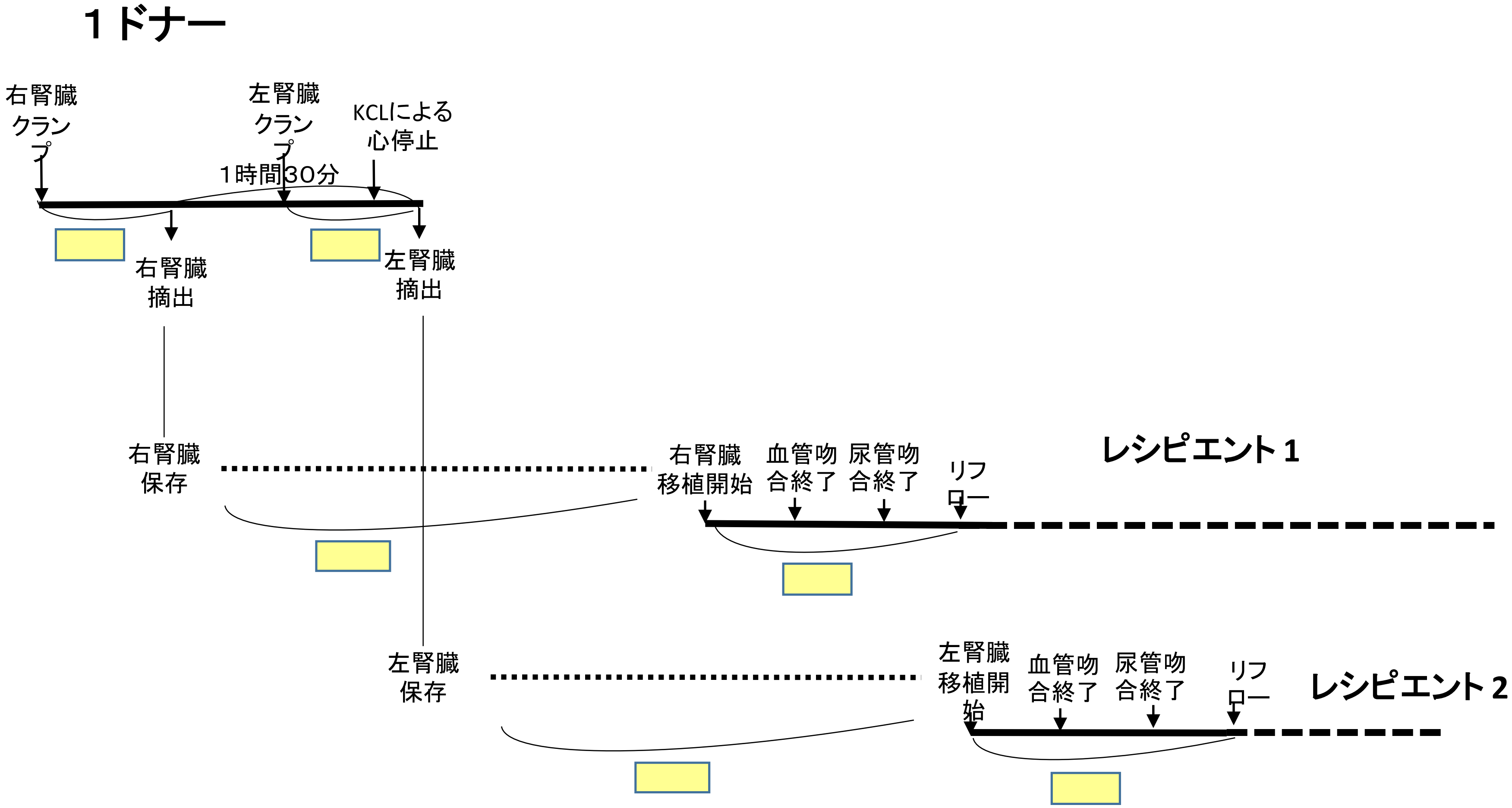
SCREEN

A Novel Machine Perfusion System Continuously from Ex-vivo to “In-situ” on Marginal liver transplantation



(TTS 2018, Madrid)

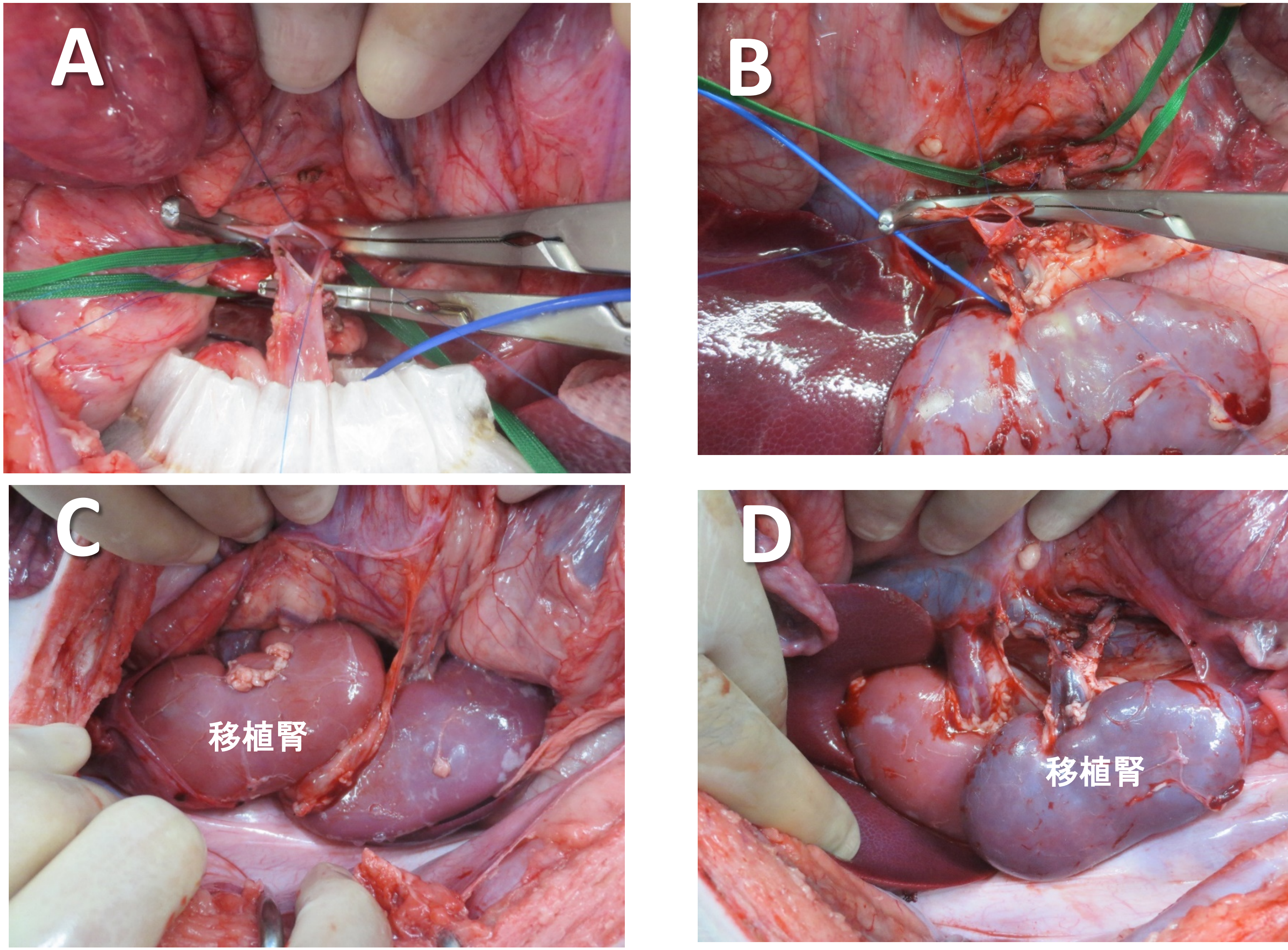
循環停止腎臓の‘蘇生還流法’開発のプロトコール



(Kobayashi E 2018)

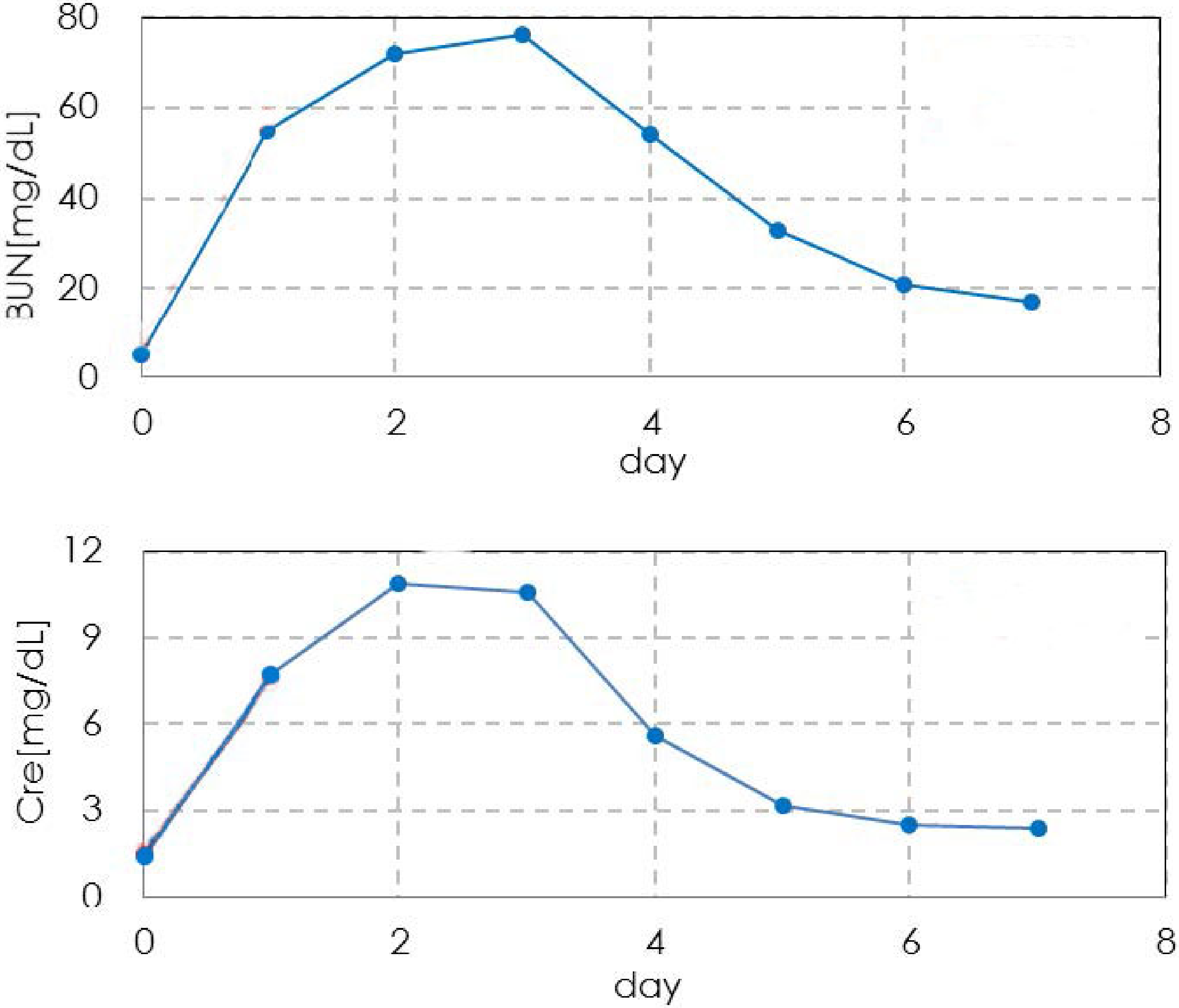
左腎移植

右腎移植

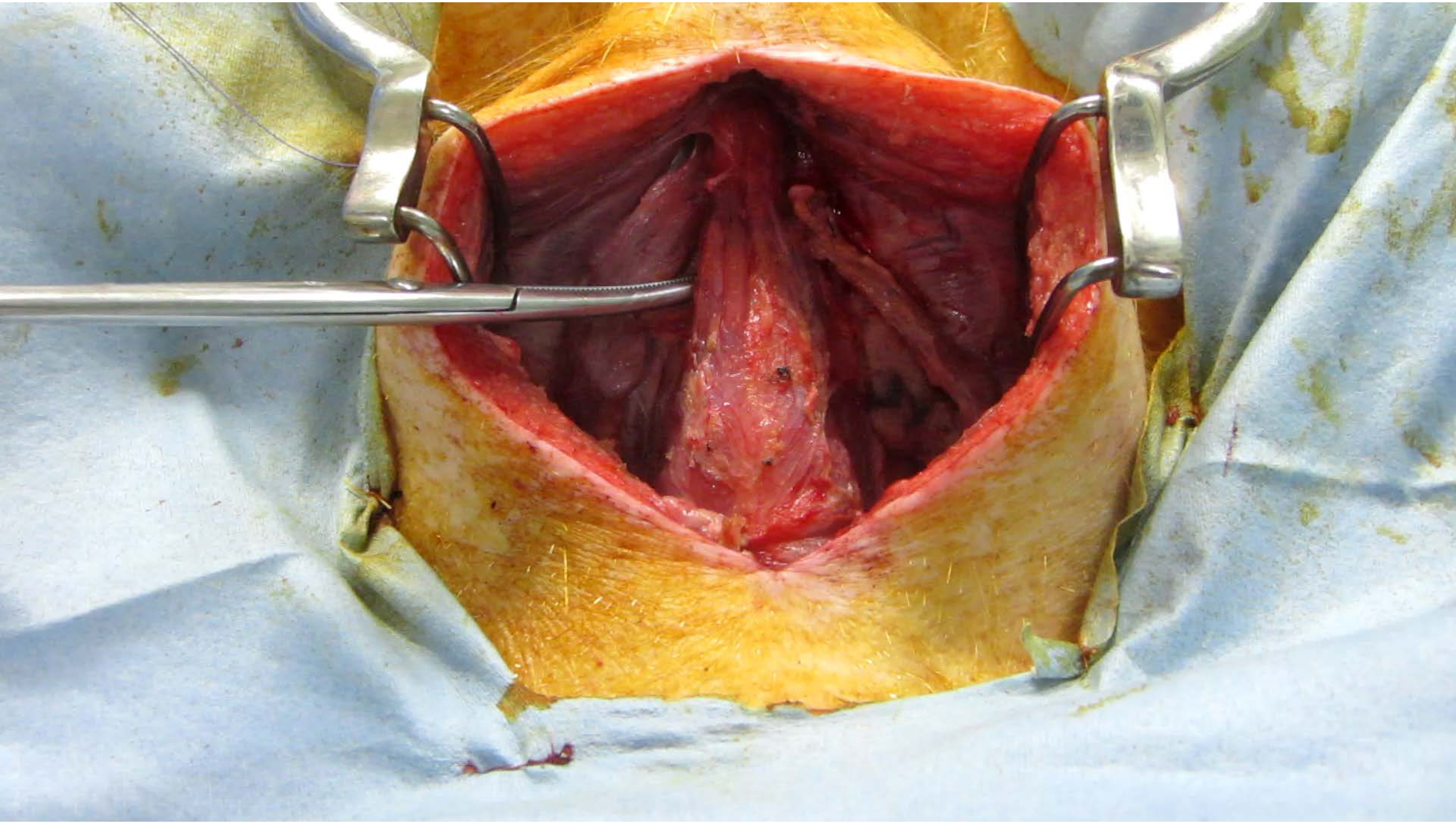


循環停止腎臓の機能回復パターン;ブタモデル

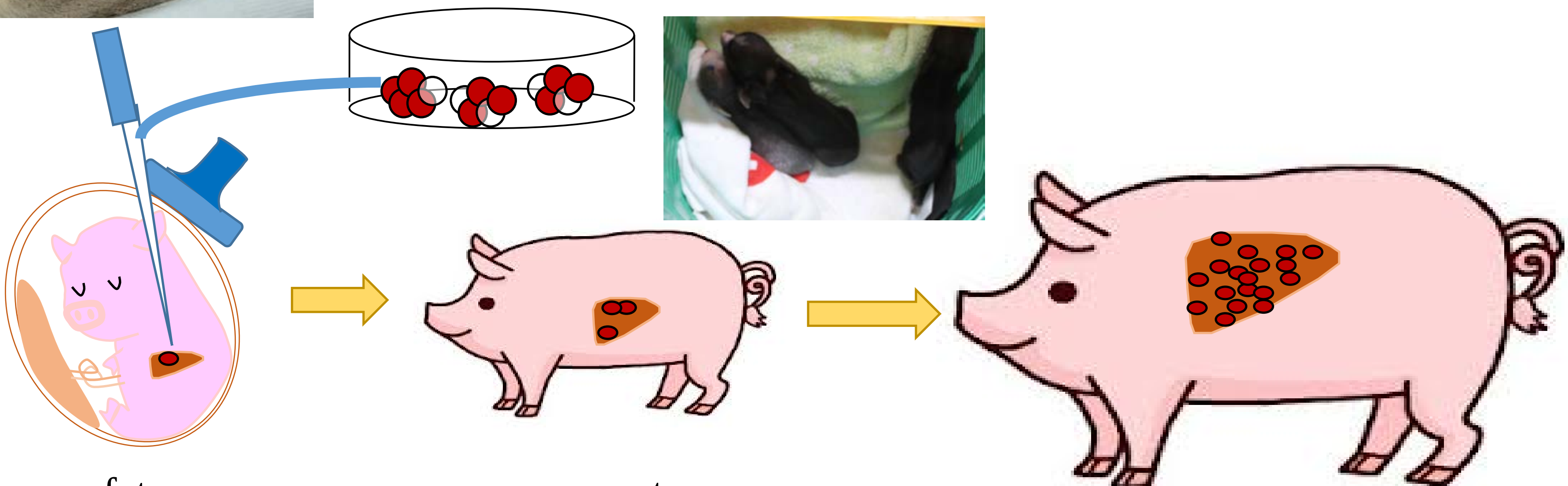
移植後の血液検査結果




Operational SCID Pigの誕生
ヒト由来の細胞・組織を数カ月
にわたって受け入れるブタ作出の技術
（Unpublished）



Actively Acquired Toleranceによるヒト細胞の増幅



（岐阜大学獣医学部との共同研究）

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.

'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 11·0 ± 0·3 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 intra-peritoneal 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).

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

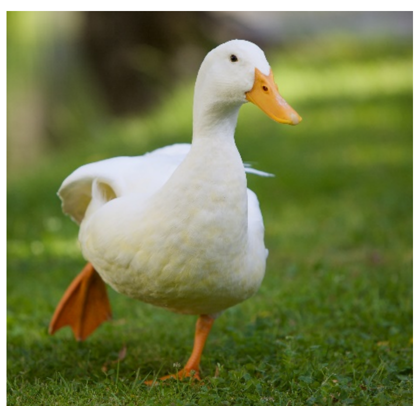
Parabionts	Controls
2*	32† 64, 32
4*	32, 64
8*	258
4, 4	128, 128
4	32, 8
64‡	64
	32, 64

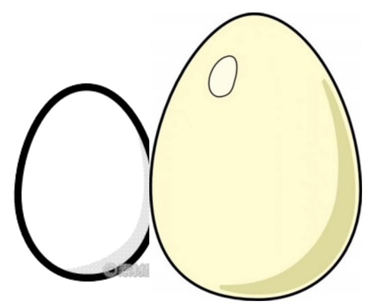
* Animal immunized by erythrocytes of embryonal parabiotic partner.
† Parabiont in which the exchange of blood was excluded by agglutination test.
‡ Exchange of blood not unequivocally demonstrated.
All parabionts or their partners were tested by agglutination immediately after hatching for the exchange 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.





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 × 10[8] nucleated cells/kg of body weight) or from tibial bone marrow (13·5 × 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

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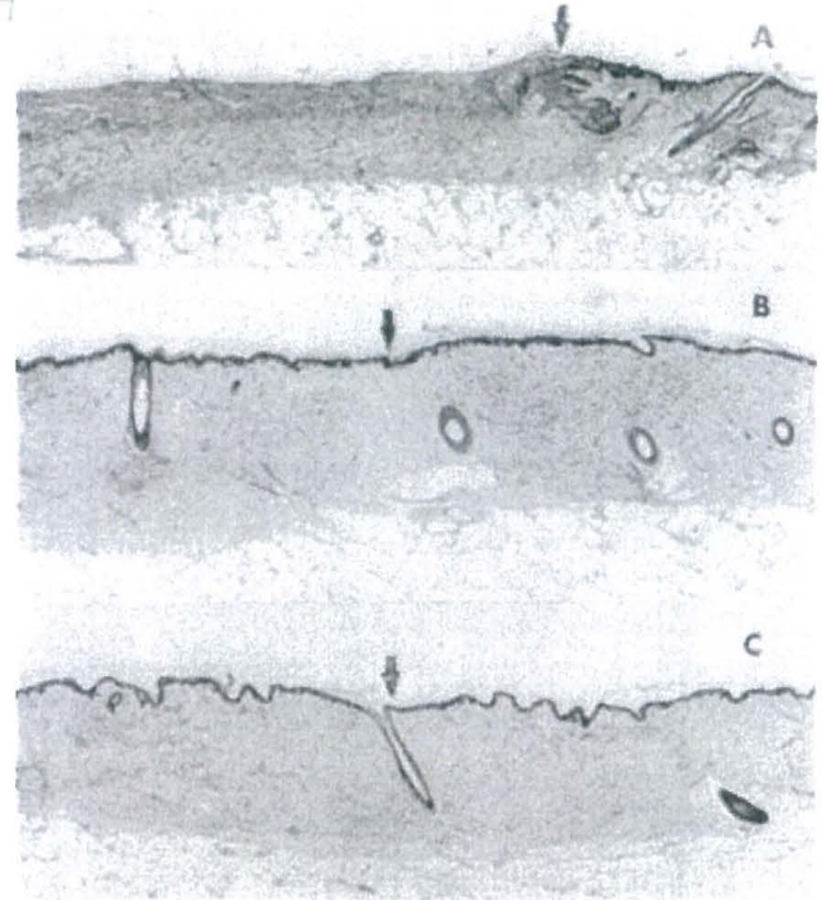

Fig. 1. Photomicrographs of biopsies from homografts on piglets treated at 60 days gestation with: A, control conventional antigens; B and C, bone marrow cell suspension from the skin graft donor (× 7·5) (arrow denotes junction of graft on left and normal skin on right). A, Conventional antigen treated 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.

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慈恵医科大学
高血圧内科　　横尾隆

明治大学　農学部
発生工学研究室　　松成ひとみ、長嶋比呂志

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自治医科大学
先端医療技術開発センター　　花園　豊

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胆管膵・移植外科　八木真太郎、上本伸二

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先端生命医科学研究所　　関根秀一、清水達也

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先端医療開発室　　許懐哲、絵野沢伸

理化学研究所
多細胞システム形成研究センター
石川潤、辻孝

岐阜大学
獣医　　森教授、高須准教授