長崎大学大学院セミナー　追加　　講演

(2018年3月27日、長崎)

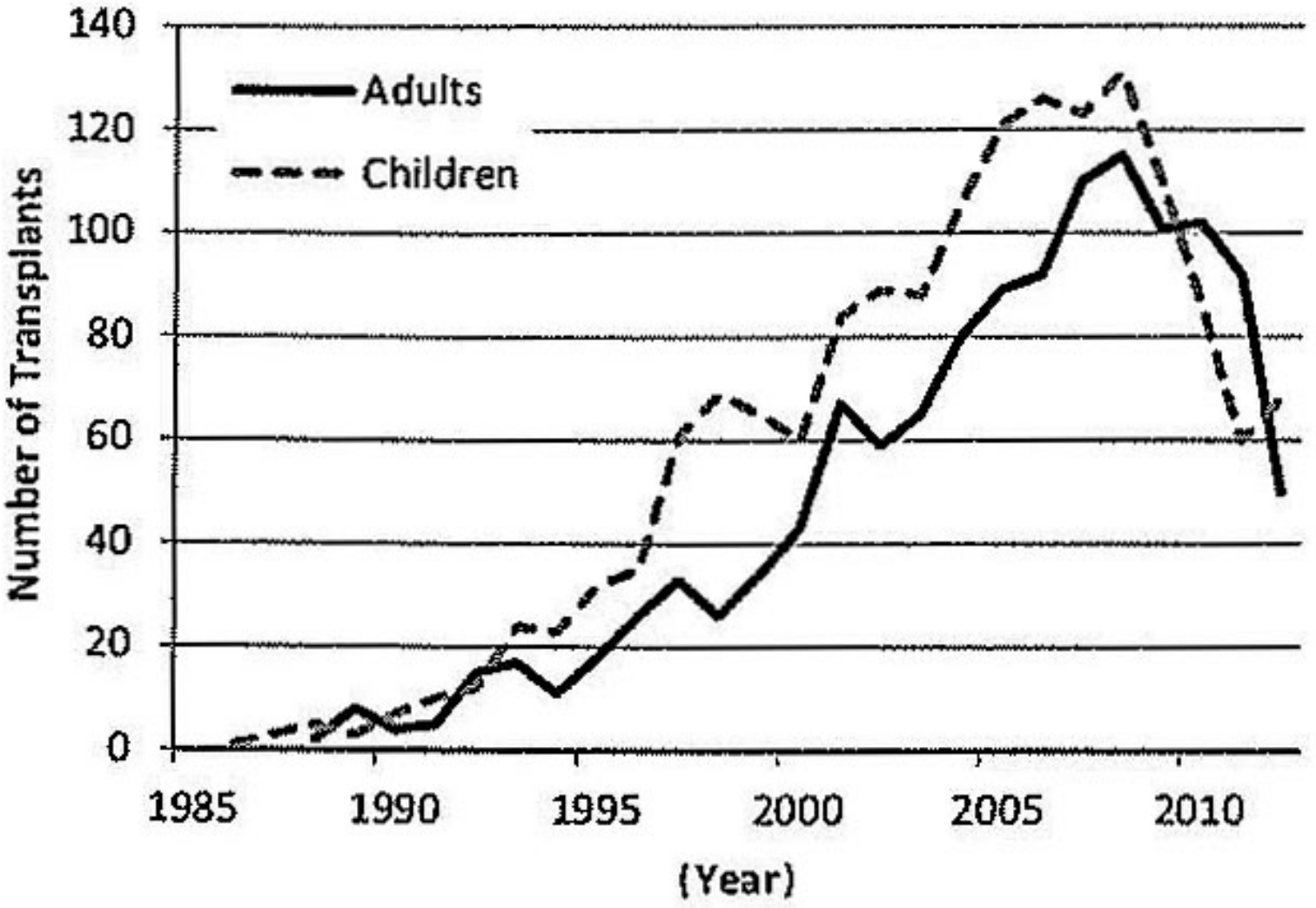
科学は技術と伴にある

―ラット小腸移植モデルの展開―

Eiji Kobayashi, MD,PhD

Keio University, School of Medicine

世界の臨床小腸移植の年次推移



Numbers of intestinal/multivisceral transplantation
(OPTN/SRTR data)

Transpl Immunol 2016 May;36:32-41

Difficulties, guidelines and review of developing an acute rejection model after rat intestinal transplantation.

Andres AM, Santamaria M, Hernandez-Oliveros F, Guerra L, Lopez S, Stringa P, Vallejo MT, Largo C, Encinas JL, Garcia de Las Heras MS, Lopez-Santamaria M, Tovar JA

Pediatric Surgery Department, La Paz University Hospital, Paseo La Castellana, 261, 28046 Madrid, Spain

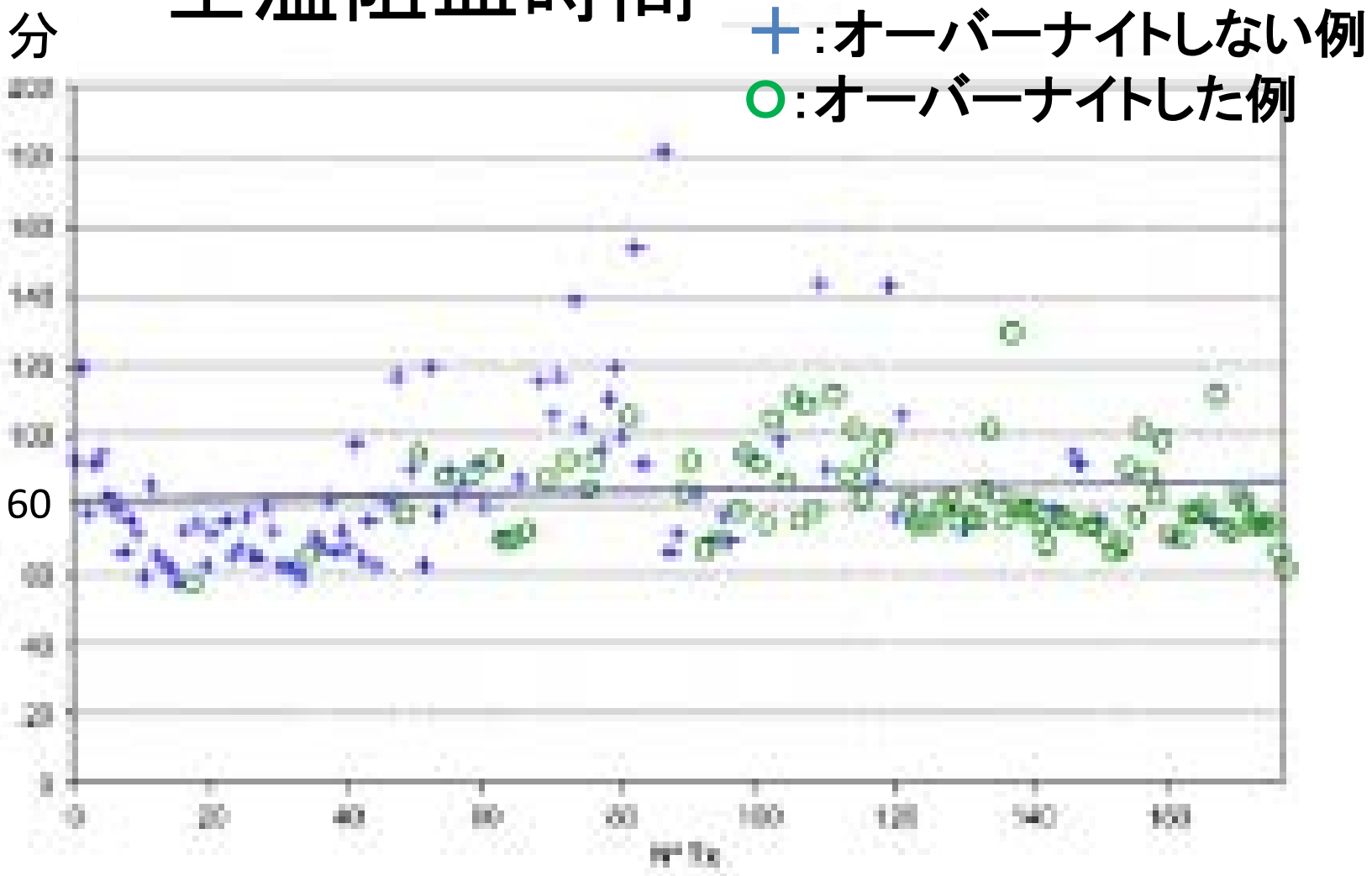
Table　Average surgicaltimes.

	Learning curve (n = 160)	Rejection study group* (n = 50)
Donor surgery	38 ± 8 min	32 ± 6 min
Venous anastomosis	18 ± 6 min	15 ± 6 min
Arterial anastomosis	16 ± 4 min	14 ± 3 min
Warm ischemia	38 ± 9 min	35 ± 7 min
Recipient surgery	98 ± 20 min	86 ± 15 min
Total transplantation	152 ± 23 min	138 ± 20 min

＊異所性に植えて術後６日以上生きたもの

（Andres MA, et al. Transplant Immunol 2016）

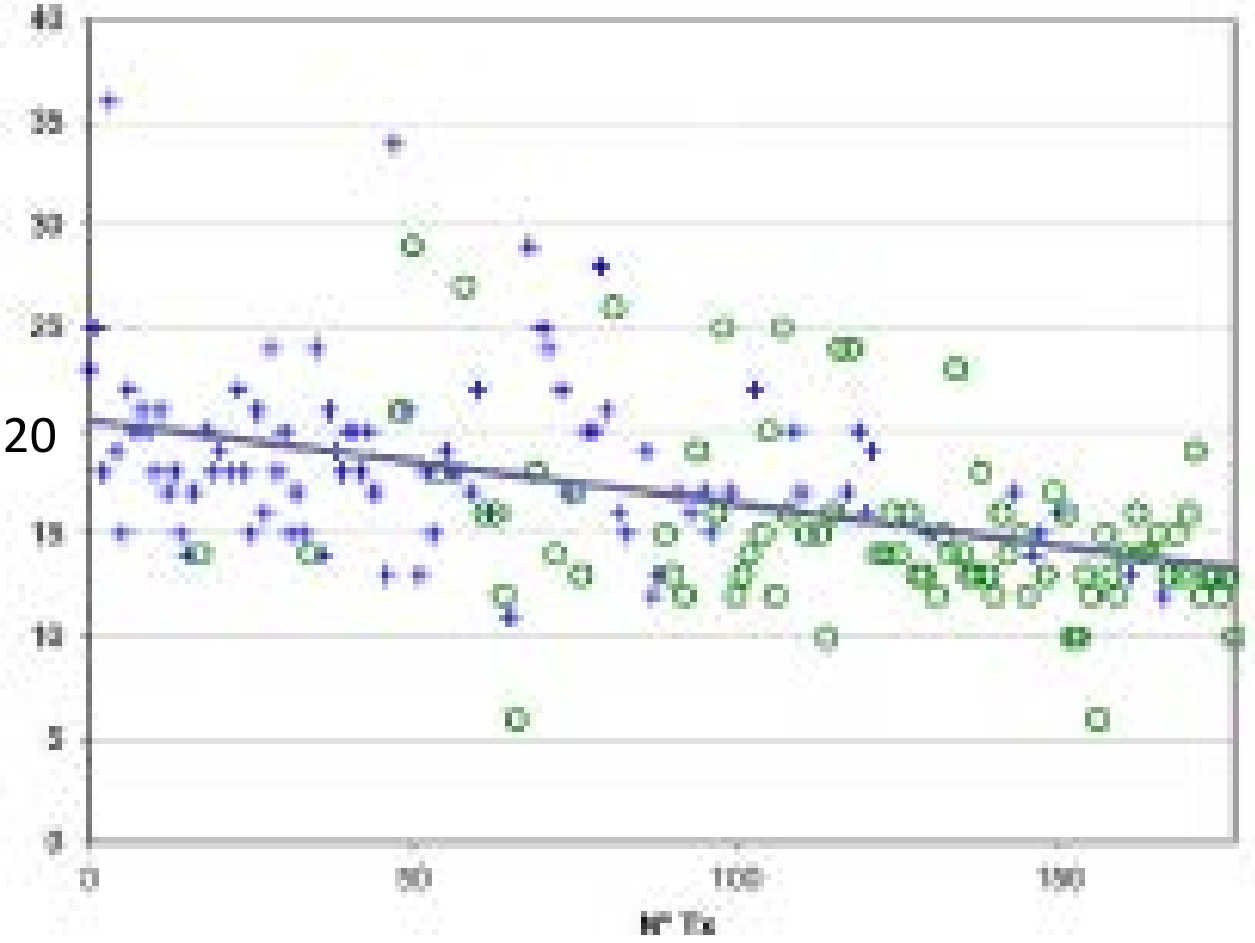
全温阻血時間



（Andres MA, et al. Transplant Immunol 2016）

動脈吻合時間

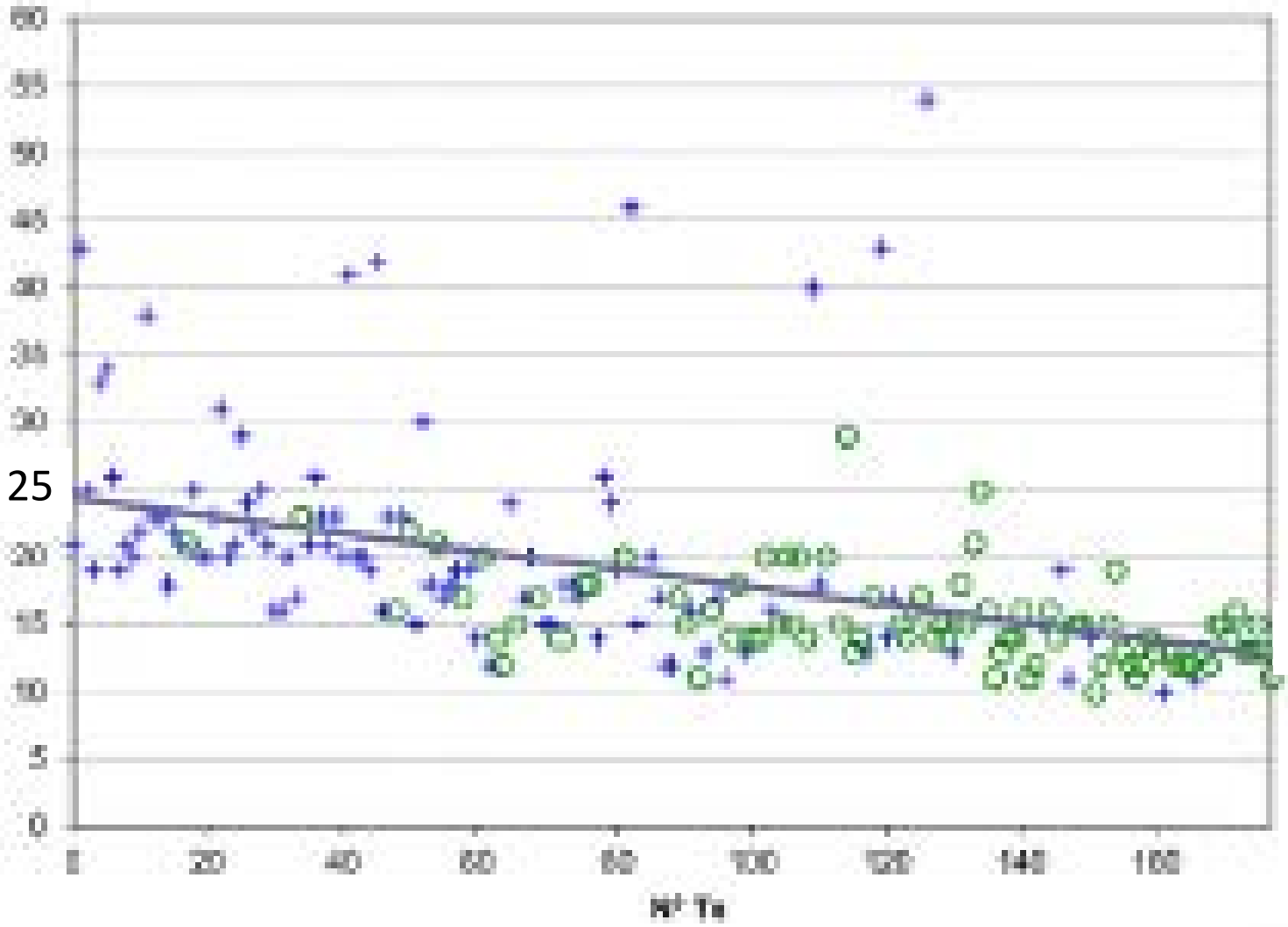
分



（Andres MA, et al. Transplant Immunol 2016）

静脈吻合時間

分



（Andres MA, et al. Transplant Immunol 2016）

Microsurgery 2007;27(4):277-81.

Teaching intestinal transplantation in the rat for medical student.

Galvão FH, Bacchella T, Cerqueira ,Machado M
Experimental Microvascular Laboratory of Transplant and Liver Surgery Discipline, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

Abstract

Technical difficulties hamper the widespread use of intestinal transplantation in rats. We evaluated the feasibility in training this microsurgical model for medical students. Thirty eight students were assessed. After information about intestinal transplantation in rats, they spontaneously agreed to be trained for this procedure. The course consisted of 4-h weekly lessons during 4-month period. The teaching process includes assessment in four phases:

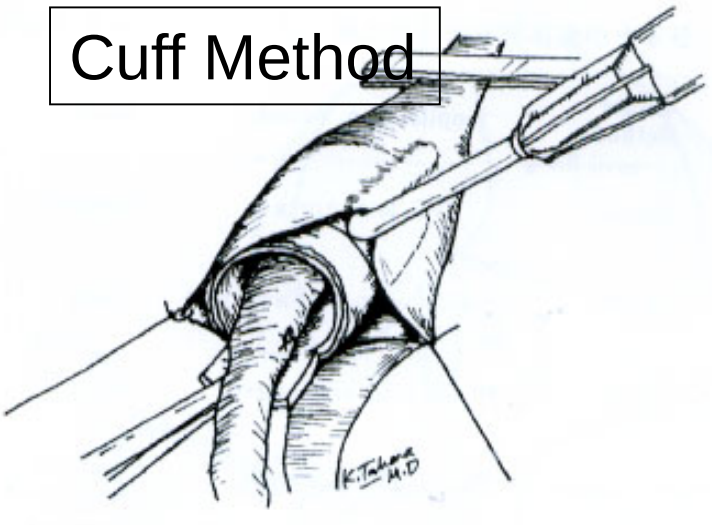
I) conception of intestinal transplantation and rat anatomy;

II) basic microsurgery training; III) donor operation; IV) donor/recipient operation. Wistar rats were used as donors and recipients in one-step small bowel transplantation. All students (100%) reached phase II, seven students (18.42%) reached phase III and two students (5.26%) reached phase IV.

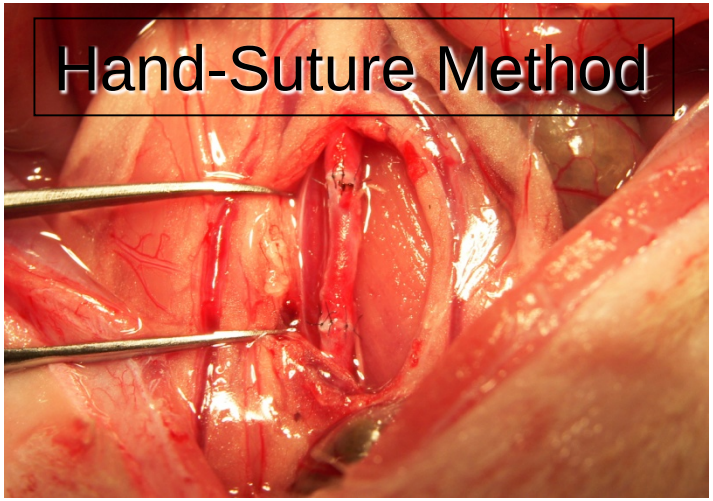
Decreased interest about the theme, lack of time and patience, frustration and/or inability were all reasons given by the student that may have contributed to the low rate of success. Medical students achieved a low rate of completion for training in rat intestinal transplantation microsurgical procedures

Microsurgery Room in Kobayashi's Labo
（2000－2009）

Cuff Method

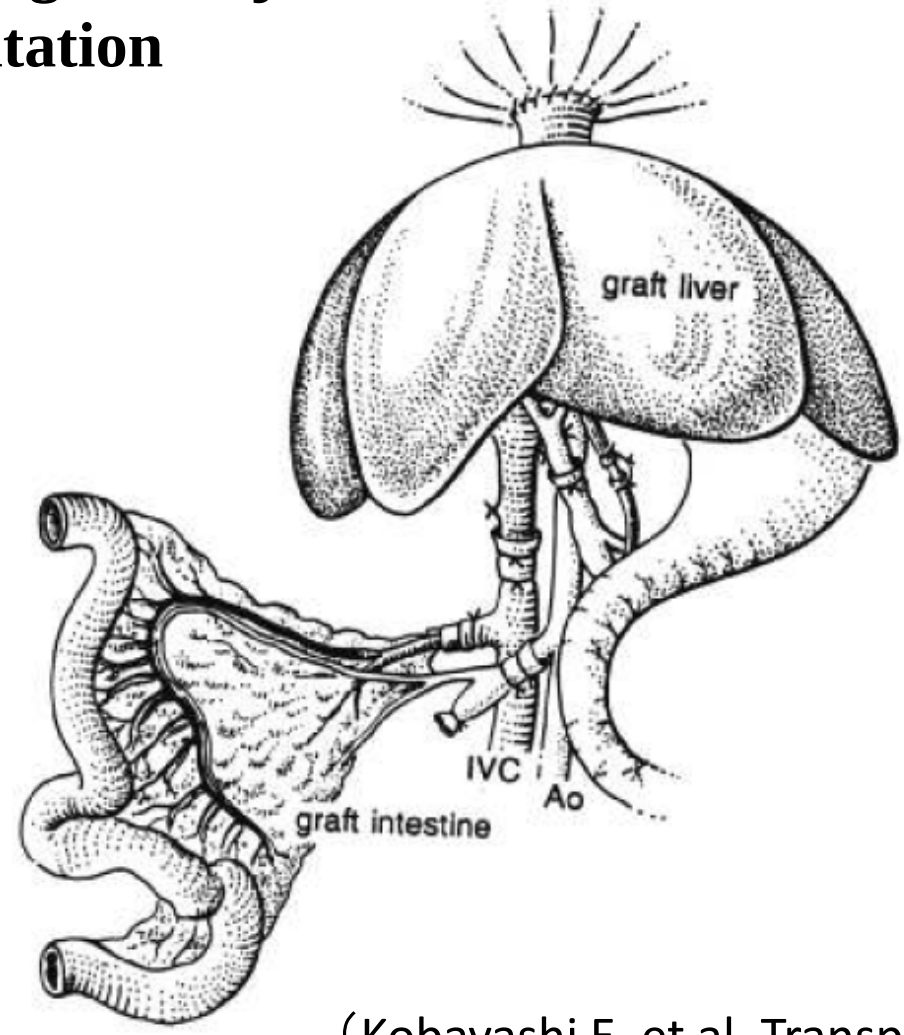


Hand-Suture Method



研究テーマは常に臨床から（1）

Prevention by Liver transplantation of the Graft-Versus-Host Reaction and Allograft Rejection in a Rat Model of Small Bowel Transplantation



（Kobayashi E, et al. Transplantation 1994）

Impact of graft length on surgical damage after intestinal transplantation in rats

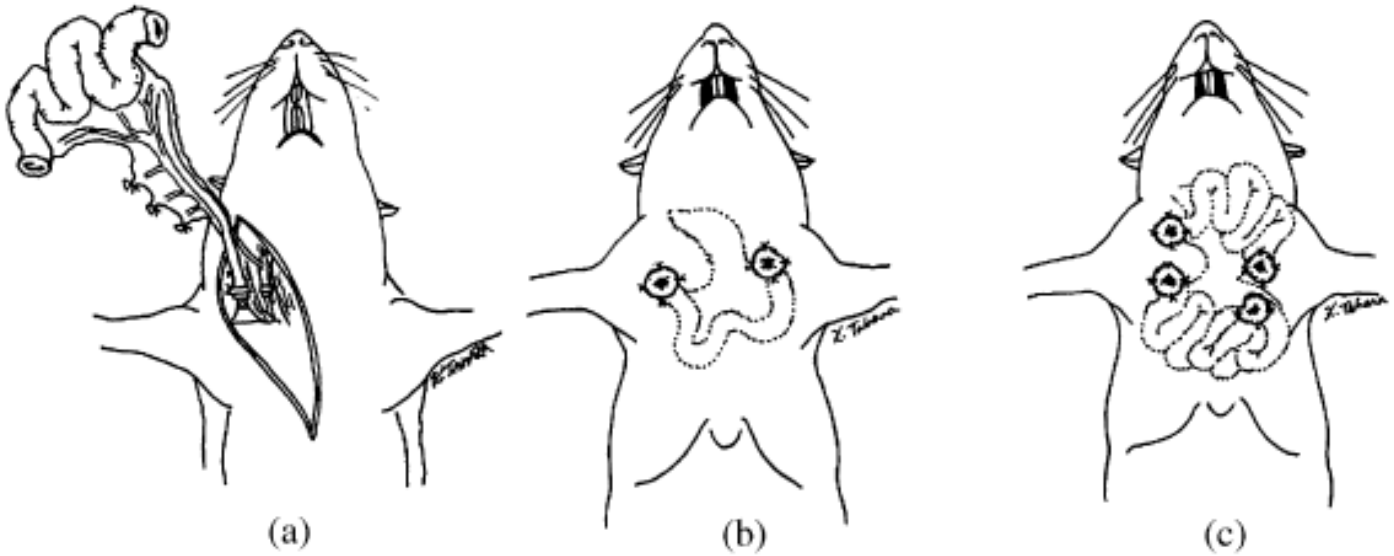


Table 1
Survival of cervical SBT rat

Group	Cuff size		Graft length	*N*	Survival (days)	Lethality (%)
	Artery	Vein				
A	20 G	16 G	Segment (15 cm)	10	>100, >100, >100, >100, >100, >100, >100, >100, >80, >60	0
B	18 G	14 G	Whole (700 cm)	11	7[a], 8[a], 9[a], 11[a], 16[a], >100, >100, >100, >100, >50, >50	45.6

[a] The rats showed severe weight loss with intact graft.

（Inoue S, et al. Transplant Immunology 2003）

研究テーマは常に臨床から（2）

Immunologic Benefits of Longer Graft in Rat Allogenic Small Bowel Transplantation

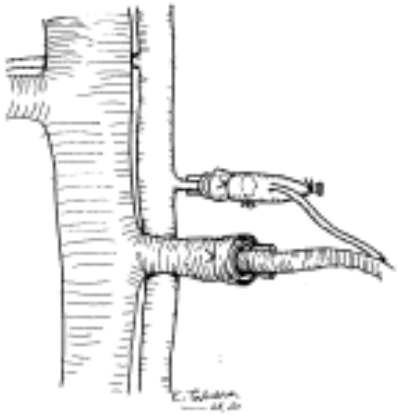
TABLE 1. Effect of graft length on recipient survival after orthotopic small-bowel transplantation (SBT) in two different models

Group	Model	Donor	Recipient	Graft length	n	Recipient survival (days)	Mean ± SD (days)	*P* value
A[c]	M&R[a]	BN	LEW	Whole (70 cm)	5	16,[d] 63, 71, 72, >80	60.4 ± 25.5	$P = 0.001$
B[c]	M&R[a]	BN	LEW	Segment (15 cm)	7	7,[d] 7,[d] 8,[d] 8,[d] 9,[d] 14,[d] 14[d]	9.6 ± 3.1	
C	M&R[a]	LEW	LEW	Segment (15 cm)	4	>80, >80, >80, >80	>80	
D[c]	Combined[b]	BN	LEW	Whole (70 cm)	8	44, 45, 47, 51, 55, 62, 63, 67	54.3 ± 8.9	$P = 0.10$
E[c]	Combined[b]	BN	LEW	Segment (15 cm)	7	29, 35, 43, 49, 52, 54, 56	45.4 ± 10.2	
F	Combined[b]	LEW	LEW	Segment (15 cm)	6	>100, >100, >100, >100, >100, >100	>100	

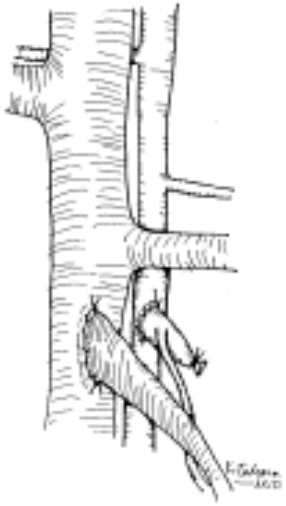
[a] Orthotopic SBT were performed using classical microsurgical technique reported by Monchik and Russell (M&R).
[b] Cuff technique was used for venous reconstruction.
[c] Tacrolimus (0.64 mg/kg/day) was administered intramuscularly from 0 to 13 postoperative days.
[d] Recipients died with intact graft.
BN, Brown Norway; LEW, Lewis.

（Fujishiro J, Tahara K, Inoue S, et al. Transplantation 2005）

Experimental Models of Rat Small Intestinal Transplantation: Cuff Method and Suture Method

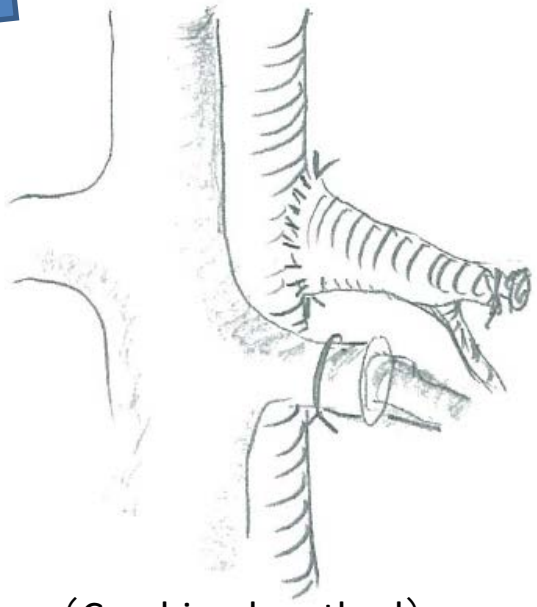


Cuff method



Suture method

（Nakao A, et al. Transplant Proc 2003）



（Combined method）

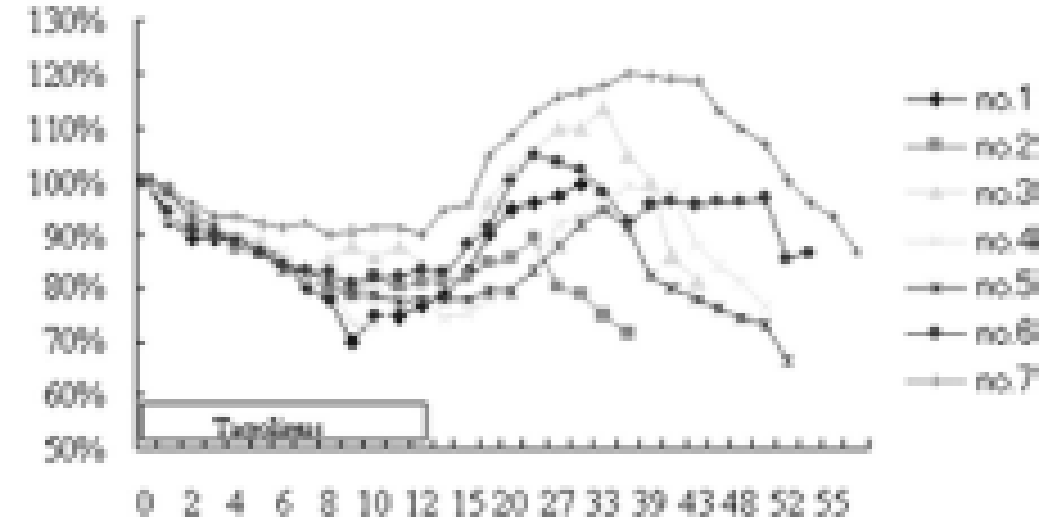
Immunologic Benefits of Longer Graft in Rat Allogenic Small Bowel Transplantation

TABLE 1. Effect of graft length on recipient survival after orthotopic small-bowel transplantation (SBT) in two different models

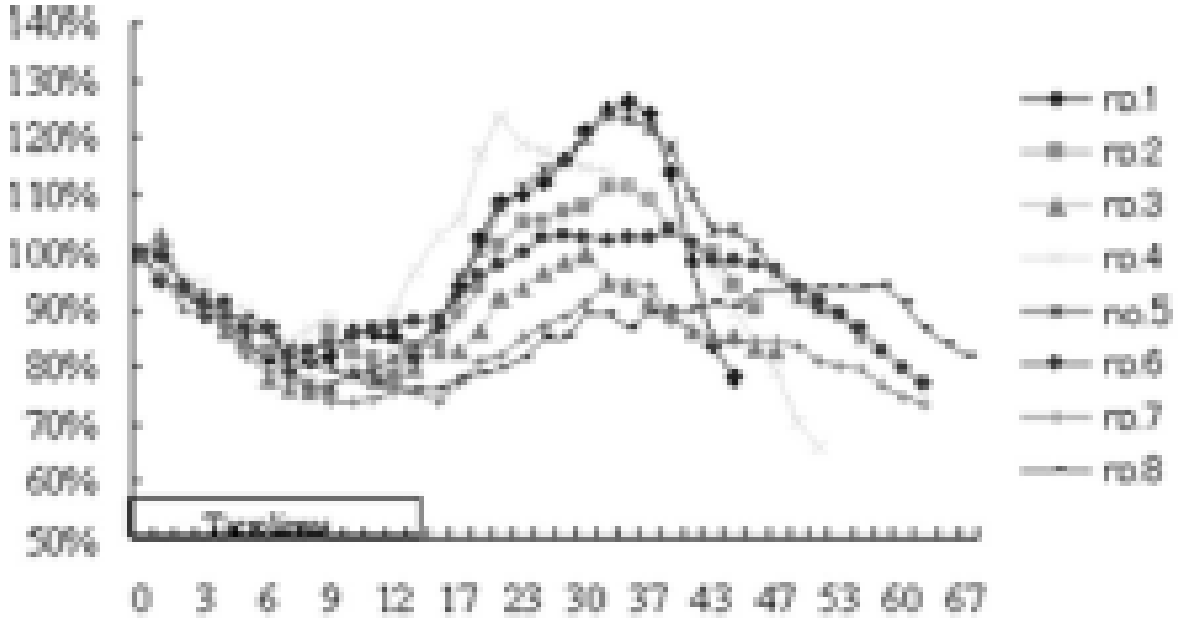
Group	Model	Donor	Recipient	Graft length	n	Recipient survival (days)	Mean ± SD (days)	P value
A[c]	M&R[a]	BN	LEW	Whole (70 cm)	5	16,[d] 63, 71, 72, >80	60.4 ± 25.5	$P = 0.001$
B[c]	M&R[a]	BN	LEW	Segment (15 cm)	7	7,[d] 7,[d] 8,[d] 8,[d] 9,[d] 14,[d] 14[d]	9.6 ± 3.1	
C	M&R[a]	LEW	LEW	Segment (15 cm)	4	>80, >80, >80, >80	>80	
D[c]	Combined[b]	BN	LEW	Whole (70 cm)	8	44, 45, 47, 51, 55, 62, 63, 67	54.3 ± 8.9	$P = 0.10$
E[c]	Combined[b]	BN	LEW	Segment (15 cm)	7	29, 35, 43, 49, 52, 54, 56	45.4 ± 10.2	
F	Combined[b]	LEW	LEW	Segment (15 cm)	6	>100, >100, >100, >100, >100, >100	>100	

[a] Orthotopic SBT were performed using classical microsurgical technique reported by Monchik and Russell (M&R).
[b] Cuff technique was used for venous reconstruction.
[c] Tacrolimus (0.64 mg/kg/day) was administered intramuscularly from 0 to 13 postoperative days.
[d] Recipients died with intact graft.
BN, Brown Norway; LEW, Lewis.

（Fujishiro J, et al. Transplantation 2005）



Segment SBT (Group E)



Whole SBT (Group D)

（Fujishiro J, et al. Transplantation 2005）

Immunologic Benefits of Longer Graft in Rat Allogenic Small Bowel Transplantation

TABLE 2. Onset of rejection[a] and suffering period[b] after whole or segmental orthotopic SBT (BN to LEW)

Group	Graft length	Onset of rejection (days)	Mean ± SD (days)	Suffering period (days)	Mean ± SD (days)
D	Whole	30, 33, 35, 37, 37, 39, 41, 60	39.0 ± 9.1	5, 7, 8, 14, 20, 21, 21, 26	15.3 ± 7.9
			N.S.		$P = 0.027$
E	Segment	27, 29, 35, 35, 41, 43, 52	37.4 ± 8.6	0, 2, 5, 6, 8, 8, 17	6.6 ± 5.5

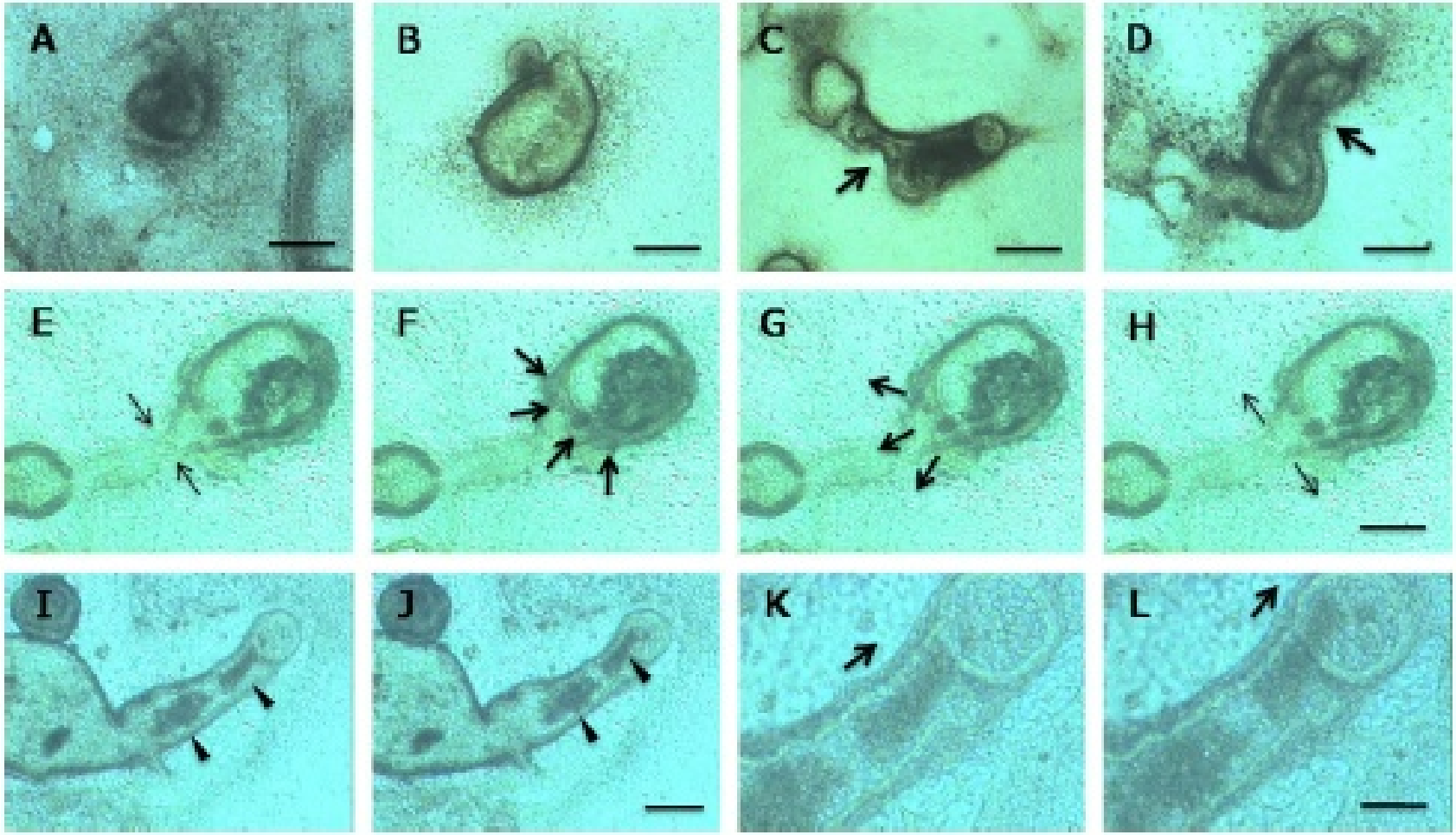
The characteristics of group D and E are shown in Table 1.
[a] Onset of rejection was defined by the day when recipients body weight start decreasing.
[b] Suffering period was defined by the period from the onset of rejection to recipient death.
SBT, small-bowel transplantation; BN, Brown Norway; LEW, Lewis.

Parameter	Short Graft (5 cm)
Necrosis	0; None
Width of villi	1; Longitudinal/transverse ratio>1
Width of proliferating zone	2; Focal disappearance
Inflammation	2; Moderate
Total score	5

Parameter	Long Graft (40 cm)
Necrosis	0; None
Width of villi	0; Normal
Width of proliferating zone	0; Normal
Inflammation	1; Mild
Total score	1

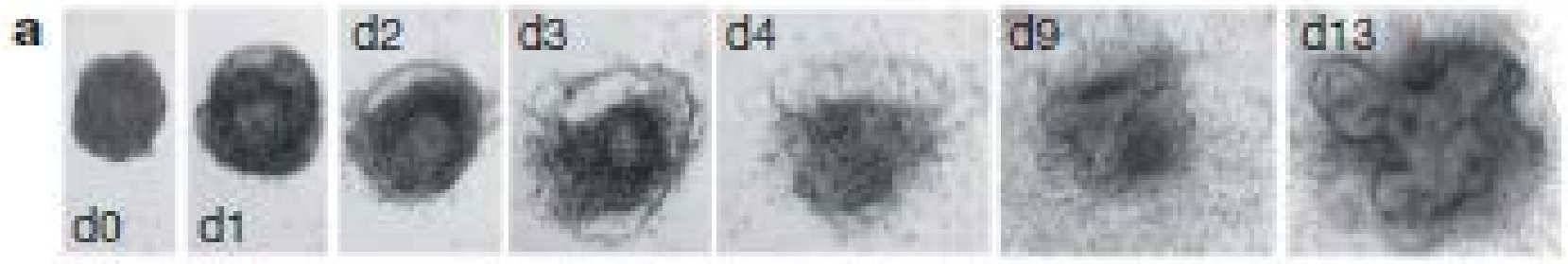
（Fujishiro J, et al. Transplantation 2005）

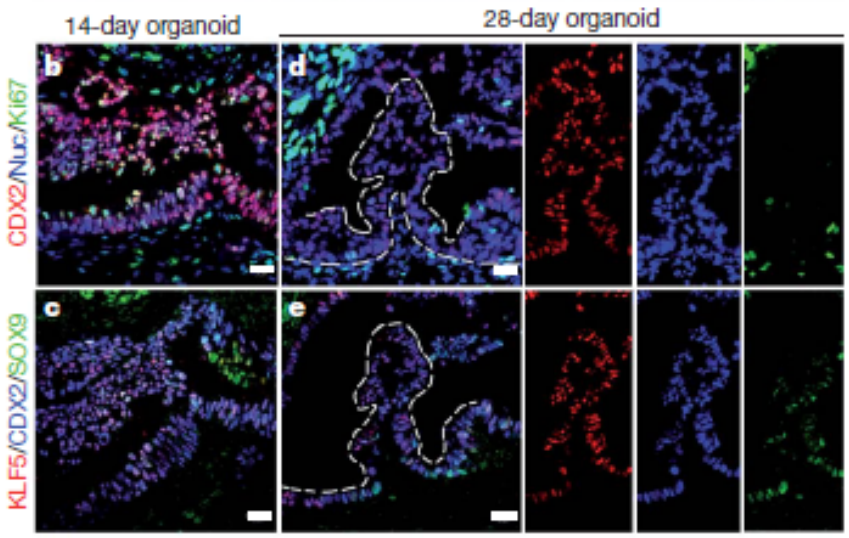
Generation of functional gut-like organ from mouse induced pluripotent stem cells

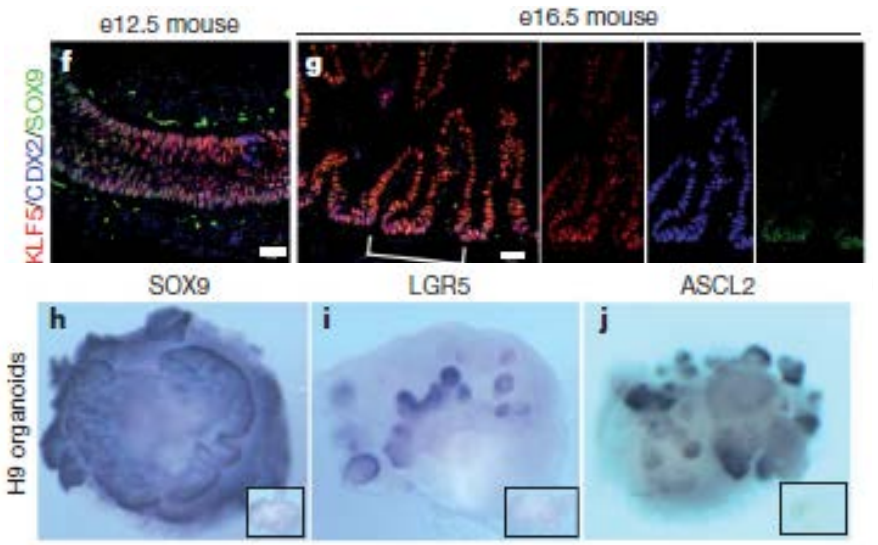


(Ueda T, et al. BBRC 391;38-42,2010)

Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro

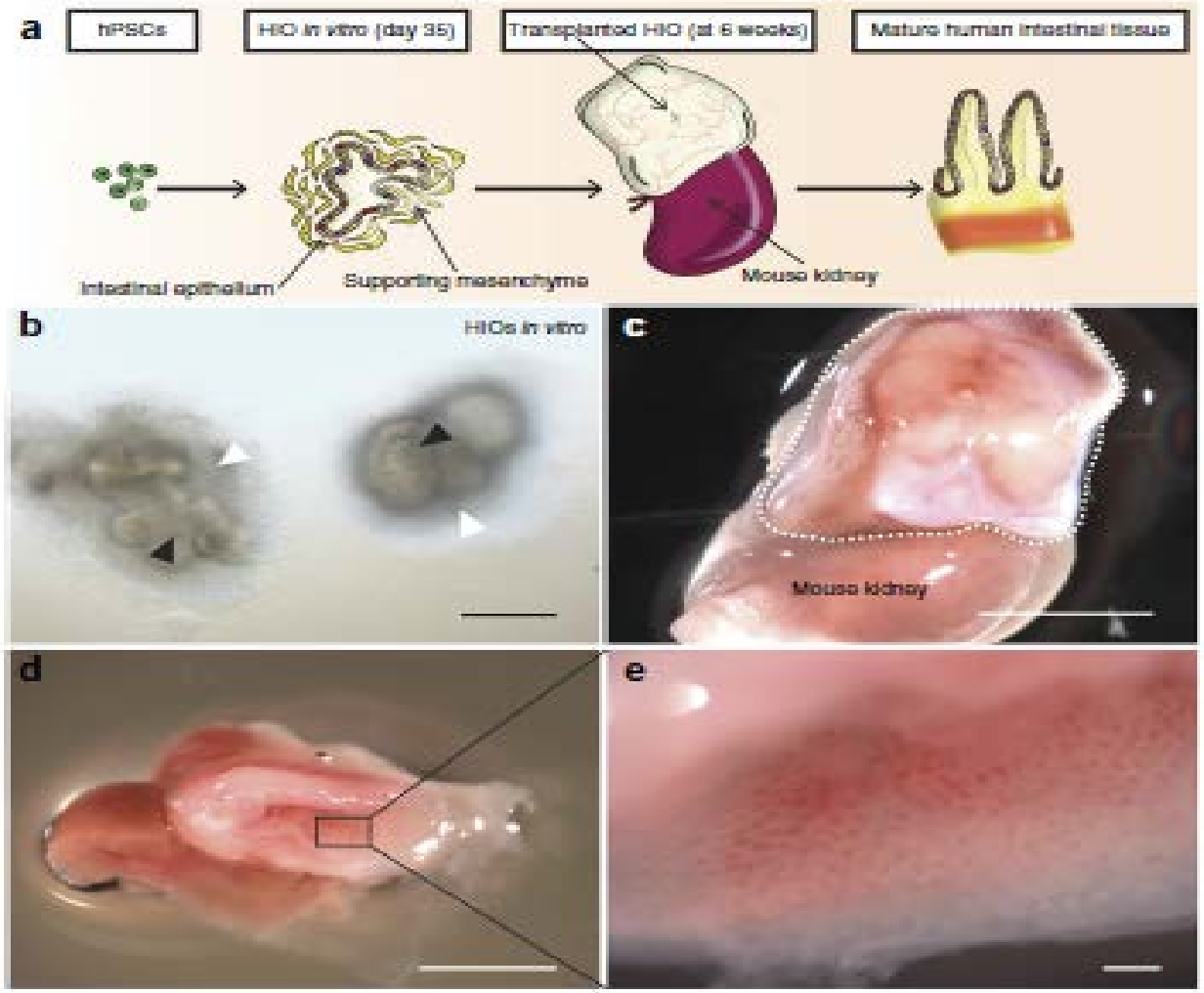






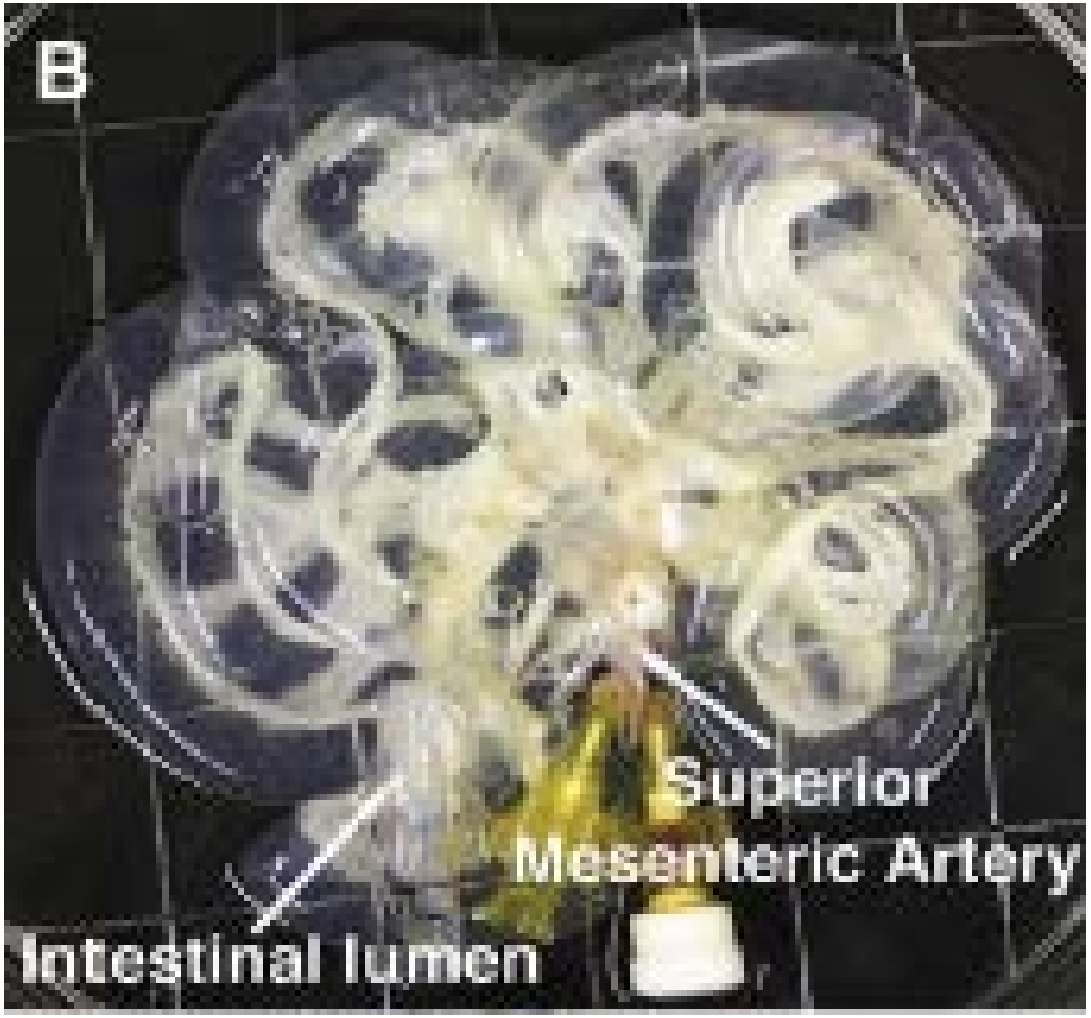
(Spence JR, et al. Nature 470;105,2011)

An *in vivo model of human small intestine using pluripotent stem cells*



(Watoson CL, et al. Nature Ned 2014)

A rat decellularized small bowel scaffold that preserves villus-crypt architecture for intestinal regeneration



(Totonelli G, et al. Biomaterials 33;3401,2012)

研究テーマは過去を勉強するところから

Effect of FK 506 on growth of transplanted newborn **rat** intestine.
Ogasa N, Maeda K, Nakamura K, Itoh H.
Transplant Proc. 1991 Dec;23(6):3255-6. No abstract available.

Successful **transplantation** of newborn **rat** intestine as a free graft.
Ogasa N, Maeda K, Nakamura K, Itoh H, Schwartz MZ.
Transplant Proc. 1992 Jun;24(3):1065-6. No abstract available.

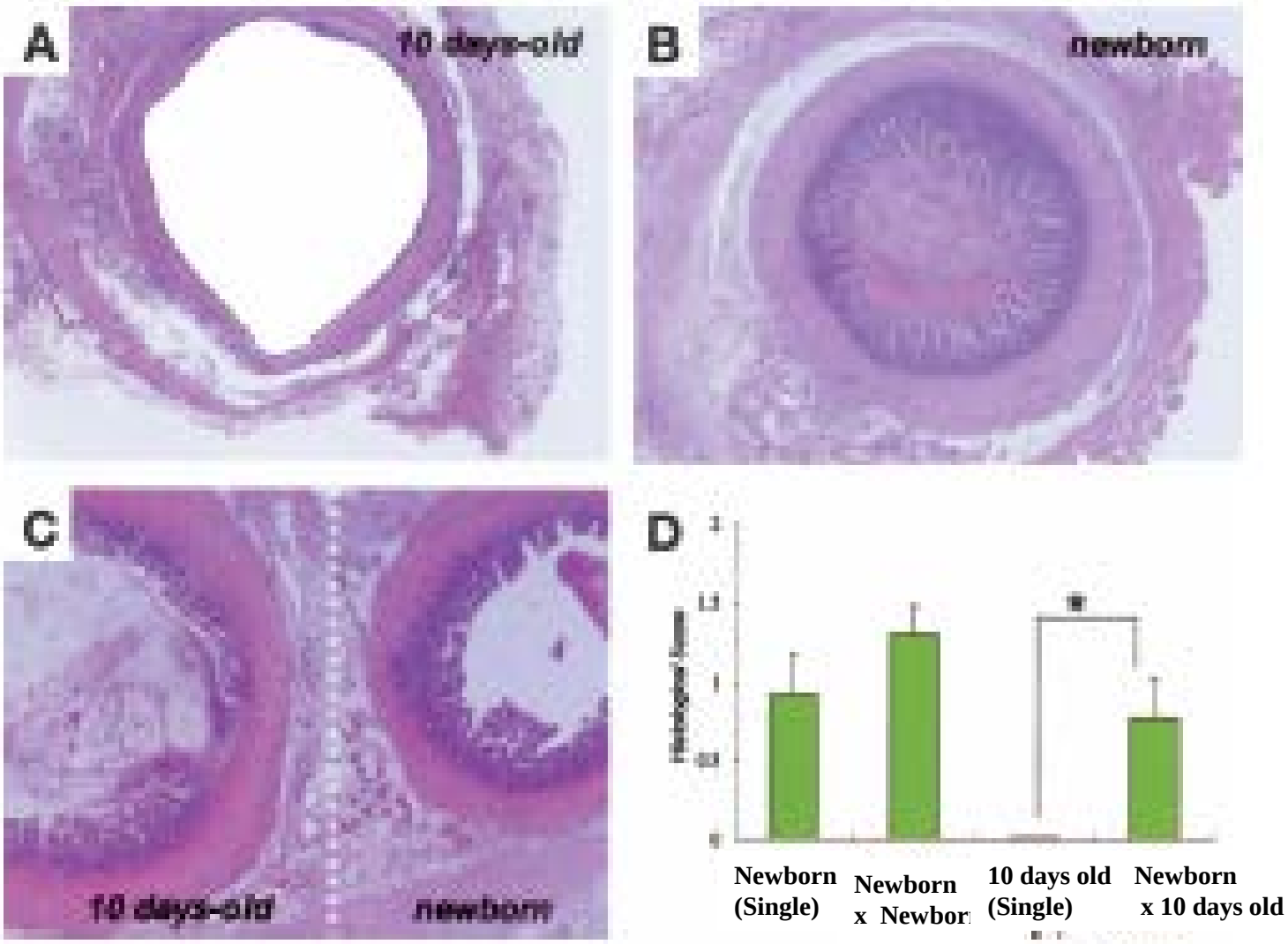
Experimental Small Bowel Transplantation Using Newborn Intestine in Rats:
I. Lipid Absorption Restored After Transplantation of Nonvascularized Graft
成長したグラフトで同所置換 (*Uchida H, et al. J Pedia Surg 1999*)

II. Revascularization of Newborn Intestine Is Independent of Vascular Endothelial Growth Factor92 75 42 25 0 %
脾臓＞小腸＞腎＞心臓＞肝（膵） *Uchida H, et al. J Pedia Surg 1999*)

III. Long-Term Cryopreservation of Rat Newborn Intestine
凍 結保存効果（抗原性の失活はない） (*Tahara K, et al. J Pedia Surg 2001*)

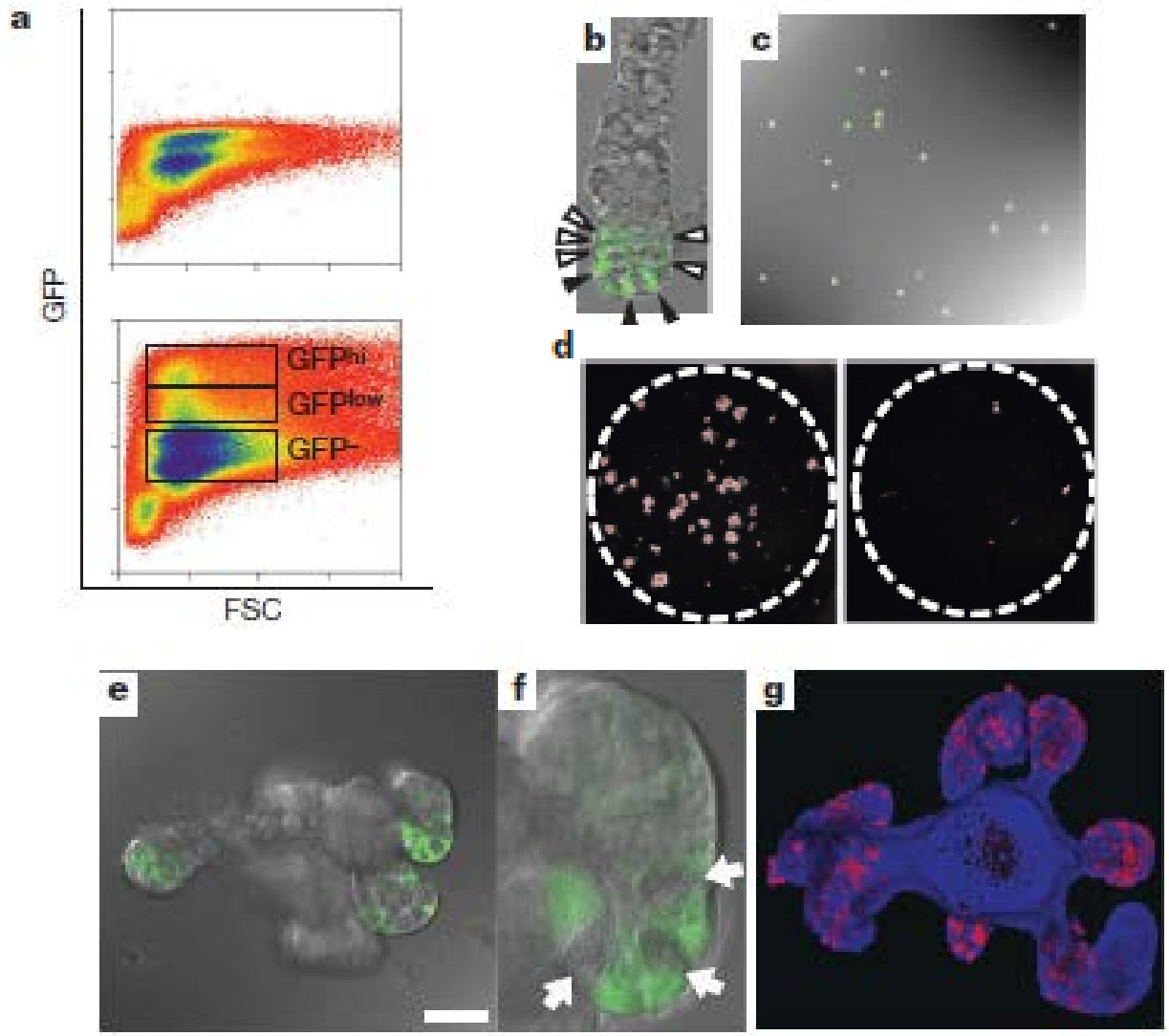
IV. Effect of Cold Preservation on Graft Neovascularization
UW, m-TOM, リンゲル液で保存効果に差なし(*Uchida H, et al. J Pedia Surg 2001*)

Regeneration of the Rat Neonatal Intestine in Transplantation



(Tahara K, et al. Ann Surg 2005)

Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche



(Sato T, et al. Nature 459;262,2009)

謝辞

水田　耕一　（自治医科大学　移植外科）
内田　広夫　（名古屋大学医学部　小児外科）
中尾　篤典　（岡山大学医歯薬学総合研究科　救急医学）
田原　和典　（成育医療センター）
井上　成一朗　（旧 筑波大学医学部）
藤城　準　（東京大学医学部附属病院小児外科）

野沢　真澄　（初代　国際実験マイクロサージャリー学会　会長）
（第五回　日本小腸移植研究会　当番世話人）