PANCREAS PROCURMENT AND PRESERVATION PRIOR TO ISLET ISOLATION

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Challenges and Emerging Opportunities

DONOR

Procurement

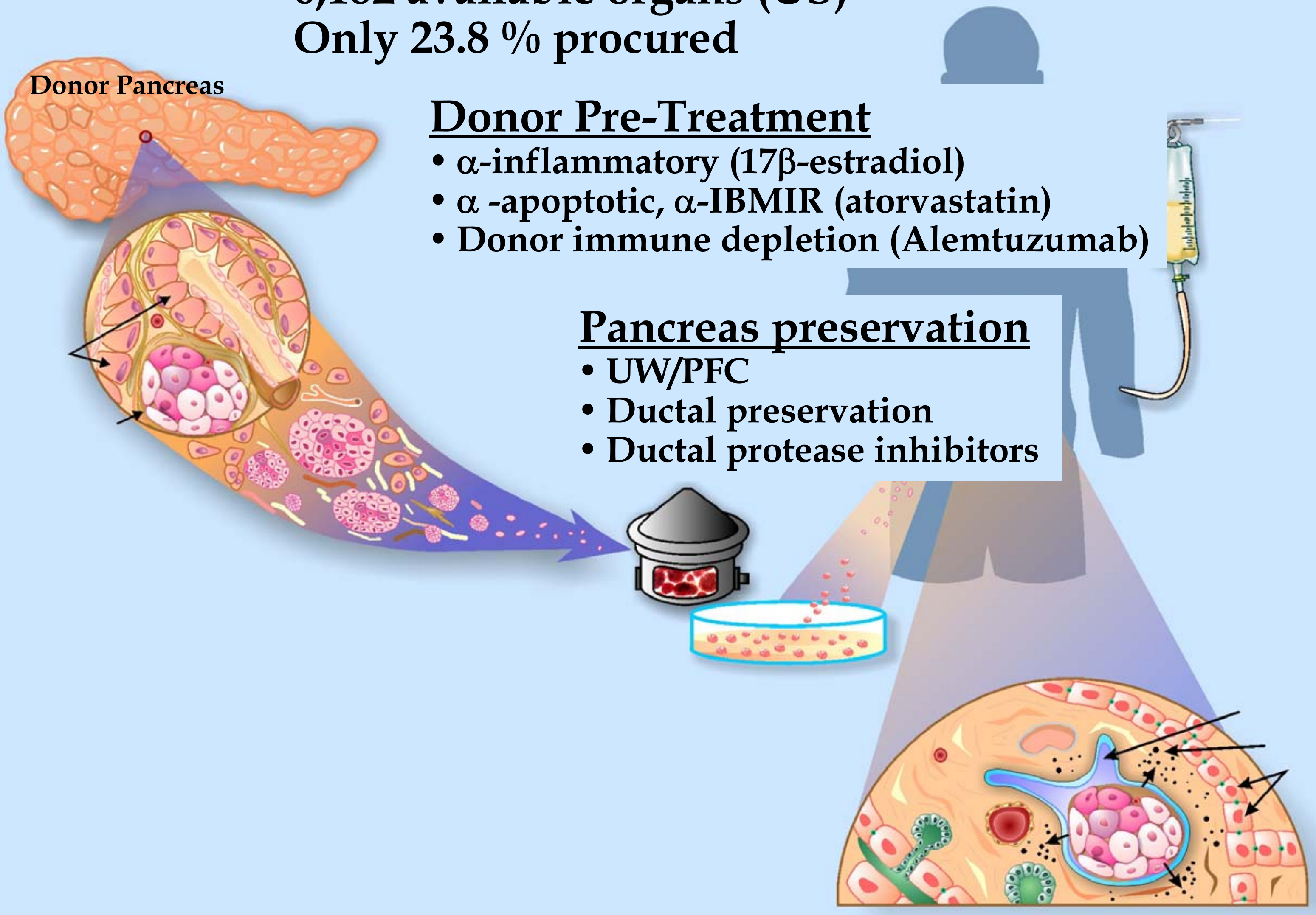
6,182 available organs (US)
Only 23.8 % procured

Donor Pre-Treatment

- α-inflammatory (17β-estradiol)
- α -apoptotic, α-IBMIR (atorvastatin)
- Donor immune depletion (Alemtuzumab)

Pancreas preservation

- UW/PFC
- Ductal preservation
- Ductal protease inhibitors



Key Parameters

1. *Donor selection*
   - *Donor factors predicting isolation outcome*
   - *Key donor parameters*
2. *Organ procurement*
3. *Pancreas Preservation*
4. *New advances and studies*

Stepwise Multivariate Logistic Regression Analysis of Donor Related Variables Predicting Isolation Success

Variable	p Value	R Value	Odds Ratio*
Donor Age (yrs)	<0.05	0.18	1.10
Body Mass Index (kg/m^2)	<0.01	0.19	1.30
Procurement Team (local/distant)	<0.01	0.21	7.04
Min Blood Glucose (mmol/L)	<0.01	-0.24	0.68
Duration of Cardiac Arrest (min)	<0.01	-0.17	0.81
Duration of Cold Storage (hrs)	<0.05	-0.13	0.86

* Odds ratio: >1 positive correlation with isolation success, <1 negative correlation

77% for predicting successful isolation

86% for predicting failed isolation

Model Accuracy: *Overall Accuracy 82%*

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VARIABLES IN ORGAN DONORS THAT AFFECT THE RECOVERY OF HUMAN ISLETS OF LANGERHANS[1]

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ZILIANG AO,[3] A.M. JAMES SHAPIRO,[3] AND NORMAN M. KNETEMAN[3,6]

Departments of Surgery, Medicine, and Health Care Quality and Outcome, and the Surgical-Medical Research Institute, University of Alberta, Edmonton, Alberta, Canada

The Standardization of Pancreatic Donors for Islet Isolations

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Donor Information

Age (yrs old)	Allocated Points	< 25	25 – 35	35 – 55	55 - 65	> 65
	20	5	10	20	10	5
CIT (hrs)	Allocated Points	0 – 3	3 – 8	8 – 12	> 12	
	15	10	15	7.5	4	
BMI	Allocated Points	< 20	20 – 24.9	25 – 30	30 - 40	> 40
	11	2	7	11	5	0
Cause of Death	Allocated Points	Suicide	Anoxia	Traumatic with Injury	Traumatic Without Injury	Cerebral Vascular
	18	0	3	8	14	18
Hospital Stay (days)	Allocated Points	0 – 4	4 - 7	7 – 14	> 14	
	5	5	3	2	0	
Amylase / Lipase (U/L)	Allocated Points	Normal	1.5 – 2x Normal	2 – 5x Normal	> 5x Normal	
	5	5	2	1		
Vasopressors	Allocated Points	Low	Moderate	High		
	4	4	2	0		
Blood Glucose	Allocated Points	Normal	High	Normal Treated	High Treated	
	4	4	2	3	1	
Procuring Team	Allocated Points	Edmonton	Distant			
	9	9	2			
Social History	Allocated Points	Drug Abuse	Promiscuous Behavior	Jail Time	Other	
	4	- 1	- 1	- 1	- 1	
Medical History	Allocated Points	Hypertension	Alcohol Abuse	Arrests > 5 min	Transfusions	Other
	5	- 2	3	- 5	- 1	- 1

Subtotal A / 100

Pancreas Physical Properties

Size	Allocated Points	Very Small	Small	Average	Large	Very Large
	- 3	- 3	- 2	0	0	- 1
Quality of Flush	Allocated Points	Very Poor	Poor	Adequate	Good	Excellent
	- 3	- 3	- 2	- 1	0	0
Pancreas Consistency	Allocated Points	Very Soft	Soft	Average	Firm	Very Firm
	- 5	- 5	- 4	0	- 1	- 3
Quality of Procurement	Allocated Points	Very Poor	Poor	Adequate	Good	Excellent
	- 3	- 3	- 2	- 1	0	0
Damage	Allocated Points	None	Acceptable	Unacceptable		
	- 3	0	- 1	- 2		
Quality of Packaging	Allocated Points	Very Poor	Poor	Adequate	Good	Excellent
	- 3	- 3	- 2	- 1	0	0
Fat Content	Allocate d Points	Very Lean	Lean	Average	Fatty	Very Fatty
	- 5	- 5	- 3	0	0	- 2

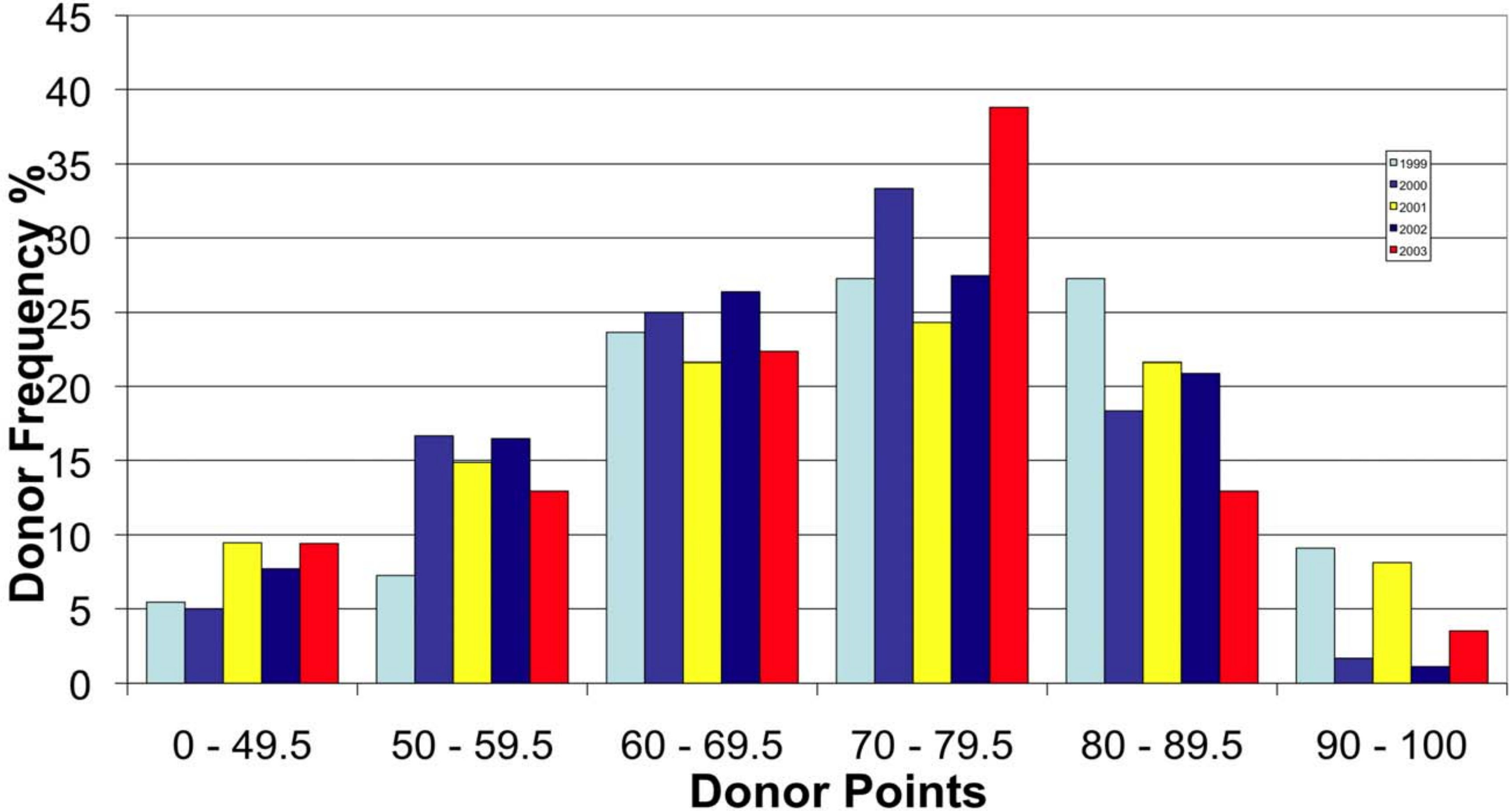
Subtotal B

Subtotal A ____________

Subtotal B ____________

Sum of Subtotals ____________

Pancreatic Donor Frequency by Year

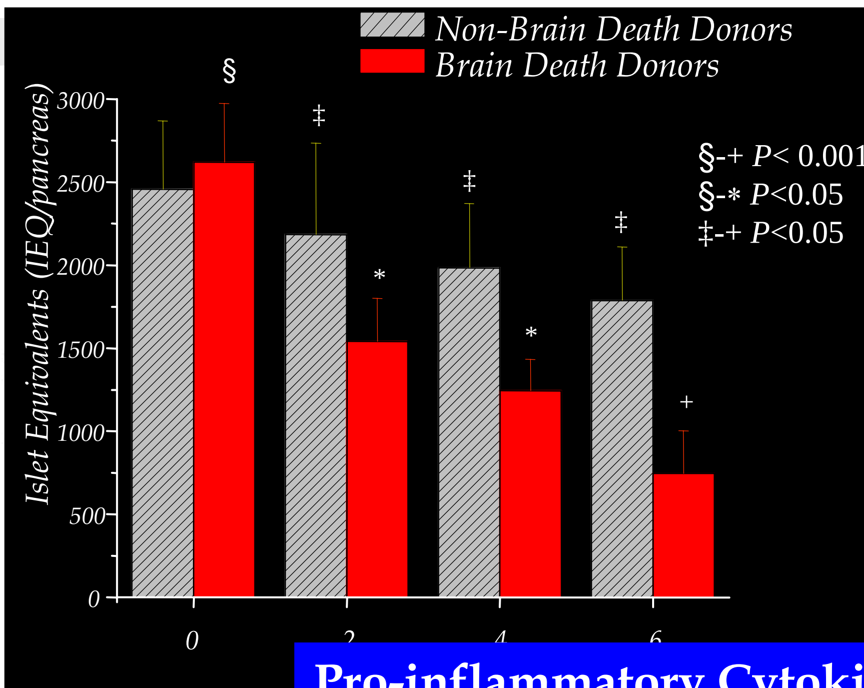


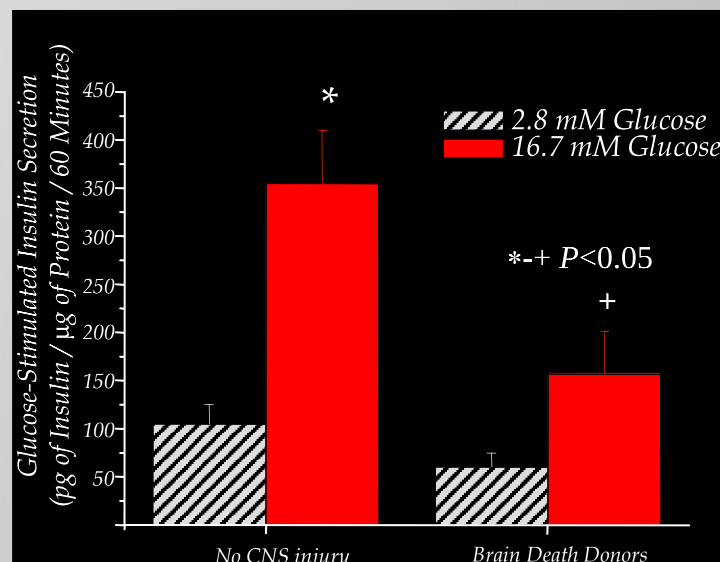
Pancreatic donor and transplant frequency for standardization of islet donors

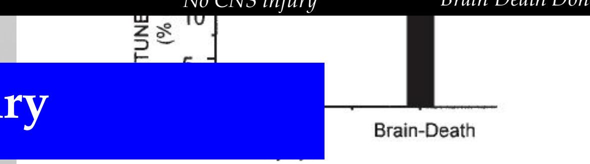
DP Range	No. of Donors	Frequency of Donors (%)	No. of Tx	Tx Frequency (%)
0 – 49.5	22	6.8	0	0.0
50 – 59.5	44	13.5	13	29.6
60 – 69.5	79	24.2	32	40.5
70 – 79.5	100	30.7	35	35.0
80 – 89.5	66	20.3	36	54.6
90 – 100	15	4.6	15	100

Brain Death Significantly Reduces Isolated Pancreatic Islet Yields and Functionality In Vitro and In Vivo After Transplantation in Rats

Juan L. Contreras,[1] Christopher Eckstein,[1] Cheryl A. Smyth,[1] Marty T. Sellers,[2] Mario Vilatoba,[1] Guadalupe Bilbao,[1] Firoz G. Rahemtulla,[3] Carlton J. Young,[1] J. Anthony Thompson,[1] Irshad H. Chaudry,[4] and Devin E. Eckhoff[1]







DIABETES, VOL. 52, DECEMBER 2003

Pancreas Recovery for Islet Isolation

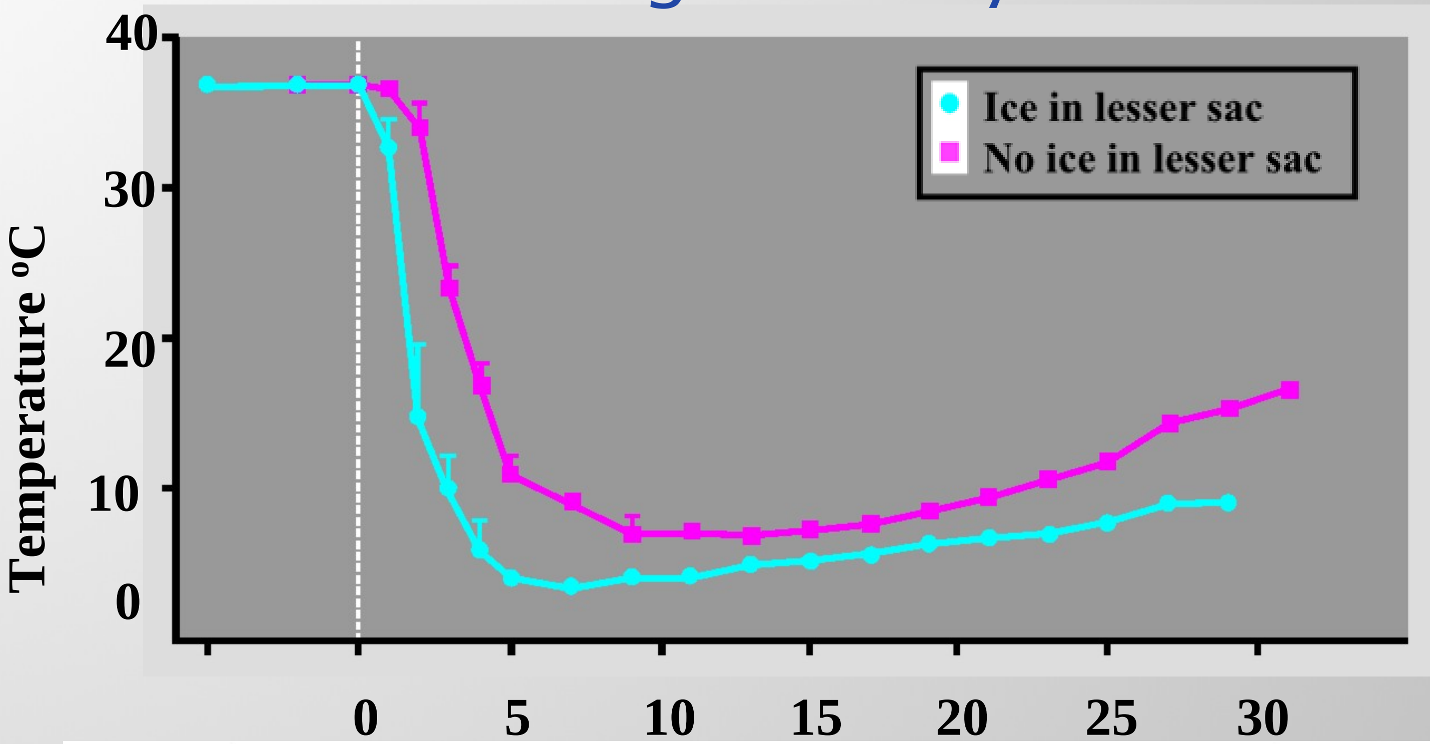
"Treat like an organ - not like a lymph node"

Handle with care - keep capsule intact

Core pancreas cooling and keep pancreas cold

En bloc with liver- keep cold

Procurement cooling affects yield and function



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EFFECT OF CORE PANCREAS TEMPERATURE DURING CADAVERIC PROCUREMENT ON HUMAN ISLET ISOLATION AND FUNCTIONAL VIABILITY[1]

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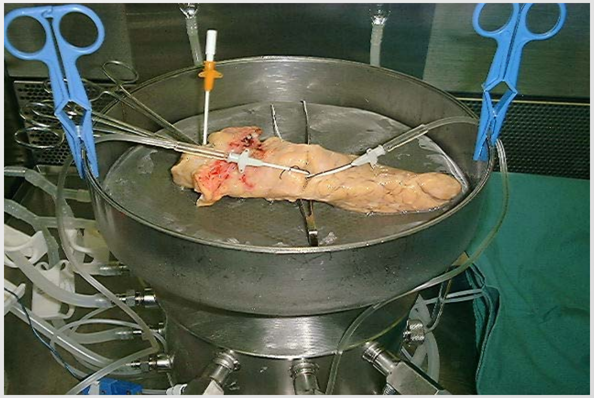
Pancreas cooling affects yield and function

	Yield	Function (SI)
Ice:	223 ± 35 IE $x10^3$	4.4 ± 0.3
No ice:	103 ± 26 IE $x10^3$	3.0 ± 0.4

$p < 0.05$

Cool the pancreas -> DOUBLE the yield!

Additional considerations for pancreas preservation before islet isolation



- Integrity of the ductal system

- Differential density between the exocrine and endocrine components

- Activity of endogenous pancreatic enzyme

Cold Ischemia is Detrimental to Human Islet Yields

- *Kneteman NM, Lakey JRT, Warnock GL, Rajotte RV. Human islet isolation after prolonged cold storage. Diab Nutr Metab 1992; 5: 33-37.*
- *Robertson GSM, Chadwick D, Thirdborough S, Swift S, Davies J, James R et al. Human islet isolation – a prospective randomised comparison of pancreatic vascular perfusion with hyperosmolar citrate or University of Wisconsin solution. Transplant 1993; 56: 550-553.*
- *Ketchum JR, Nicolae M, Jahr H, Friedman A, Naji A, Barker CF et al. Analysis of donor age and cold ischaemia as factors in cadaveric human islet isolation. Transplant Proc 1994; 26: 596-597.*
- *Benhamou PY, Watt PC, Mullen Y, Ingles S, Watanabe Y, Normura Y et al. Human islet isolation in 104 consecutive cases. Transplant 1994; 57: 1804-1810.*
- *Lakey JRT, Rajotte RV, Warnock GL, Kneteman NM. Human pancreas preservation prior to islet isolation – cold ischaemic tolerance. Transplant 1995; 59: 689-694.*

0041-1337/95/5905-689$03.00/0
TRANSPLANTATION
Vol. 59, 689–694, No. 5, March 15, 1995

HUMAN PANCREAS PRESERVATION PRIOR TO ISLET ISOLATION

COLD ISCHEMIC TOLERANCE[1]

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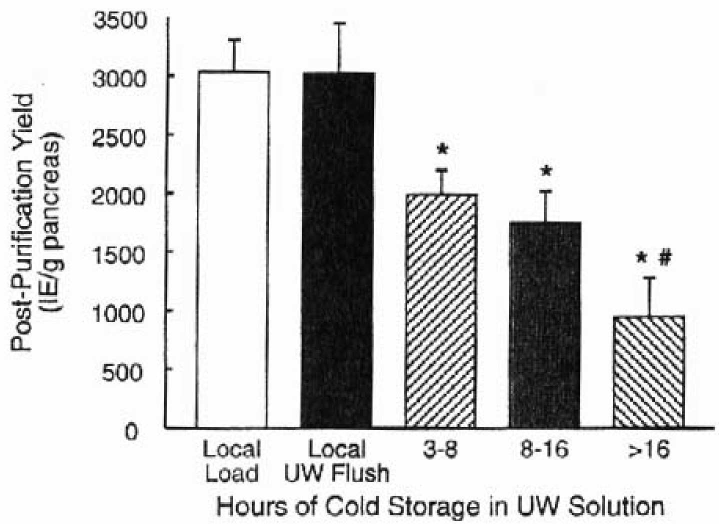


FIG. 2. Postpurification islet yields from human cadaver pancreases as grouped: local pancreases loaded with collagenase solution (Local Load), local pancreases in situ flushed with UW solution (Local UW Flush), and distantly procured pancreases subdivided by the hr of cold storage prior to islet isolation (3–8, 8–16, and >16 hr). *$P<0.05$ vs. Local UW group; #$P<0.05$ vs. 8–16-hr group.

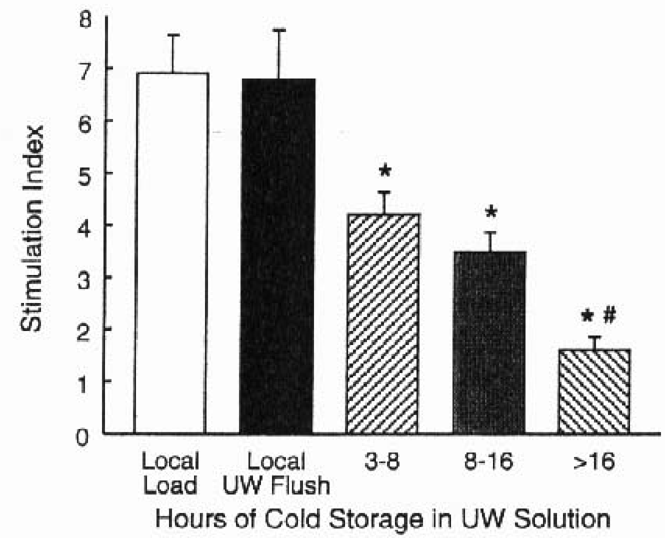


FIG. 4. Islet recovery (>100,000 IE of >50% purity) and in vitro viability (glucose perifusion, SI > 2) from cadaver donor pancreases loaded with collagenase solution after primary pancreatectomy or pancreases flushed and stored in UW solution prior to islet isolation.

Clinical Outcomes and Insulin Secretion After Islet Transplantation With the Edmonton Protocol

Edmond A. Ryan,[1] Jonathan R.T. Lakey,[2] Ray V. Rajotte,[2] Gregory S. Korbutt,[2] Tatsuya Kin,[2] Sharleen Imes,[4] Alex Rabinovitch,[1] John F. Elliott,[3] David Bigam,[2] Norman M. Kneteman,[2] Garth L. Warnock,[2] Ingrid Larsen,[4] and A.M. James Shapiro[2]

DIABETES, VOL. 50, APRIL 2001

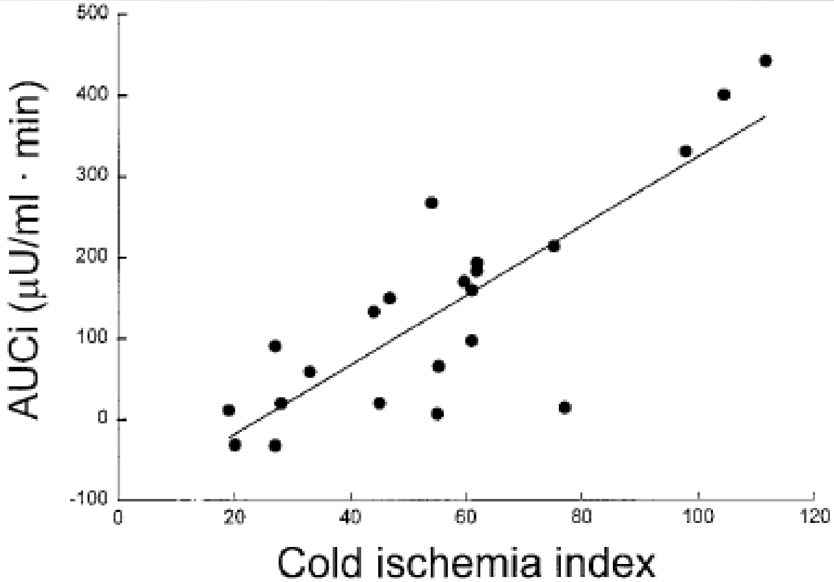


FIG. 6. *A*: Relationship of AUCi as derived from the IVGTT in subjects at midtransplant ($n = 10$) and at 1 ($n = 3$) or 3 months ($n = 9$) post–islet transplant and the number of islets transplanted. Data include results after the first and subsequent transplants; $r = 0.587$, $P = 0.004$. *B*: Relationship of AUCi, as derived from the IVGTT in subjects at midtransplant ($n = 10$) and at 1 ($n = 3$) or 3 months ($n = 9$) post–islet transplant and the ischemia index (islet numbers $\times 10^{-3}$/cold ischemia time); $r = 0.828$, $P < 0.001$.

Cold Storage < 2 hrs also Deleterious to Human Islet Yields

- *London NJ, Lake SP, Wilson J, Bassett D, Toomey P, Bell PR et al. A simple method for the release of islets by controlled collagenase digestion of the human pancreas. Transplant 1990; 49: 1109-1113.*

Two-layer cold storage method

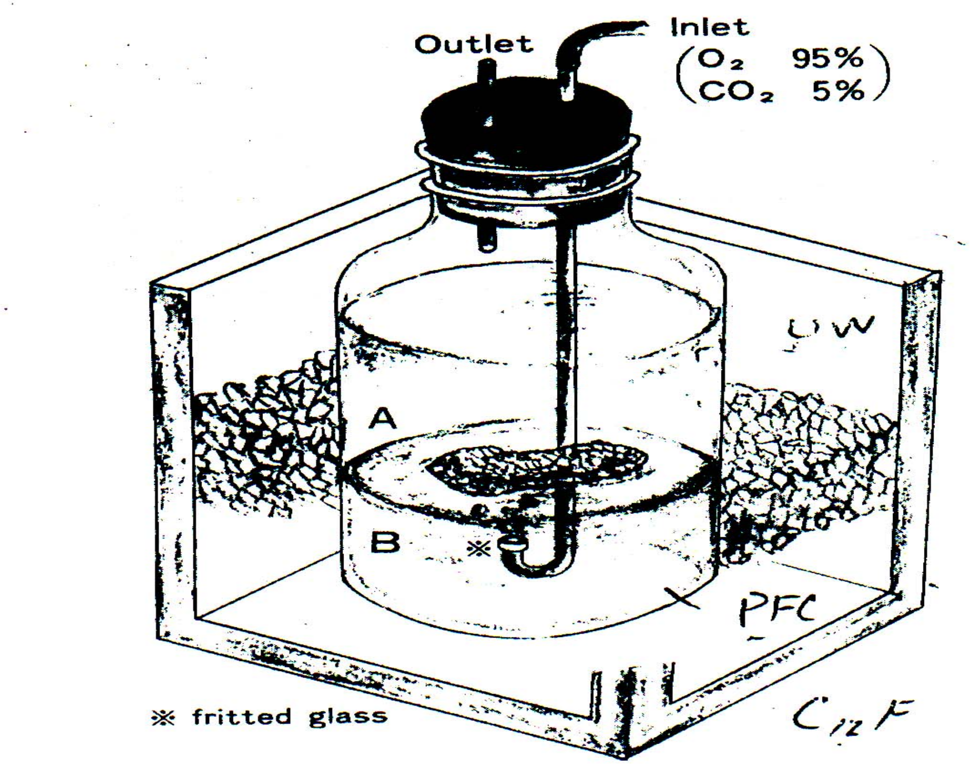
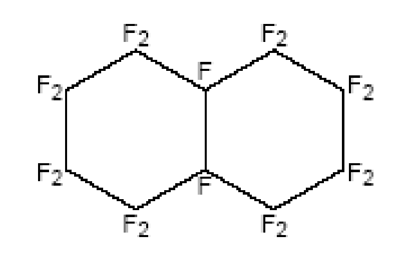


FIGURE 1. Two-layer cold storage method. Oxygenation was continued through the fritted glass to PFC throughout the storage period, the pancreas graft being surrounded by cold EC and PFC in a styrofoam box packed in ice. (A) Euro-Collins' solution; (B) perfluorochemical.

Kuroda Y et al. A new, simple method for cold storage of the pancreas using perfluorochemical. Transplantation 1988; 46: 457

Perfluorochemical (PFC)



Perfluorodecalin (Perfluorocarbon)

Molecular weight 462
Density 1.96 g/ml
Particle <0.2um
Clear, colorless, odorless, immiscible with aqueous systems

Hyper oxygen carrier---High specific gravity (1.93 g/ml)

A simple passive process
20-25 times greater than in either water or plasma

Release O2 into surrounding tissue effectively

Primarily being developed as an artificial blood substitute

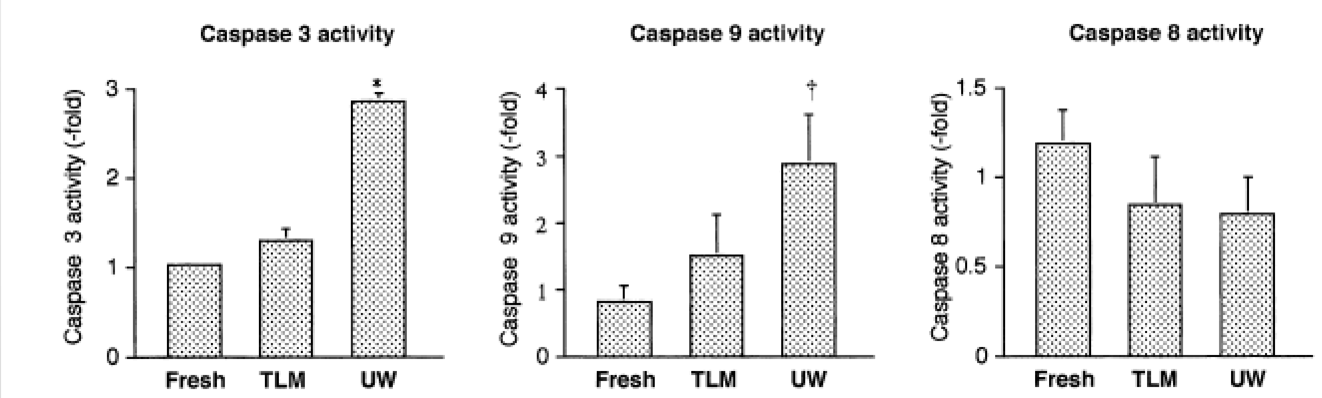
Table 1. Solubilities of oxygen and carbon dioxide in PFC liquids in mmol l^{-1} at standard temperature and pressure

Liquid	Oxygen	Carbon dioxide
Water	2.2	57
Perfluorotributylamine	35.2	123
Perfluorodecalin	35.5	125
Bis(perfluorohexyl)ethene	37.9	159
Perfluorotripropylamine	39.6	146
Perfluoro-octyl bromide	44.0	185
Bis(perfluorobutyl)ethene	44.0	203

Pancreas preservation by the 2-layer cold storage method before islet isolation protects isolated islets against apoptosis through the mitochondrial pathway

Takeru Matsuda, MD, PhD, Yasuyuki Suzuki, MD, PhD, Yasuki Tanioka, MD, PhD,
Hirochika Toyama, MD, Keitaro Kakinoki, MD, Kunihiko Hiraoka, MD, PhD,
Yasuhiro Fujino, MD, PhD, *and* Yoshikazu Kuroda, MD, PhD, *Kobe, Japan*

(Surgery 2003;134:437-45.)



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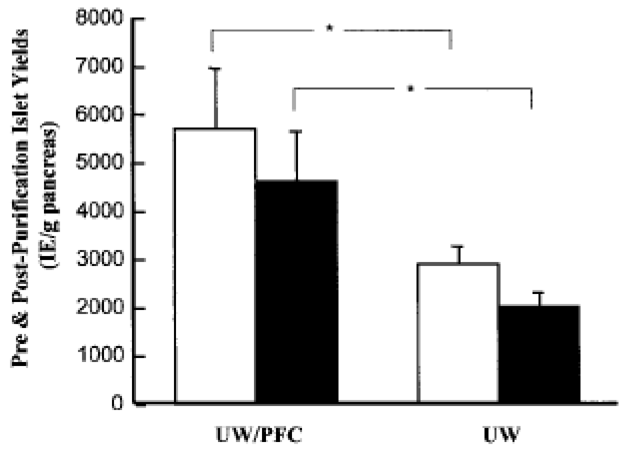
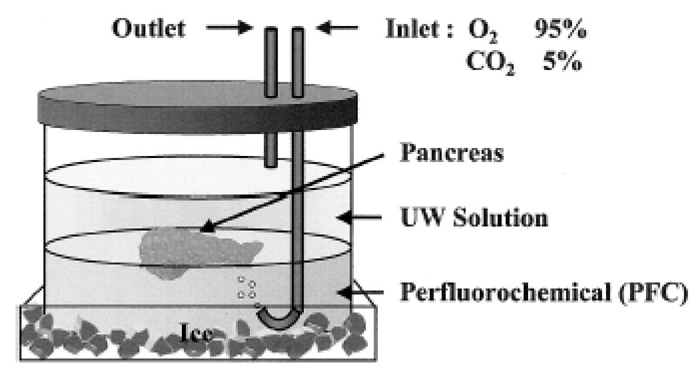
HUMAN ISLET TRANSPLANTATION FROM PANCREASES WITH PROLONGED COLD ISCHEMIA USING ADDITIONAL PRESERVATION BY THE TWO-LAYER (UW SOLUTION/PERFLUOROCHEMICAL) COLD-STORAGE METHOD

TOSHIAKI TSUJIMURA,[1,4] YOSHIKAZU KURODA,[4] TATSUYA KIN,[4] JOSE G. AVILA,[4] RAY V. RAJOTTE,[1,2,3] GREGORY S. KORBUTT,[1,2] EDMOND A. RYAN,[3] A. M. JAMES SHAPIRO,[1,2] AND JONATHAN R. T. LAKEY[1,2,5]

TABLE 2. Human islet isolation variables

Group	n	Islet recovery ($\times 10^3$ IE)	Trapped islets (%)	Purity (%)	Insulin release (stimulation index)	Cellular composition (% of total)		Insulin content (Insulin/DNA)	Successful isolation (%)
						β cell	α cell		
UW/PFC	7	349.2 ± 44.1[a]	7.0 ± 5.5	70.0 ± 5.3	3.3 ± 0.7	26.6 ± 5.9	7.1 ± 2.2	2.3 ± 0.9	71 (5/7)
UW	14	214.1 ± 31.0	5.1 ± 0.8	71.8 ± 4.3	2.2 ± 0.3	23.6 ± 3.7	13.3 ± 2.9	1.6 ± 0.4	36 (5/14)

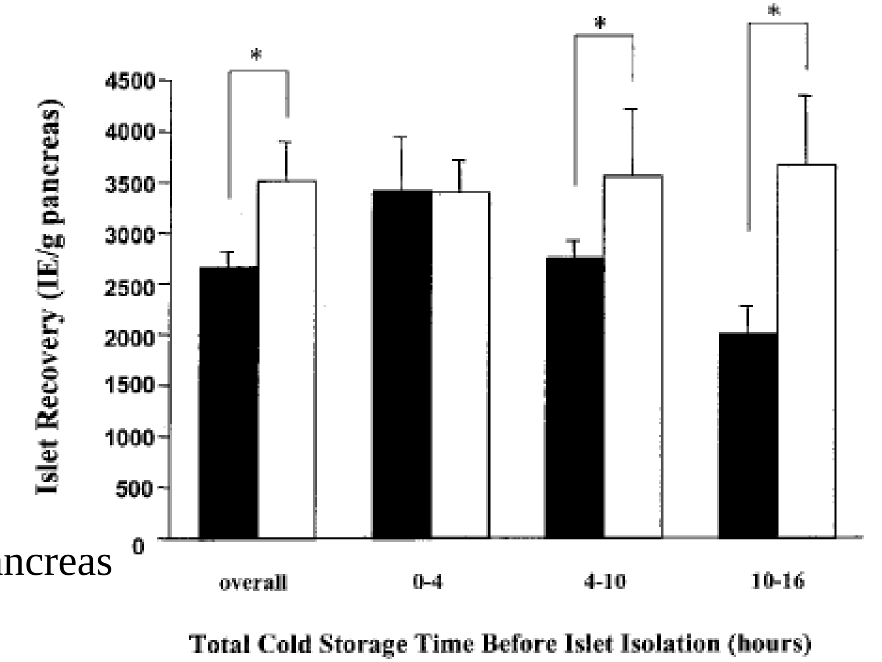


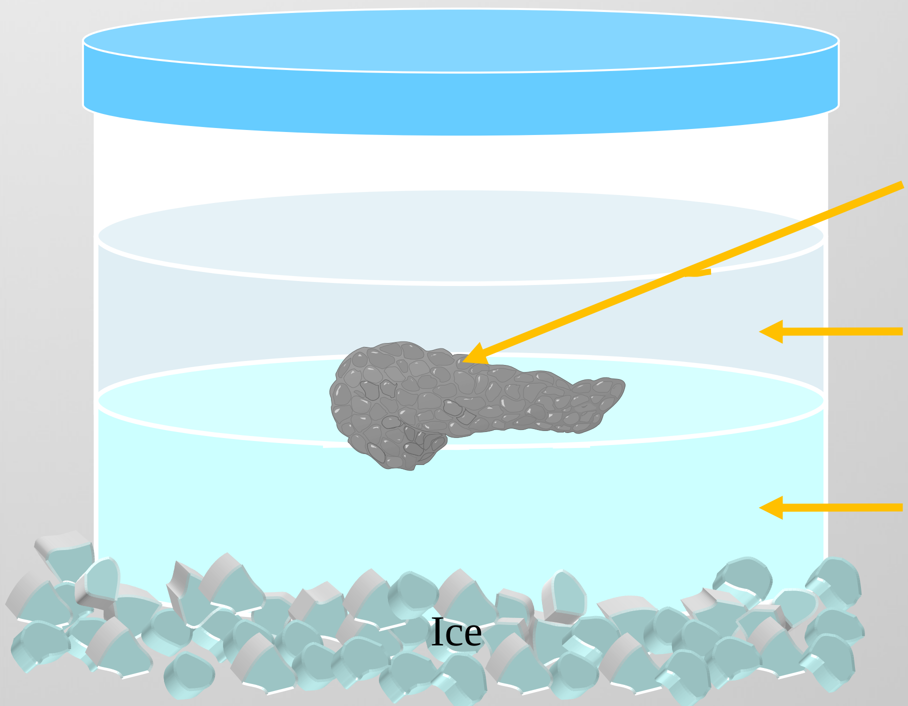


Influence of Pancreas Preservation on Human Islet Isolation Outcomes: Impact of the Two-Layer Method

Toshiaki Tsujimura,[1,2] Yoshikazu Kuroda,[2] Jose G. Avila,[1] Tatsuya Kin,[1] Jose Oberholzer,[3] A. M. James Shapiro,[3] and Jonathan R. T. Lakey[1,3,4]

(*Transplantation* 2004;78: 96–100)

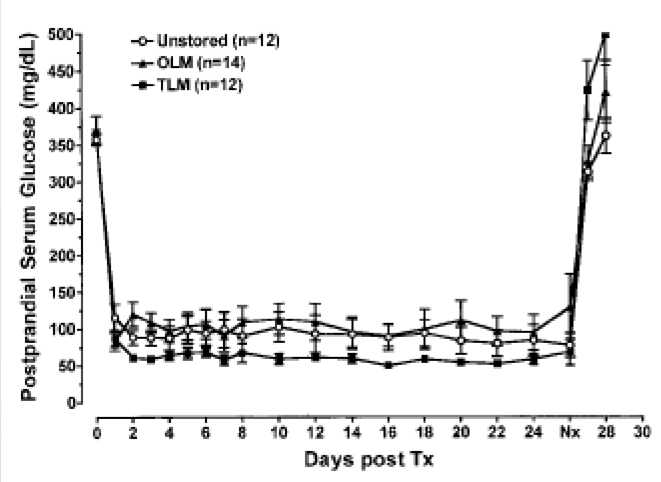




Successful Pancreas Preservation by a Perfluorocarbon-Based One-Layer Method For Subsequent Pig Islet Isolation

Daniel Brandhorst, Marcus Iken, Mathias D. Brendel, Reinhard G. Bretzel, and Heide Brandhorst

(*Transplantation* 2005;79: 433–437)

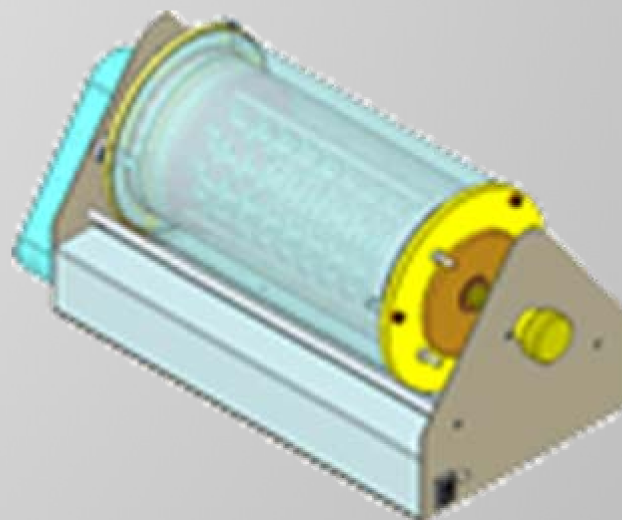


					Insulin release (percent of content)		
Storage condition	n	Formazan production (OD/mg protein)	ATP content (μg/mg protein)	Insulin content (U/mg protein)	Low glucose (2.8 mM)	High glucose (20 mM)	Stimulation index (20 mM/ 2.8 mM)
Unstored	6	1.53±0.25	0.85±0.13	1.46±0.17	8.4±3.2	17.2±5.6	2.47±0.36
OLM	8	1.44±0.08	0.91±0.18	1.97±0.14[a,b]	3.9±0.9	6.7±1.4[a]	2.27±0.57
TLM	10	1.38±0.11	0.94±0.41	1.40±0.12	6.2±1.5	10.4±2.7	1.81±0.20[a]

Organ Preservation Containers

HUG Box Cooler

BioRep Inc.



Hering et al., U Minn

Ricordi et al., DRI, Miami

Islet Isolation and Transplantation Outcomes of Pancreas Preserved with University of Wisconsin Solution Versus Two-Layer Method Using Preoxygenated Perfluorocarbon

Tatsuya Kin, Mohammadreza Mirbolooki, Payam Salehi, Manabu Tsukada, Doug O'Gorman, Sharleen Imes, Edmond A. Ryan, A. M. James Shapiro, and Jonathan R. T. Lakey

Transplantation • Volume 82, Number 10, November 27, 2006

TABLE 3. Pancreatic tissue energy after cold preservation

	TLM	UW
ATP (nmol/mg protein)	10.4±1.5	8.9±1.2
ADP (nmol/mg protein)	3.5±0.5	3.3±0.5
ADP/ATP	0.96±0.26	0.82±0.16
Energy charge	0.64±0.04	0.60±0.03

TABLE 4. Postpurification islet yield stratified by storage time

CIT	TLM	UW	*P* value (*t* test)
≤4 hours	276±93	222±30	NS
4 to ≤8 hours	234±34	218±23	NS
>8 h	275±27	263±46	NS
P value (ANOVA)	NS	NS	

- *No beneficial effect of TLM on islet isolation and transplantation outcomes was observed when pre-oxygenated perfluorocarbon was utilized*
- *Discrepancies between the present results and our previous studies may be related to the difference in method of oxygenation (pre-charge versus continuous bubbling)*

TABLE 5. Islet transplant outcome

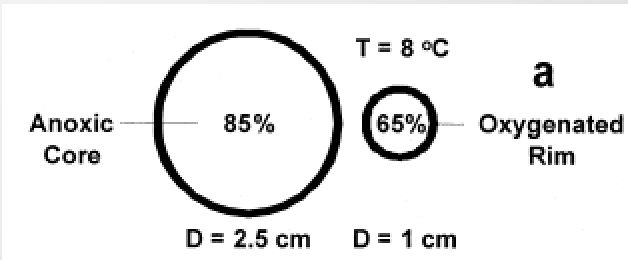
	TLM	UW
Islet mass transplanted (IE/kg)	5,5432±308	5,270±235
Viability (%)	84.2±2.4	86.5±1.5
Stimulation index	3.0±0.4	3.0±0.6
Insulin usage before first transplant (U/kg/d)	0.57±0.05	0.62±0.04
Insulin usage after first transplant (U/kg/d)	0.27±0.04	0.25±0.04
Percent decrease in insulin (%)	54.3±6.3	58.8±4.9
Insulin usage before second transplant (U/kg/d)	0.36±0.04	0.41±0.06[a]
Insulin usage after second transplant (U/kg/d)	0.17±0.04	0.26±0.06[b]
Percent decrease in insulin (%)	59.0±9.1	43.0±10.3[a]

75 pancreata were preserved by TLM
91 pancreata were preserved in UW

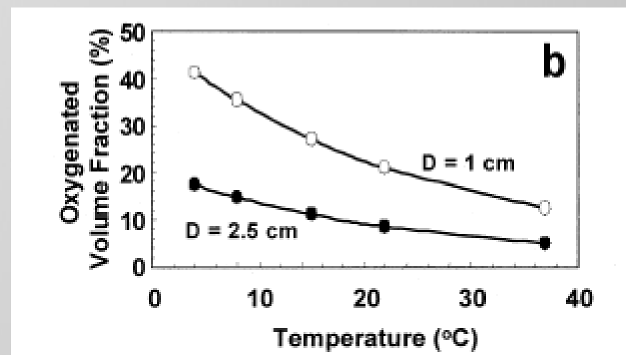
Pancreas Oxygenation Is Limited During Preservation With the Two-Layer Method

K.K. Papas, B.J. Hering, L. Gunther, M.J. Rappel, C.K. Colton, and E.S. Avgoustiniatos

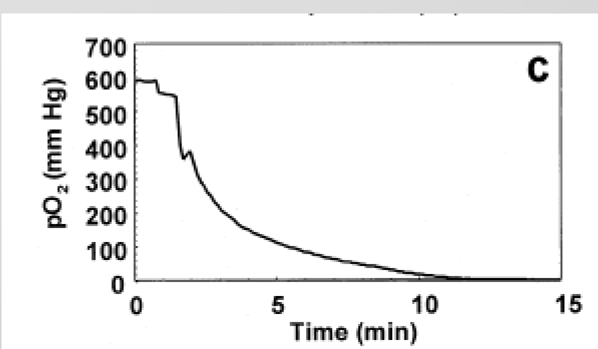
Transplantation Proceedings, 37, 3501–3504 (2005)



The TLM oxygenates only a small volume fraction (15%) of a 2.5-cm-thick pancreas.



The PO_2 is zero at the core of a preserved canine, porcine, or human pancreas.



Elevated ATP levels achieved with the TLM indicate enhanced oxygenation, but must be compared to some physiological standard for evaluation of the extent of oxygenation.

Novel Preservation Strategies

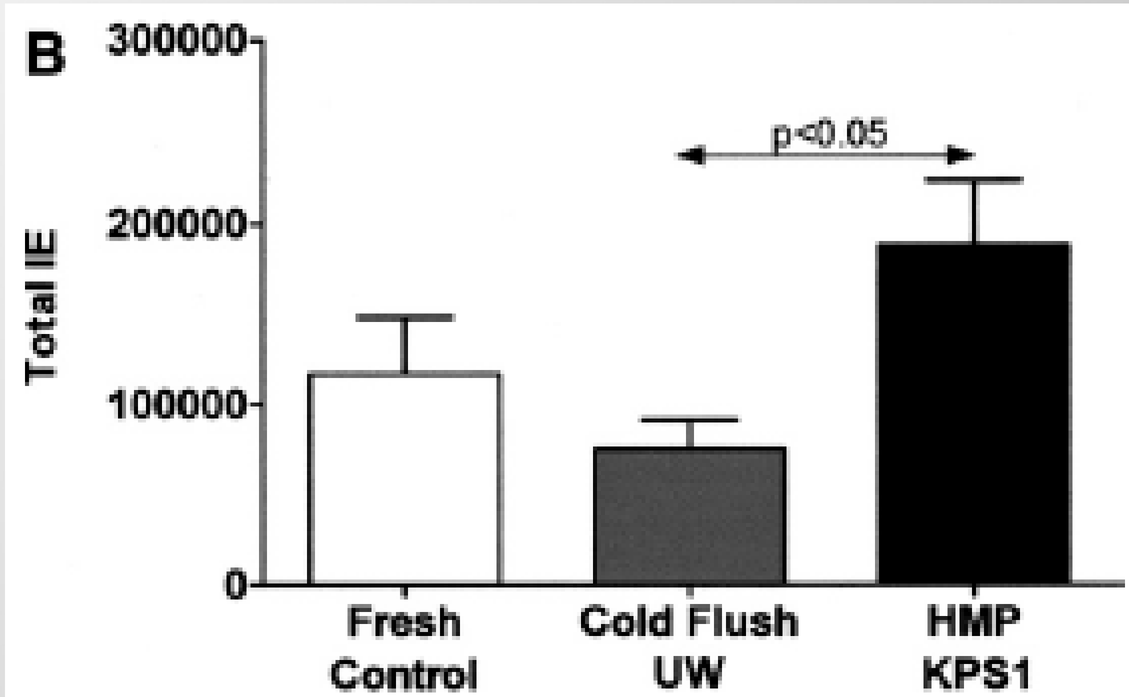
- *Continuous Perfusion*

- *Intraductal delivery*
  - *UW solution*
  - *Glutamine/Nicotinamide*

- *Continuous Oxygenation*

Twenty-Four Hour Hypothermic Machine Perfusion Preservation of Porcine Pancreas Facilitates Processing for Islet Isolation

M.J. Taylor, S. Baicu, B. Leman, E. Greene, A. Vazquez, and J. Brassil



Edema

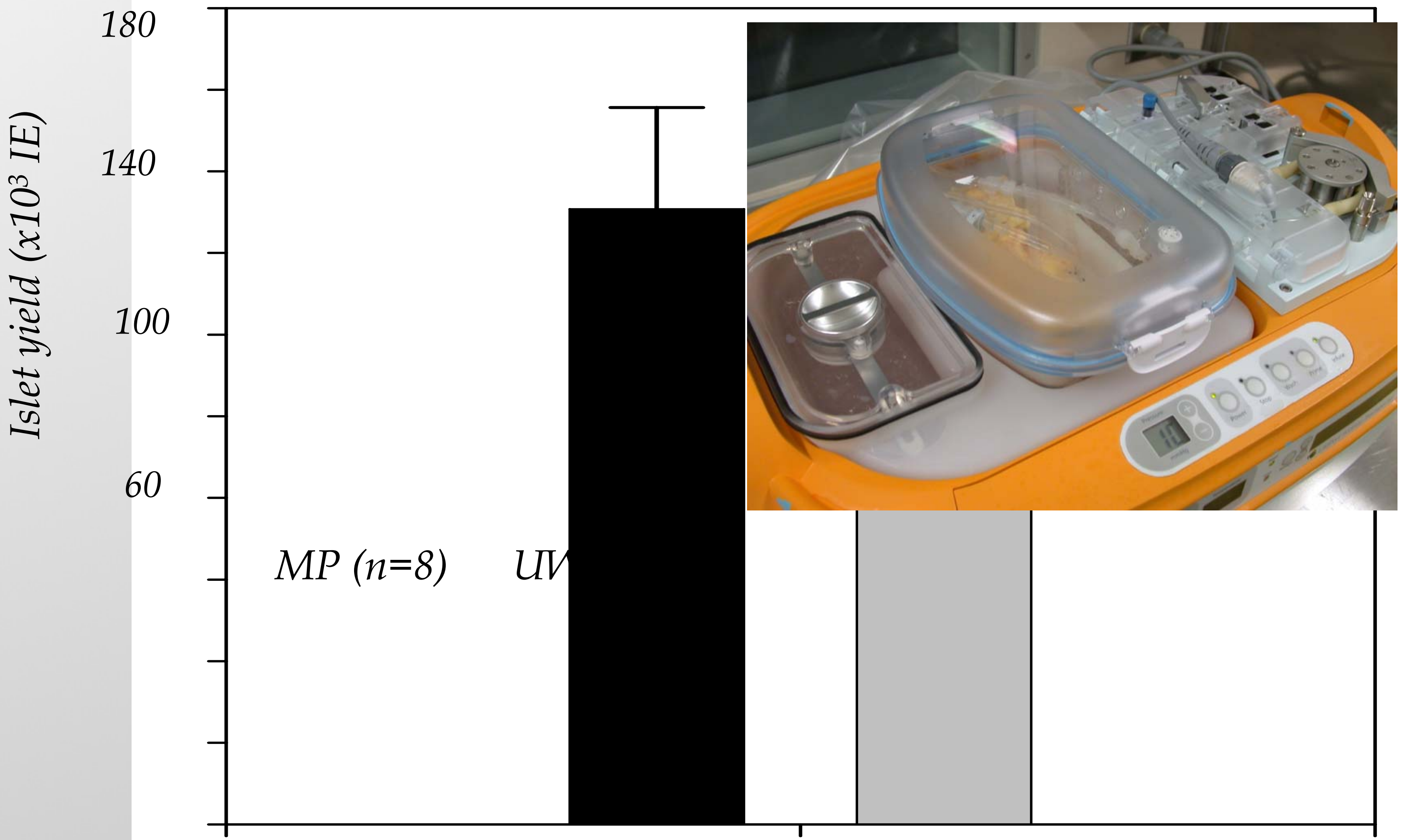
Endothelial damage

Oxygen free radicals

Expensive and Heavy

Islet Yield after Mechanical Perfusion

Improved yield, but not statistically significant
P=0.27



American Journal of Transplantation 2006; 6: 2060–2068
Blackwell Munksgaard

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Improved Human Islet Isolation Using Nicotinamide

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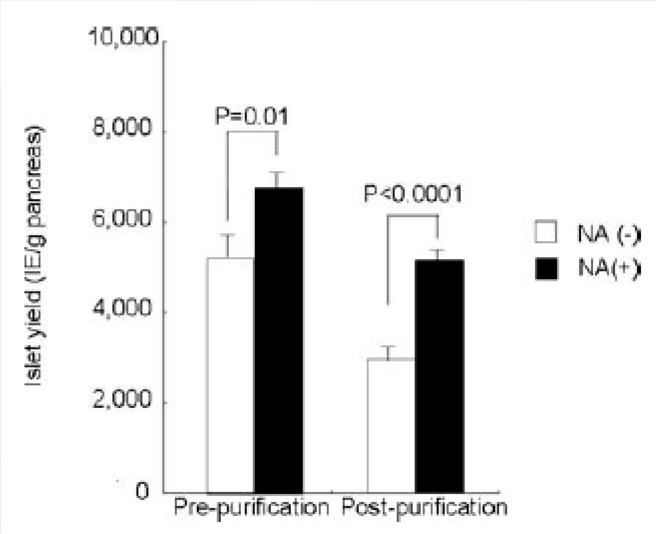
for the separation of islets and more effective immunosuppressive strategies (1–4). Improved islet isolation has been key to the evolution of this procedure (5–8). However, it is still difficult to consistently produce adequate islet num-



n=102, 10 mM NA(−) (n = 47) or NA(+) (n = 55)

Table 5: Human islet and isolation variables

Group	Viability (%)	Stimulation index	Preparation transplanted (%)	Successful isolation (%)
Group I (n = 36)				
UW + NA(−)	85.5 ± 1.8	2.2 ± 0.3	25	22
Group II (n = 19)				
UW + NA(+)	91.7 ± 1.4	2.1 ± 0.3	47	58
Group III (n = 11)				
TLM + NA(−)	91.6 ± 1.5	2.3 ± 0.3	45	55
Group IV (n = 36)				
TLM + NA(+)	94.4 ± 0.7	2.0 ± 0.3	69	72

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Intra-Ductal Glutamine Administration Reduces Oxidative Injury During Human Pancreatic Islet Isolation

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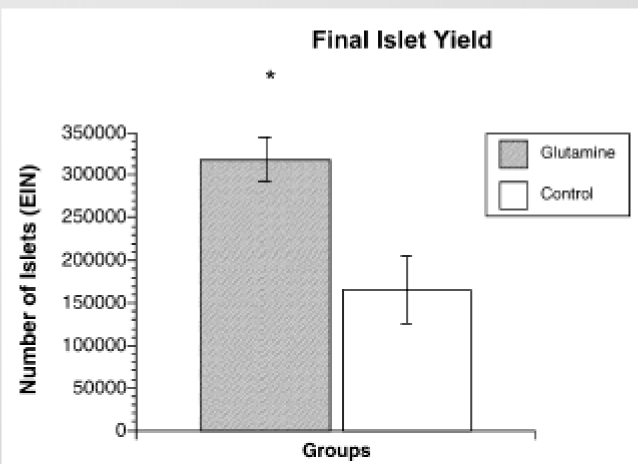
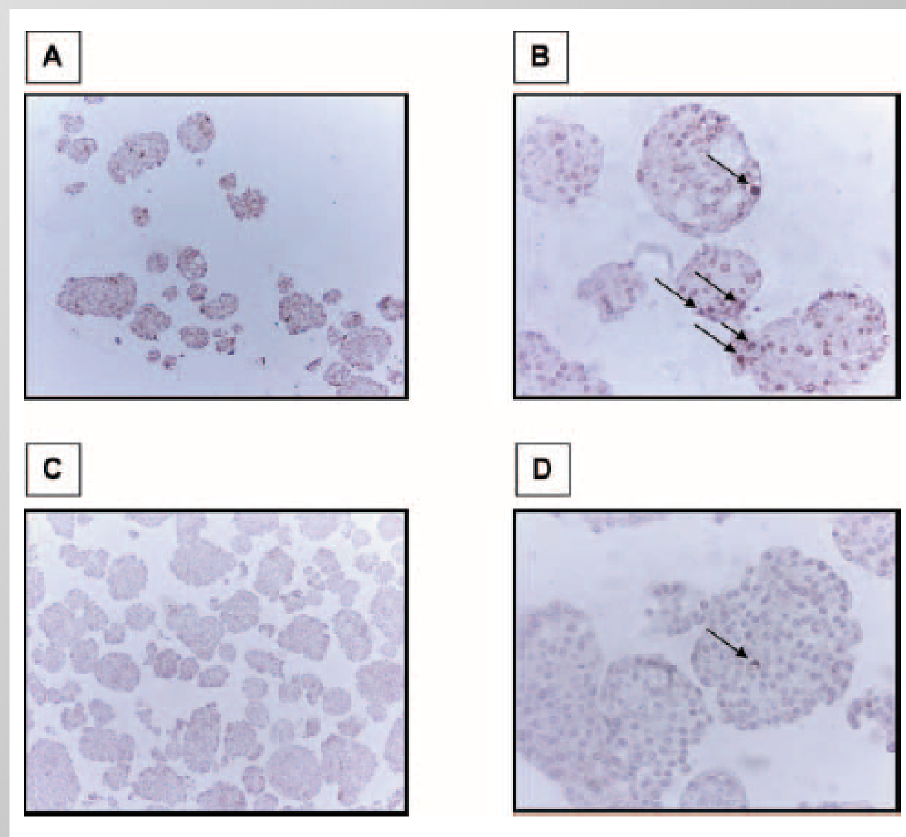
Introduction

In the transplant setting, islets of Langerhans are faced with various types of stress related to the isolation and transplantation procedure, which trigger a cascade of cell

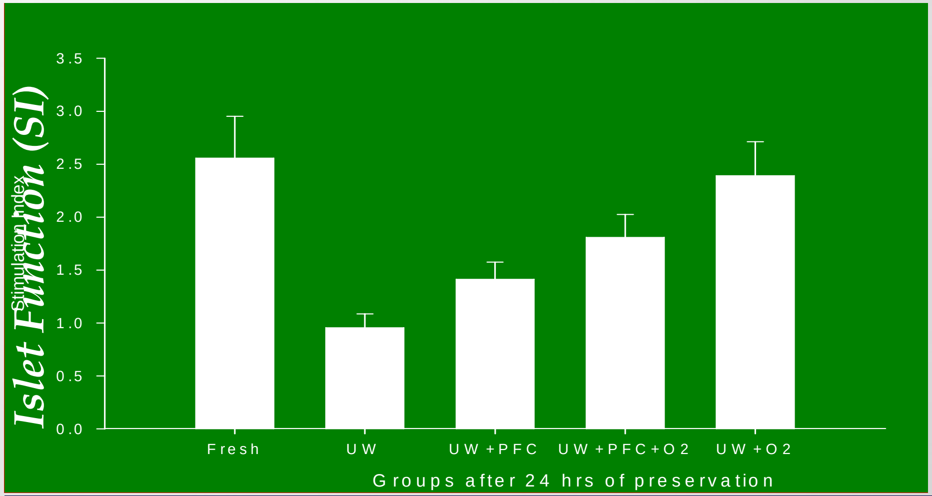


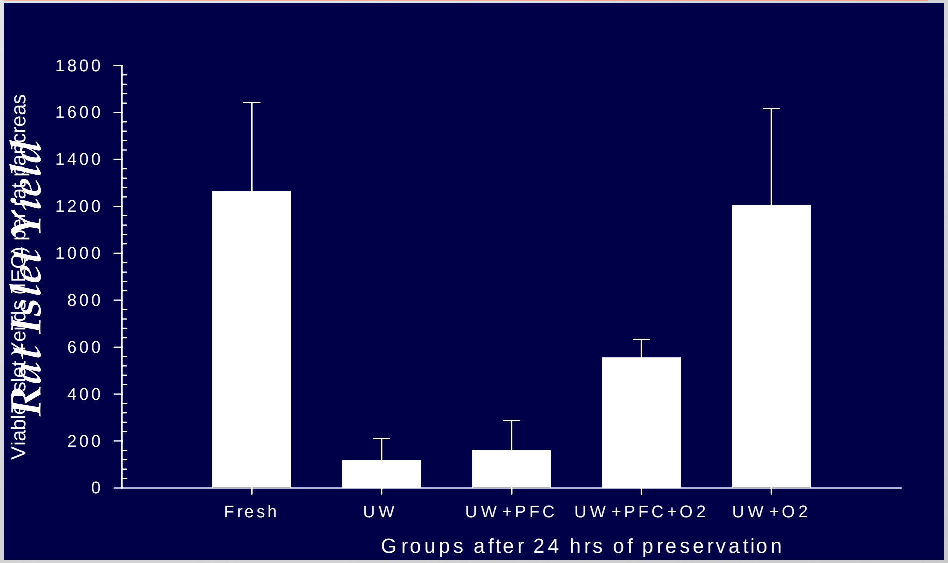


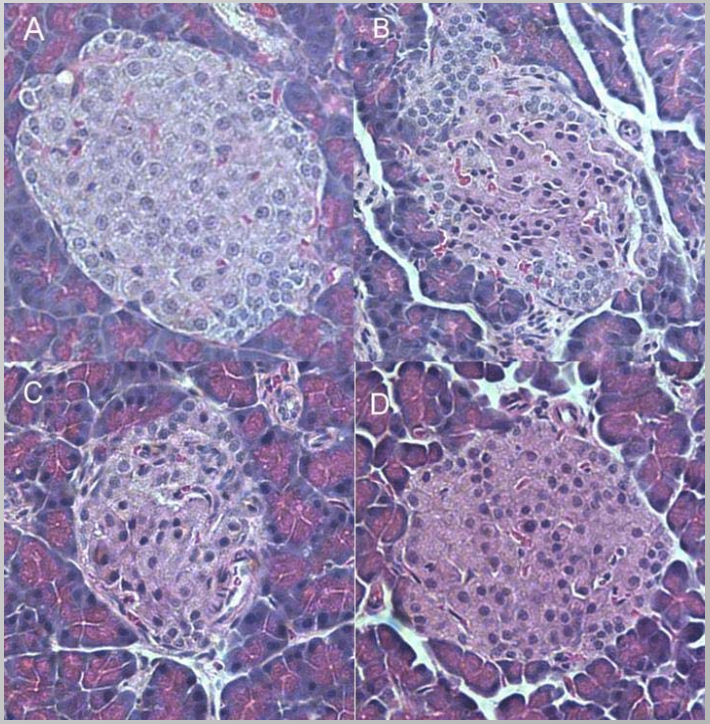
BUBBLING OF OXYGEN IN THE UNIVERSITY OF WISCONSIN SOLUTION DURING PANCREAS PRESERVATION IMPROVES ISLETS SURVIVAL.

Mirbolooki, et al.,
Department of Surgery, University of Alberta, Edmonton, AB Canada.

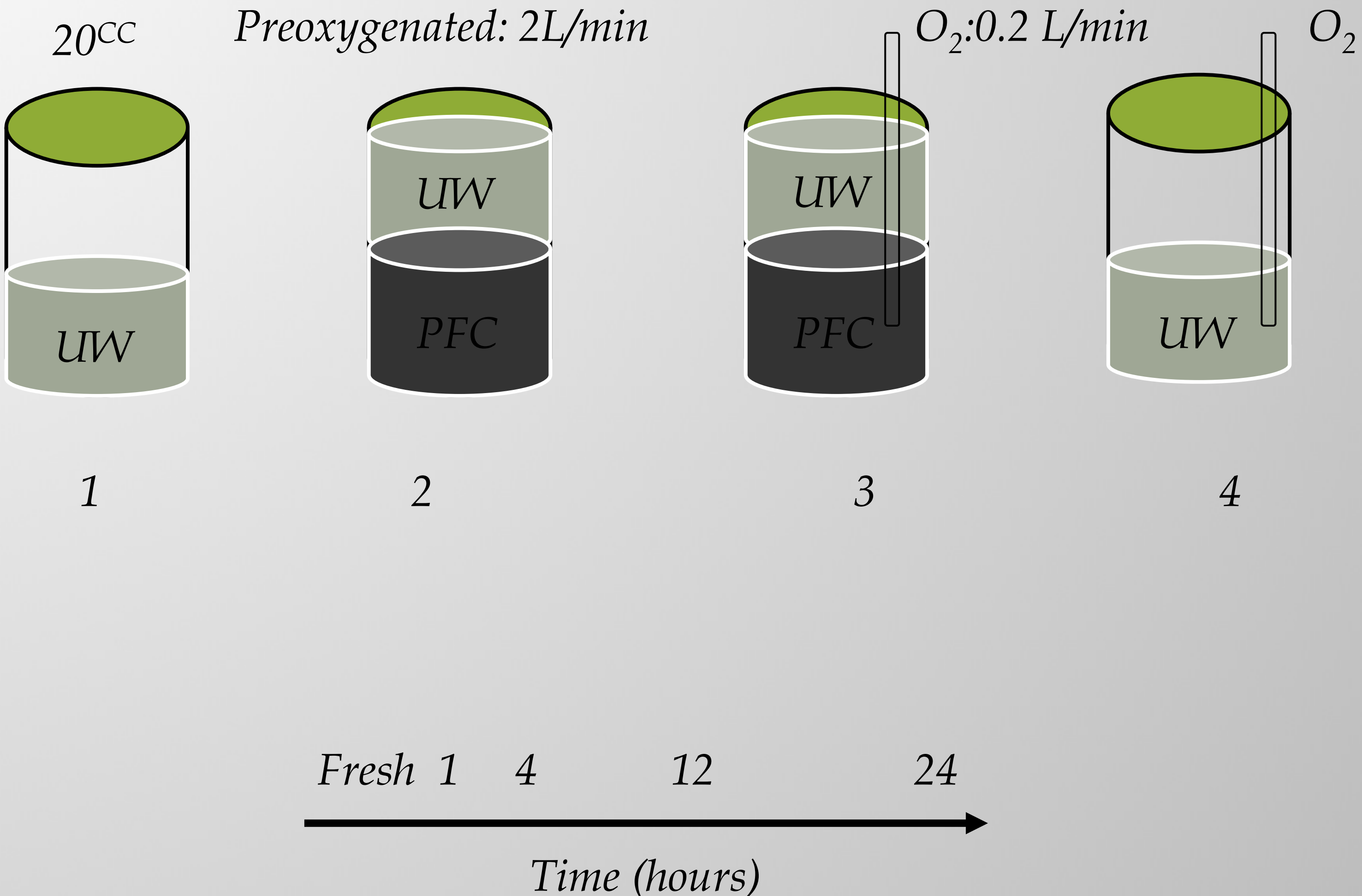
CTS Meeting, Banff 2007



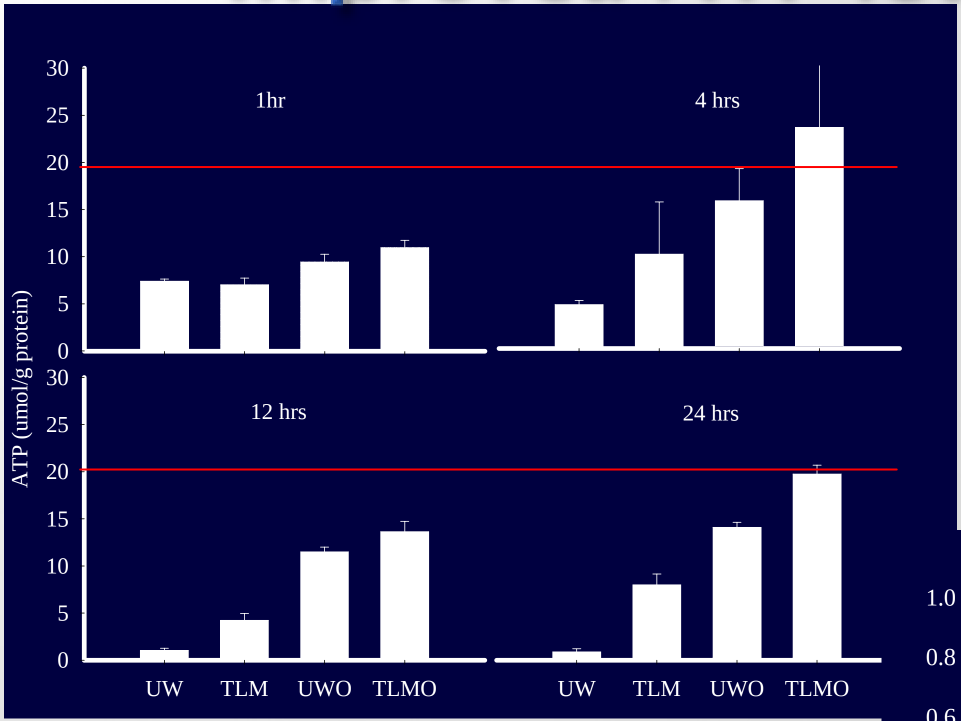




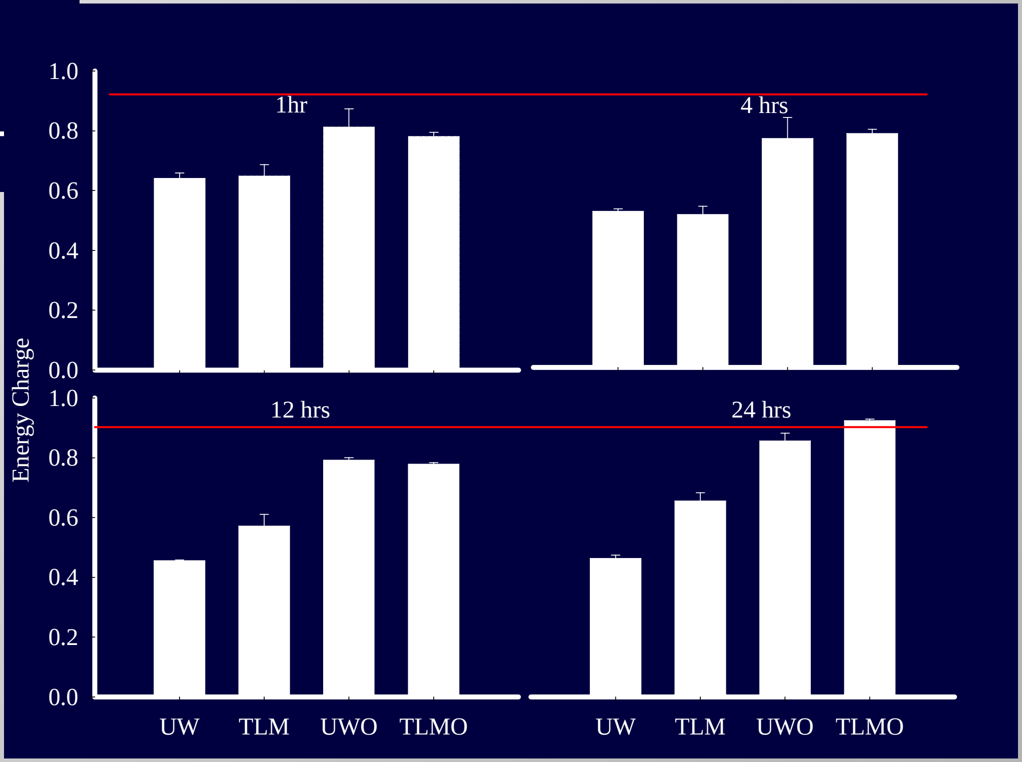
Experimental Plan



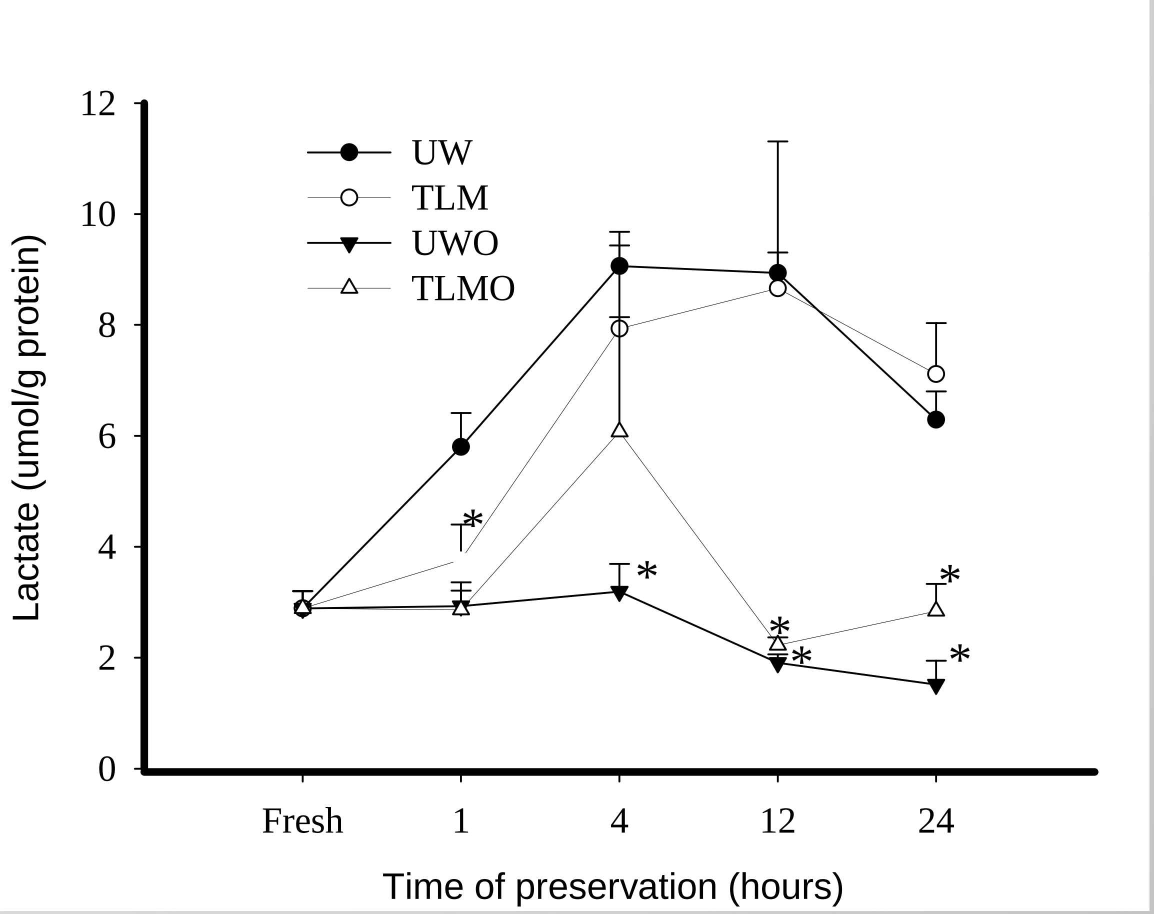
Improved ATP levels and Function



Continuous bubbling of oxygen improves ATP production and Energy parameters significantly

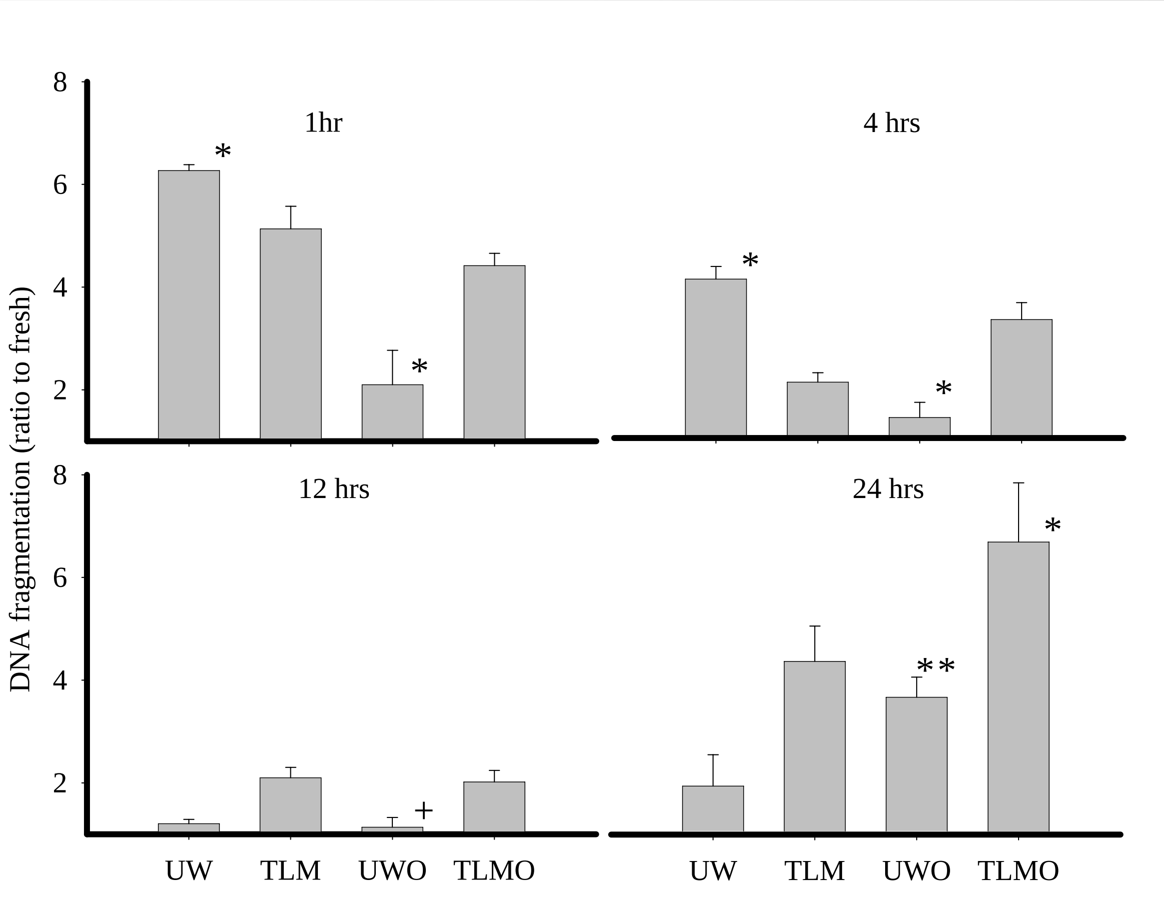


Bubbling of oxygen reverse anaerobic to aerobic metabolism



Lactate level of pancreatic tissue during 24 hours of rat pancreas preservation.
** p<0.001 as compared to UW group*

Continuous bubbling of oxygen prevents apoptosis



DNA fragmentation level of pancreatic tissue during 24 hours of rat pancreas preservation.
** p<0.01 as compared to other groups, + P<0.001 as compared to TLM and TLMO, ** p<0.01 as compared to UW*

Summary

- *Donor factors can predict islet isolation success*
- *Pancreas procurement is a critical step in islet success*
- *Pancreas preservation should be minimized*
- *Benefits of two layer preservation need to be fully elucidated*
- *Novel strategies will allow improved recovery and islet function*