- ICPIT 93
  - First slide please: TITLE
    - Thank you, chairman, ladies and gentleman
    - We studied islet isolation efficacy in dogs
  - Next slide: VARIABILITY OF ISOLATION OUTCOME
    - The variability of isolation outcome is a major problem hampering human islet transplantation
    - And difficult to analyse, due to the intertwined effects of numerous variables, like:
      - predonation events
      - donor & pancreas characteristics
        - such as the islet content of the pancreas
      - organ preservation conditions
      - and isolation methods
    - Knowledge of the variability due to INTRINSIC factors like the islet content of the individual pancreas
      is essential for analysis of the EXtrinsic factors
  - Next slide: PARAMETERS FOR ISOLATION EFFICACY
    - In the past, variability of the pancreatic islet content was, indirectly, calculated in:
      by estimating islet yield from insulin recovery from the pancreas
      - However, because ínsulin recovery may not accurately reflect íslet yield,
        this method has been largely superseeded by the current method of :
    - islet sizing—to assess final yield
    - As yet, however, no attempts have been made to compare yield with the islet volume of the pancreas
  - Next slide: OUR STUDY

- We therefore studied the impact of interindividual differences in pancreatic islet content and other donor characteristics on isolation outcome
- and compared the assessment of isolation efficacy by morphometry and insulin extraction
- Results from 31 consecutive islet isolations from the splenic dog pancreas, are reported

Next slide: ISOLATION METHOD

- Isolation from the splenic pancreas was performed by
  -collagenase digestion
  -decantation of the collagenase solution, and
  -dispersion of tissue in UW solution by syringing and screening
  -The islets were purified in density gradients
- Samples were taken from the pancreas, the digest, and from the pure and rest fractions of the gradients

Next slide: ASSESSMENT

- For assessment samples were compared:
  - by morphometry to assess islet volume and size
    - For sizing on H&E stained sections of the pancreas we used a grid
    - During isolation the mean Ø of DTZ stained islets was used
      - islets entrapped in acinar fragments
        - roughly estimated
        less than 20% of total islet volume-
        were not counted
  - Extraction was performed for assessment of insulin and amylase recovery
  - And microscopy, to compare ß-cell granulation
- Islet function, was studied by perifusion

- Next slide: DONOR CHARACT
  - This table summarizes the donor characteristics
    - Animal wt ranged from 9-18 kg:
      thus, varied 2-fold
    - Age: 8-67 mo, varied 8-fold
    - And the fractional islet volume in the pancreas averaged 1.6 %, and varied 3-fold
- Next slide: Fig BODY WT & AGE vs ISLET
  - Body wt and age correlated with the islet content of the pancreas
    - the islet content íncreased with wt
    - and décreased with age
  - After standardizing the units for islet-volume, body wt, and age: multiple regression demonstrated
    similar—though opposite—effects of Wt and age on the islet content
    - A 100% change of Wt or age resulted in a 70% change of the islet volume of the pancreas
    - A similar effect was evident on islet yield in the digest
  - Together Wt and age explained 50% of the variability of the islet volume in the pancreas, and digest
- Next slide: IMPACT ISLET ON OUTCOME
  - The islet content of the pancreas had a similar impact on islet yield in the digest
    - Again 50% of the variance in islet yield was explained by differences in the fractional islet volume of the pancreas
- Next slide: TABLE RECOVERY TISSUE etc

- We next compared recovery of tissue, amylase, insulin and islet volume in the Digest and the Pure and combined Pure & Rest fractions of the gradients
- Listed are the actual values and percentages (the bold values). We will concentrate on the percentages
- Tissue, amylase, and insulin recovery amounted to 80-90%,
  Islet recovery however was 49%
  Suggesting that we had underestimated the proportion of entrapped islets during sizing.
- This explanation is supported by the distribution of insulin and islets in the gradients. Recovery in the gradients was expressed as a percentage of the digest content
  - Insulin recovery in the pure fraction (36%) was about half of the recovery in the combined pure and rest fractions (83%)
  - In contrast, most of the recovered islets in pure & rest fractions (64%) was located in the pure fraction (53%)
  - thus, the distribution of insulin again indicated a high proportion of entrapped islets in the acinar fraction of the gradients
- Purity amounted to 94%
- And the ratio of insulin and islet measures:
  1.5 in the pancreas and 1.8 after purification,
  indicated no loss of insulin from islets

Next slide: RCM

- These results were confirmed by reflection contrast microscopy of sections of the pancreas (A) and purified islets from this pancreas (B), stained by the immuno-gold method for insulin
  - beta-cell granulation was similar

- granulation of other non-stained cells, probably alpha-cells, is also evident in these micrographs; note a small vessel, and zymogen granules in the pancreatic section

Next slide: CORRELATION ANALYSIS: PANCREAS VS DIGEST

- We further compared morphometry and insulin extraction by correlation analysis
  - The islet content of the pancreas correlated well with islet and insulin yield in the digest suspension
  - Similar findings were obtained for pancreatic insulin

Next slide: DIGEST VS PURE ISLET/INSULIN

- Next, we compared islet and insulin in the digest and pure fractions of the gradients
  - The islet content of the digest (both Top panels) correlated well with islet yield (A) and insulin (B) in the pure fraction,
    The insulin content of the digest, however (bottom panels) did clearly nót correlate with islet and insulin yield in the pure fraction
  - Demonstrating the impact of entrapped islets on assessment by insulin measures

Next slide: ISLET SIZE

- Size distributions of islets in the pancreas, the digest and pure suspensions were similar.
  And the average diameter demonstrated no fragmentration

Next slide: PERIFUSION

- Viability of the overnight cultered islets was examined by perifusion

- Aliquots were perifused in parallel, initially with low glucose, and from zero minutes with 2.5 to 10 mM glucose.
  A 10-fold insulin release during physiological glucose stimulation indicated full functional activity.

- Last slide please: WE CONCLUDE
  - We conclude, that the variability of islet and insulin yield, and size of isolated islets, may be attributed to a large extent to the variability of the native endocrine pancreas
  - Isolation efficacy was best documented by morphometry of both the isolated and native islet population
  - Additional insulin extraction allowed quantitation of entrapped islets—which we had underestimated during sizing—and further documented preservation of ß-cell granulation during isolation
  - Similar studies should facilitate analysis of other factors affecting isolation outcome in man