

BACKGROUND

The relaxin-like peptide hormone insulin-like peptide 3 (INSL3; previously known as relaxin-like factor, RLF) is a major circulating hormone in the male. It is produced almost exclusively by the Leydig cells of the testis, with anorchid men having undetectable circulating levels of the hormone. INSL3 is made by both fetal and adult-type populations of Leydig cells, but only when these have attained their mature phenotype. Various studies have indicated that the *Ins3* gene and protein are expressed constitutively once Leydig cells are mature, thus making INSL3 an excellent marker of Leydig cell differentiation status. Mice whose *Ins3* gene has been deleted are characterized by cryptorchidism and secondary complications. If the cryptorchidism is surgically corrected at birth, there appear to be no further phenotypic consequences of the mutation in the male. In female mice, however, disruption of the *Ins3* gene leads to a reduction in fertility and increased apoptosis in both steroidogenic and germ cells. INSL3 may be acting in the adult as a survival or antiapoptotic factor vis-a-vis germ cells.¹

The receptor for INSL3 has been identified as the novel G-protein coupled receptor (GPCR) LGR8, also known as *Great*. This receptor is structurally closely related to the GPCR LGR7, which is now shown to be the principal receptor for relaxin in all reproductive tissues. Like the relaxin-LGR7 system, INSL3 appears to act through its receptor to induce an intracellular increase in cAMP, both in transfected cells overexpressing cloned *Lgr8*, and in gubernacular and prostate cells with naturally expressed receptors.² Knockout mice (*Great* mice), whose *Lgr8* gene is deleted, have an identical cryptorchid phenotype to that of the *Ins3* knockout mice, and further breeding experiments have conclusively shown that no other ligand is able to activate LGR8, nor is any other receptor able to respond to INSL3. It has been shown that LGR8 expressed by the cells of the fetal gubernacular ligament, which attaches the testis ventrally to the inguinal body wall, responds to INSL3 by causing a growth and condensation of the ligament, effectively retaining the testis in the inguinal region during the first, so-called transabdominal phase of testicular descent.³ In adults, it was demonstrated that INSL3 has a modulating effect on bone metabolism and LGR8 gene mutations may be linked with human osteoporosis.⁴

The INSL3-LGR8 system appears to be evolutionarily relatively modern. There is no evidence for such a system in any submammalian vertebrate or invertebrate, and it would appear to have evolved parallel to the evolution of the relaxin (as opposed to relaxin-3)-LGR7 system in female physiology. Relaxin-3 is now recognized as being the ancestral hormone, is expressed predominantly in the brain, and acts through

another class of GPCR (GPCR135). Thus, although the LGR7/LGR8 subfamily of GPCRs appears to be quite old, its association with relaxin and INSL3 seems to have evolved together with the development of classical mammalian traits such as a scrotal testis and viviparity.⁵

References:

1. Ivell, R. & Bathgate, R.A.D.: Biol. Reprod. 67:699-705, 2002
2. Kumagai, J. et al: J. Biol. Chem. 277:31283-6, 2002
3. Scott, D.J. et al: Ann. N. Y. Acad. Sci. 1041:13-16, 2005
4. Ferlin, A. et al: Ann. N. Y. Acad. Sci. 1160:215-8, 2009
5. Feng, S. et al: Ann. N. Y. Acad. Sci. 1160:197-204, 2009

TECHNICAL INFORMATION

Source:

INSL3 Antibody is a rabbit antibody raised against a short peptide from human INSL3 sequence.

Specificity and Sensitivity:

This antibody detects endogenous levels of INSL3 proteins without cross-reactivity with other related proteins.

Storage Buffer: PBS and 30% glycerol

Storage:

Store at -20°C for at least one year. Store at 4°C for frequent use. Avoid repeated freeze-thaw cycles.

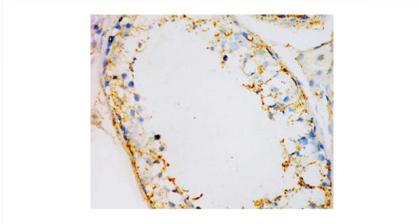
APPLICATIONS

| Application: | *Dilution: |
|--------------|------------|
| WB | 1:1000 |
| IP | n/d |
| IHC | 1:50-200 |
| ICC | n/d |
| FACS | n/d |

**Optimal dilutions must be determined by end user.*



QUALITY CONTROL DATA



Immunohistochemical analysis of paraffin-embedded human testis tissue using INSL3 Antibody.

