Immotile Short-tail Sperm

- an autosomal recessive disease
- first detected in Finnish Yorkshire boar in 1987

Andersson et al. 2000

- defect is expressed in males resulting in **total infertility** due to short tailed and immotile spermatozoa
- specifically affects the axoneme structure of sperm flagella; cilia in other tissues appear unaffected
- manifested during spermiogenesis
- affect spermatogenesis at the spermatid elongation phase (Figure 1)

Sukura et al. 2002

- ISTS syndrome express the short tail characteristic sometimes with rudimentary or coiled tails and have lower sperm counts
- Approximately 5% of spermatozoa from affected boars have tails of normal length, but none are motile
- Sperm heads appear to develop normally, but cytoplasmic droplets were abundant

Sukura et al. 2002

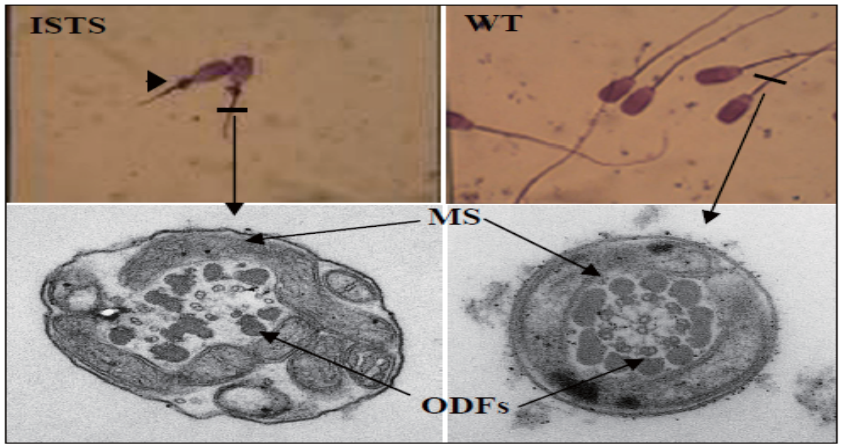


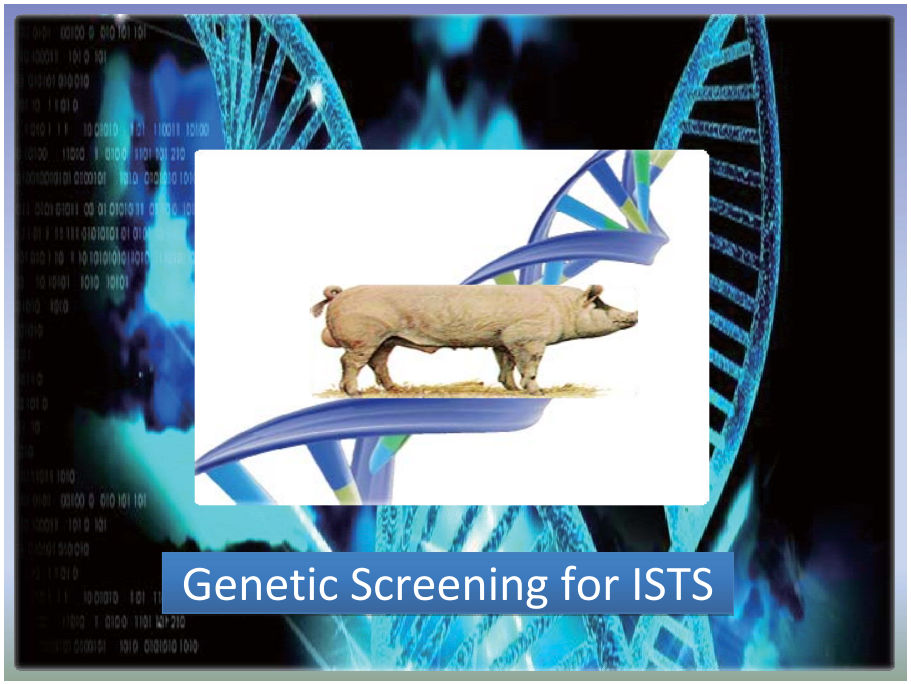
Figure 1. Structure of sperm of ISTS and normal (WT) boars. Sperm tail of ISTS affected boars is short and the proximal droplet (arrowhead) is retained in the neck region. The axoneme and accessory structures (MS, ODFs, indicated by arrows) of the ISTS flagella are also disorganized.

- ISTS disorder appears to be sperm specific, since no adverse effects on respiratory function or female reproduction have been identified

Andersson et al. 2000

SPEF2/KPL2 GENE

- Sperm flagellar protein 2
- Found in porcine chromosome 16 and contains 43 exons and spans 197.5 kb
- important for correct sperm flagella development



Genetic Screening

- The causal mutation for the ISTS defect is a recent L1 insertion within the SPEF2 gene in chromosome 16.
- Primers :

  Forward (withinexon30) – GGCAATATCAAGGTCTTTCCA
  Reverse Primer (within insertion) – GTGCCCGTAGTTCAGATGG
  Reverse Primer (beyond the insertion) – GCAGGAGAGGAGAATGACCA

Sironen et al., 2007

- The insertion was found to be homozygous only in ISTS-affected boars and heterozygous in carrier pigs (Figure 2)

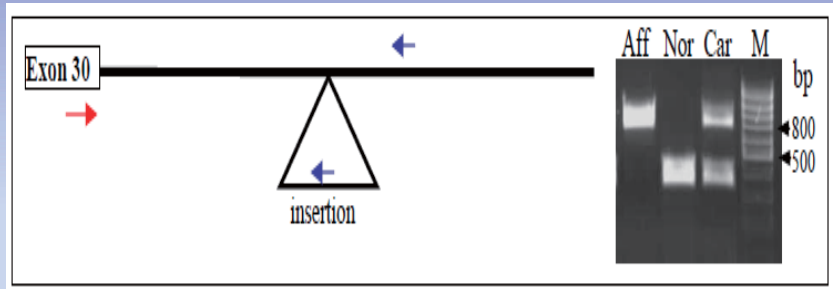


Figure 2. The PCR based test used for gene assisted selection of ISTS. The forward primer within *KPL2* exon 30 is indicated by a red arrow and reverse primers within the insertion and intron 30 just after the insertion by blue arrows. In ISTS-affected animals (Aff) only a fragment of 863 bp is detected. In samples of unaffected animals (Nor) a fragment of 354 bp is present. Both fragments are detected in ISTS carrier animals (Car). M = molecular marker.

Sironen et al., 2007

- the mutation appears to be specific to the Finnish Yorkshire, since the *KPL2* insertion has not been detected in samples from individuals of the Duroc, Hampshire, Landrace or Danish Yorkshire breeds

Sironen et al., 2007

- **Implication of KPL2 and its function**

✓ The ISTS defect is caused by an active L1 insertion within *KPL2* intron 30, resulting in aberrant splicing of exon 30

✓ *Kpl2* expression correlates with ciliated cell differentiation and dynein expression *in vitro*

✓ in ISTS-affected pigs the lack of the long form of KPL2 only affects the structure and function of the sperm tail, indicating a specific functional importance of at least one isoform of KPL2 in the testis

✓ KPL2 may be involved in axoneme development

✓ the axonemal structure of flagella was also disorganized in ISTS-affected boars with the implication that it is affected by the mutated *KPL2* either during protein delivery or mediated via accessory structures