VIABILITY OF FRESH vs CULTERED ISLETS FOR TRANSPLANTS

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Pig islet work is problematic

- Pig islets are generally difficult to
  - isolate intact
  - recover during purification
  - preserve during in vitro culture
- Transplants of pig islets have been shown* both
  - not to cure STZ-diabetes in nude mice, or
  - only after ~ 2months,
  - or within days

? What about the role of islet viability ... —>

* Heald et al. Acta Diabetol 35: 238, 1998
Davalli et al. Diabetes 44: 104, 1995
Brandhorst et al. Exp Clin Endocrinol 103: 3, 1995

The role of the viability of isolated islets, in the outcome of culture or grafting...

- .... has received little attention
- We isolated islets from 12 large sows by Liberase digestion, dispersion in cold UWS, and OptiPrep-UWS* purification
- The viability of islets was studied both immediately after isolation ('fresh islets'), and after culture for 1 day or 1 week (in RPMI + pig serum, 37°C), by comparing:
  - islet recovery
  - acridine orange / propidium iodide staining (AOPI)
  - grafting ~ 750, ~1500, or ~3000 IEQs SRC in nude mice

* Working OptiPrep-UWS will be produced by Nycomed within months

ISOLATION AND CULTURE RESULTS

	FRESH ISLETS	1-DAY CULTURE	1-WEEK CULTURE
ISLET # (IEQs)	1924 ± 346		
PURITY (%)	96 ± 2	~ 100	~ 100
RECOVERY (%)	(100)	21 ± 4	11 ± 2
DIAMETER (µm)	162 ± 13	124 ± 6	113 ± 4
VIABILITY (% AO)*	83 ± 2	87 ± 2	90 ± 2

* viability staining of fresh islets by AOPI does not really help in predicting recovery during culture

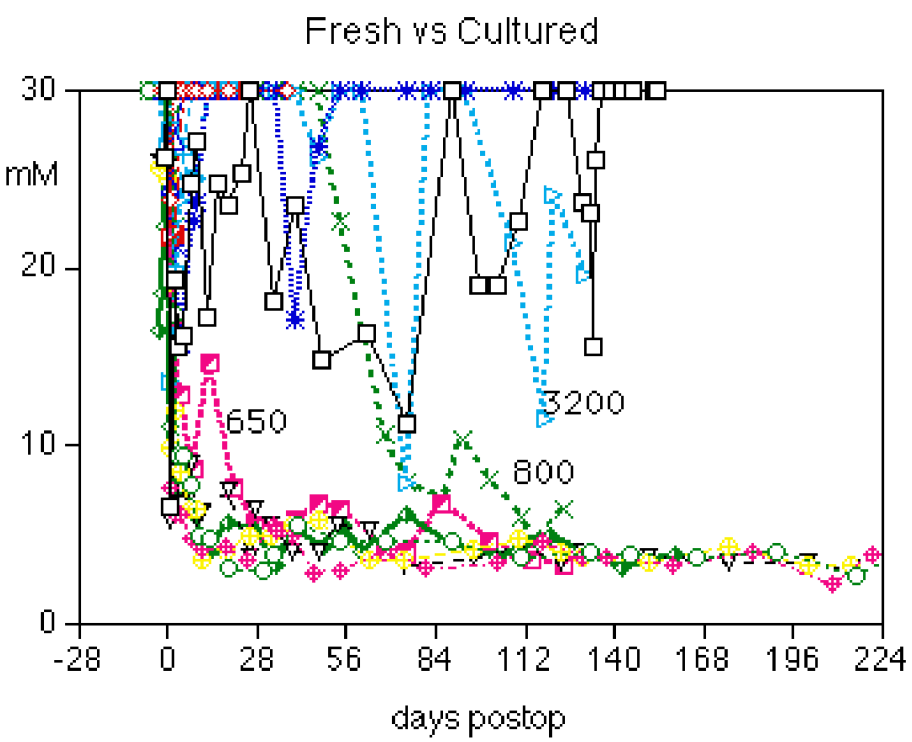
TRANSPLANT RESULTS OF FRESH VS 1 DAY OR 1 WEEK CULTERED ISLETS

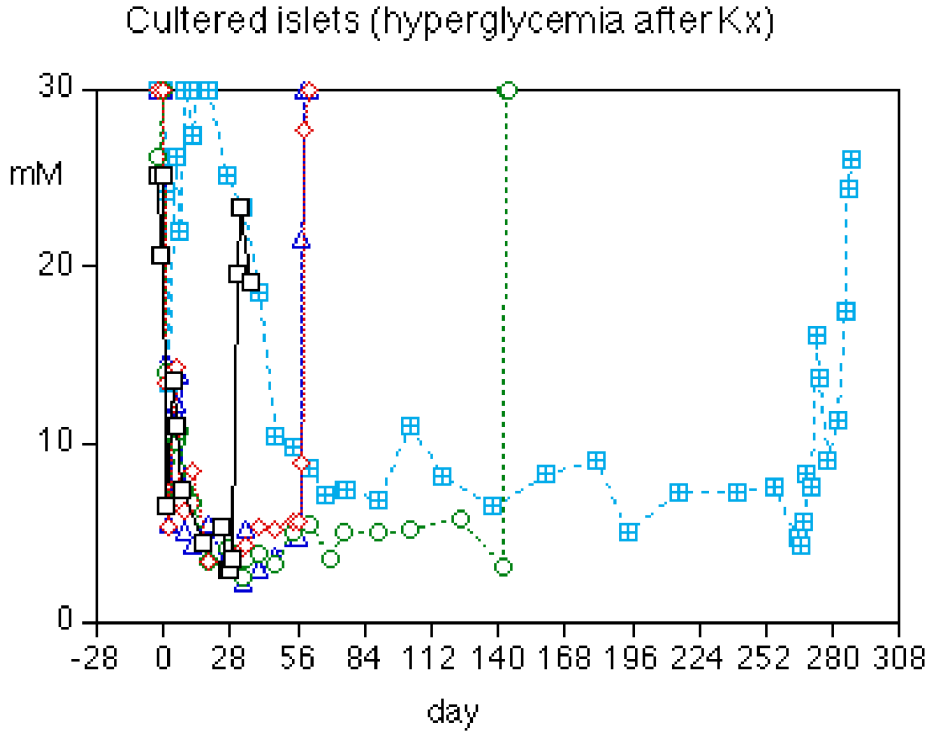
CULTURE	DOSE	MICE*	FAILURE		SUCCESS (<10.7 mM)				
		n	*n*	Glycemia	*n*	Days to normo		Glycemia mM	
NO	1500	14	13**	> 24 mM	1	1		7.9	
	3000	4	4**	> 25 mM					
1 DAY	750	8	3	> 25 mM	5	27	(8 - 61)	6.6	(4.2 - 10.1)
	1500	9			9	15	(1 - 53)	6.1	(4.1 - 10.6)
1 WEEK	750	4			4	56	(21 - 111)	7.4	(5.4 - 8.5)
	1500	9			9	2	(1 - 4)	5.0	(4.2 - 6.2)

* Swiss nu/nu mice received STZ 220 mg/kg at 5-7 days before Tx
normal random blood glucose in 15 animals: 5.3 - 10.7

** Glycemia usually > DL of 28 mM; usually killed at ~ 3 wk for severe diabetic complications

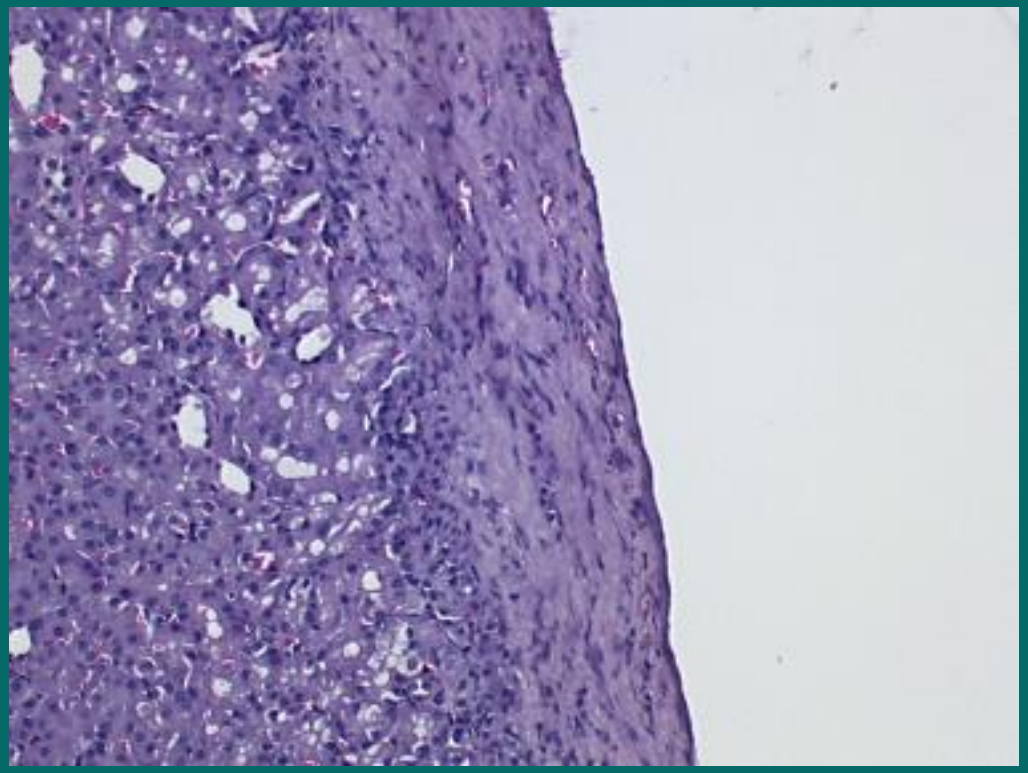
GRAPHS

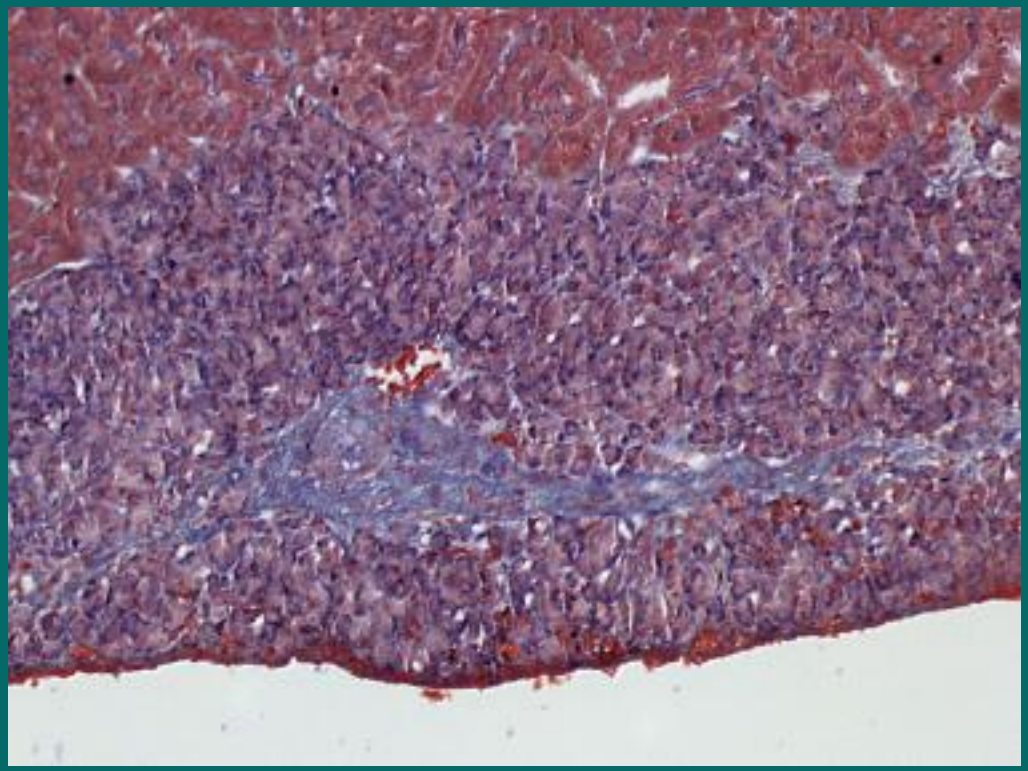

Fresh vs Cultured
30
mM
20
10
0
650
3200
800
-28
0
28
56
84
112
140
168
196
224
days postop


Cultered islets (hyperglycemia after Kx)
30
mM
20
10
0
-28
0
28
56
84
112
140
168
196
224
252
280
308
day

HISTOLOGY

- Substantial scarring and ~ no islets found in fresh islet recipients
- Well-preserved islets and little scarring in recipients of cultured islets





WE CONCLUDE:

- Culture of the islets before transplantation markedly improved the viability of the graft

- Disintegration of part of the fresh islets
  - may not only reduce the effective islet dose
  - but also hamper the engraftment of viable islets
    - because of scarring, and
    - because the cellular debris probably will attract macrophages and induce the release of harmful cytokines