MARKEDLY IMPROVED OUTCOME OF ADULT PORCINE ISLET ISOLATION, PURIFICATION, AND CULTURE USING LIBERASE-PI VERSUS COLLAGENASE-P, AND A NOVEL GRADIENT OF OPTIPREP IN UW SOLUTION.

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Barrier on the way of pig-to-man islet Tx

- The marked fragility, rapid dissociation, and loss of pig islets during isolation and especially the purification process* is a well-known barrier on the way to xenotransplantation
- Hypothesis: islet yield, integrity, and viability are endangered by:
  - the use of crude collagenase preps for digestion
  - the changing conditions generally introduced by the use of several different solutions during the digestion and further isolation and purification steps

*reported e.g. by Edmonton

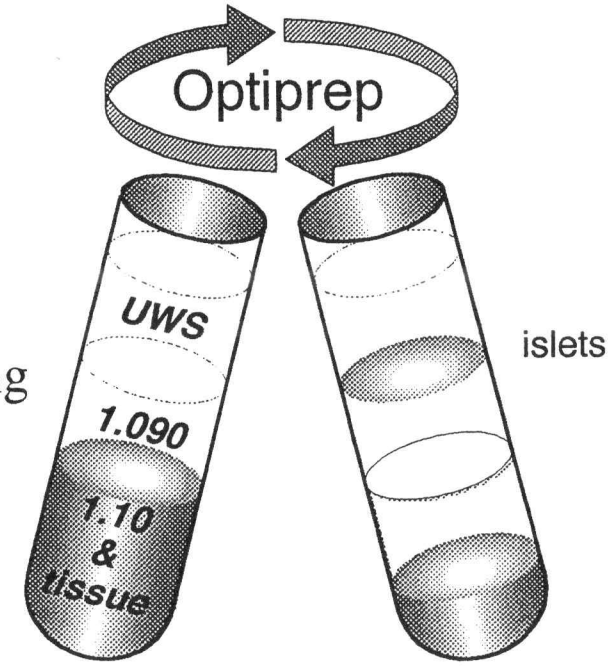
DESIGN

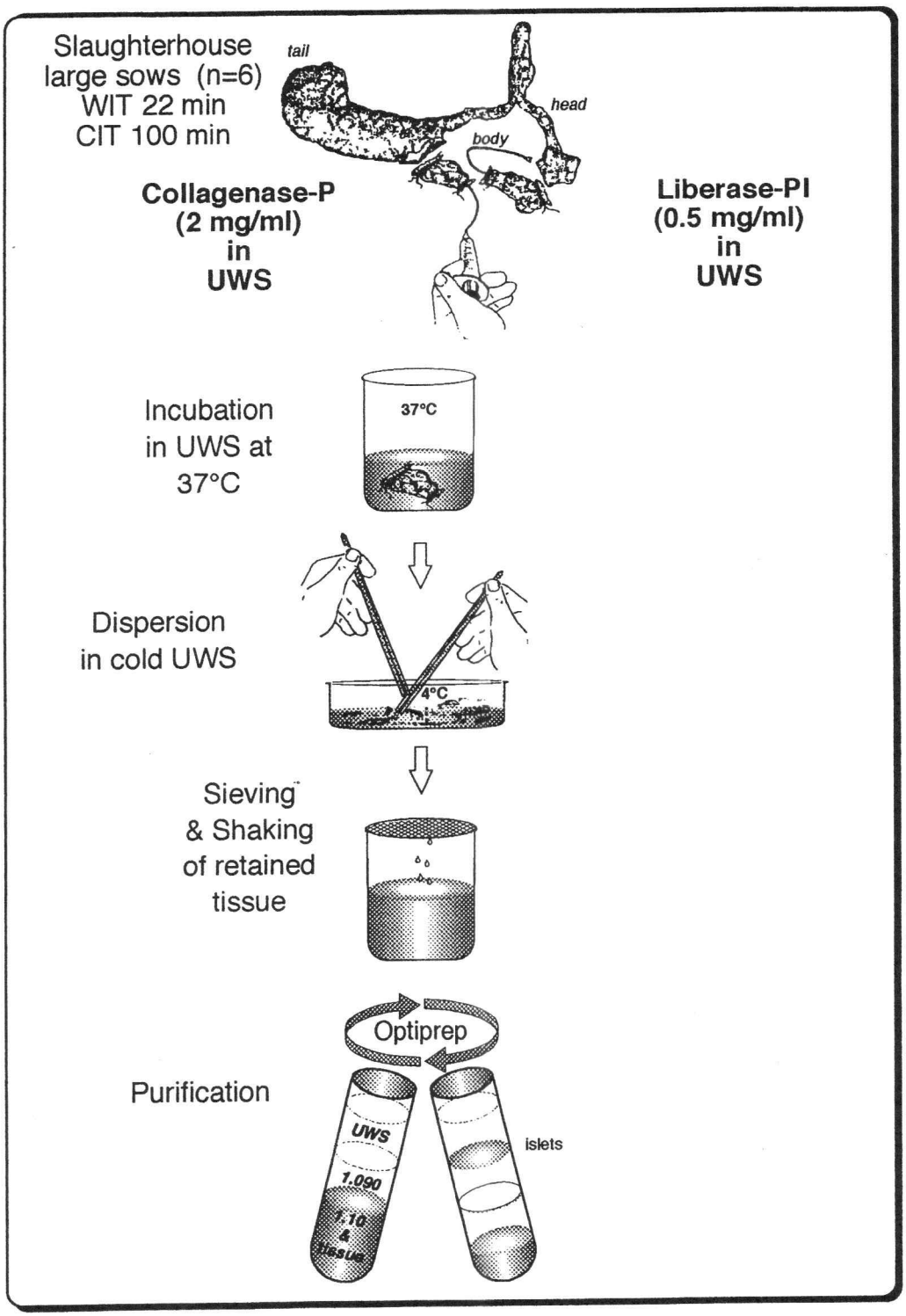
- We aimed at improving the preservation of pig islets by keeping the islets in the University of Wisconsin solution (UWS) both during isolation and subsequent purification in a novel iodixanol* density gradient in UWS.
- We compared the novel purified Liberase-PI enzyme blend vs convential collagenase-P for islet isolation by in parallel processing of two, paired, segments of the pancreas of large sows in 6 consecutive experiments.

*Optiprep™; Nycomed Pharma

RECIPE FOR OPTIPREP-UWS GRADIENT

- Optiprep (Nycomed) is a 60% iodixanol in water solution
- Working Optiprep Solution (WOP) is prepared by mixing equal volumes of
  - ◇ Optiprep
  - ◇ a 2-times concentrated UWS
- The bottom solution is prepared by mixing 10 ml WOP and 20 ml digest (in UWS)
  - ◇ Density is ~ 1.1 g/ml
  - ◇ Osmolality is ~380 mOsm
- The bottom is over-layered with a 1.090 density solution of Optiprep in UWS
  - ◇ a mixture of 10 ml WOP and 26.4 ml UWS
- The gradient is topped with UWS




Slaughterhouse
large sows (n=6)
WIT 22 min
CIT 100 min
tail
head
body
Collagenase-P
(2 mg/ml)
in
UWS
Liberase-PI
(0.5 mg/ml)
in
UWS
Incubation
in UWS at
37°C
37°C
Dispersion
in cold UWS
4°C
Sieving
& Shaking
of retained
tissue
Purification
Optiprep
UWS
1.090
islets

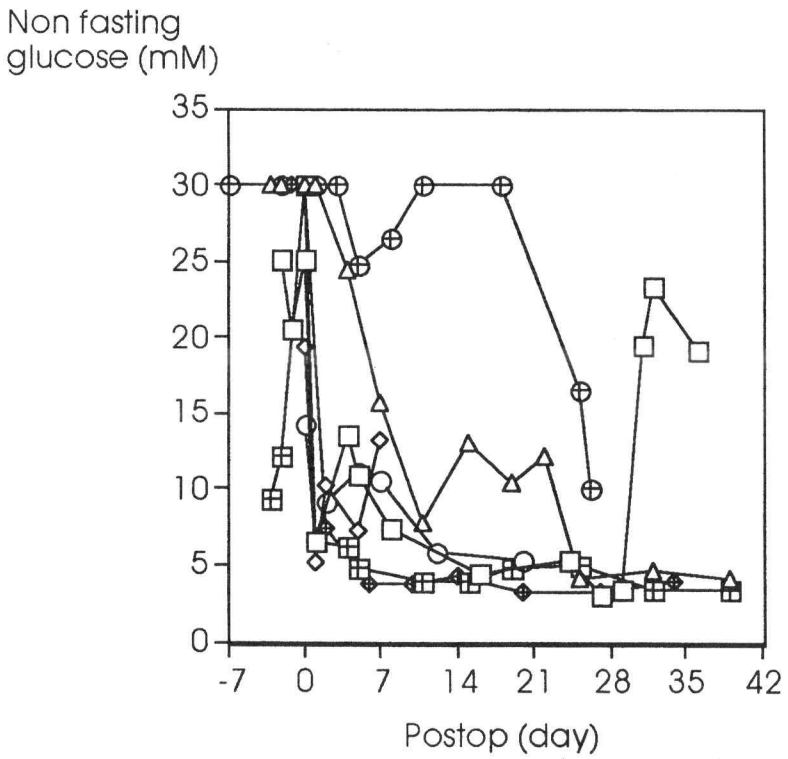
RESULTS

	LIBERASE	COLL-P
DIGEST		
Yield (IEQs/g)	2529 ± 783	2359 ± 511
Size (μm)	151 ± 20	160 ± 12
PURIFIED		
Yield (IEQs/g)	2448 ± 392	1319 ± 224*
Size (μm)	185 ± 16	140 ± 8*
Purity (%)	>95%	>95%
Viability (%)†	90 ± 2	79 ± 4*
CULTURE (RPMI 24h)		
Yield (% digest)	24 ± 9	14 ± 8*

† acridine orange - propidium iodide staining

* $P < 0.05$

Pig (500–2500 IEQs) to nude mice (SRC)



Viability of cultured (liberase) islets was corroborated by non-fasting normoglycemia >4 wk posttransplant (~ 1000 IEQs under kidney capsule) in 5/6 nude mice

IN CONCLUSION

- Islet integrity and viability are markedly improved by Liberase digestion (vs Collagenase-P), and the continuous use of UWS throughout isolation and purification in our novel Optiprep-UWS gradient.
- The Optiprep-UWS gradient allows the complete recovery of highly purified, viable, pig islets
- Continuous preservation of cell viability in UWS during isolation and purification, and absence of trypsin in Liberase appear to be key factors in the successful outcome