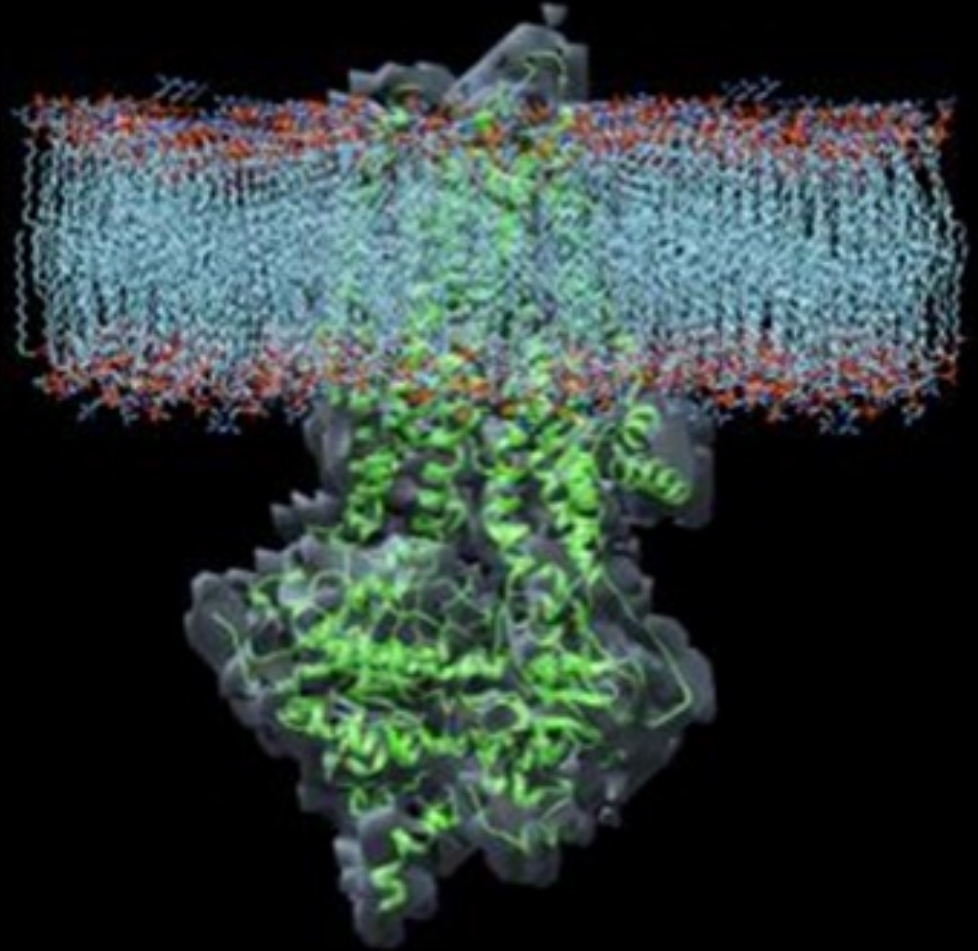


Molecular evidence supports hormonal treatment of cryptorchidism to prevent infertility

Faruk Hadziselimovic



Historical context

John Hunter (1786) congenital imperfection

Bland-Sutton (1910) inherently abnormal

Sohval (1954) dysgenetic testis

Charny (1952) primary defect

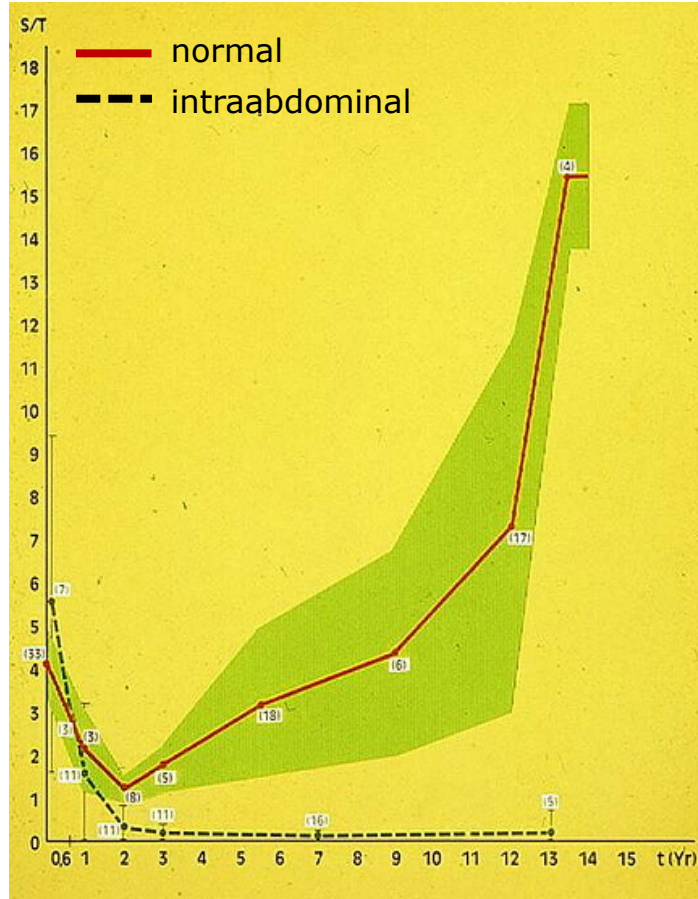
Scott (1961) inherently bilateral testicular defect

Mancini et al. (1965) spermatogonia defective

Scorer & Farrington. (1971) germ cell incapable of responding to hormonal stimulation

Nistal et al. (2000) germ cells- anomalous genetic constitution

Developmental arrest and maturation of germ cells

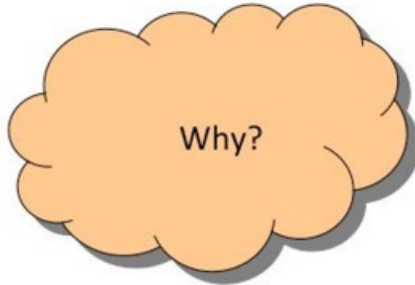


- The critical step of gonocyte-Ad spermatogia differentiation fails and is not triggered by surgery alone

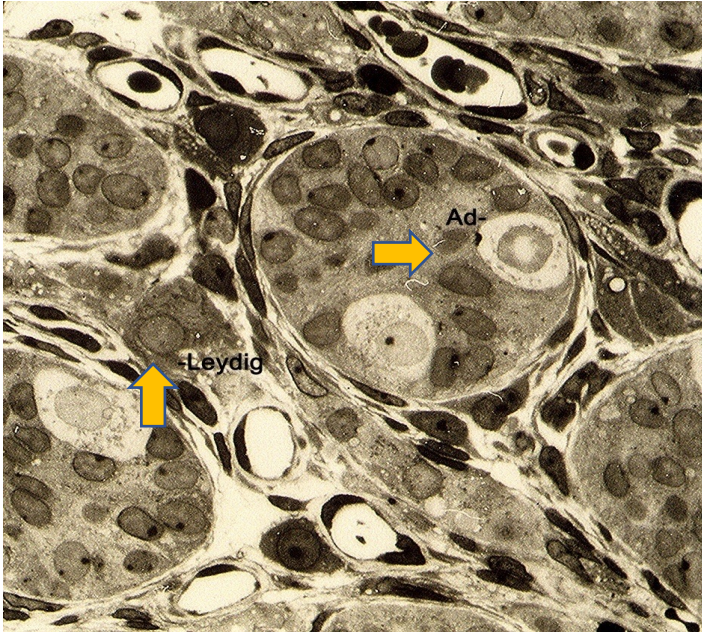
Early surgery is insufficient

36% cryptorchid boys **< 6 months** with a successful surgery and normal germ cell number at surgery, developed infertility.

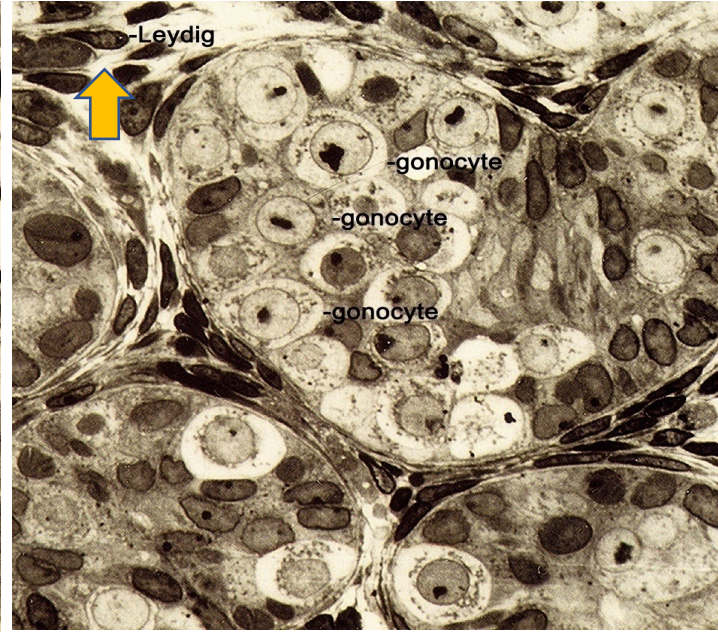
Hadziselimovic F, Herzog B., Lancet; Oct 6, 358;1156-7: 2001



The histology of cryptorchidism

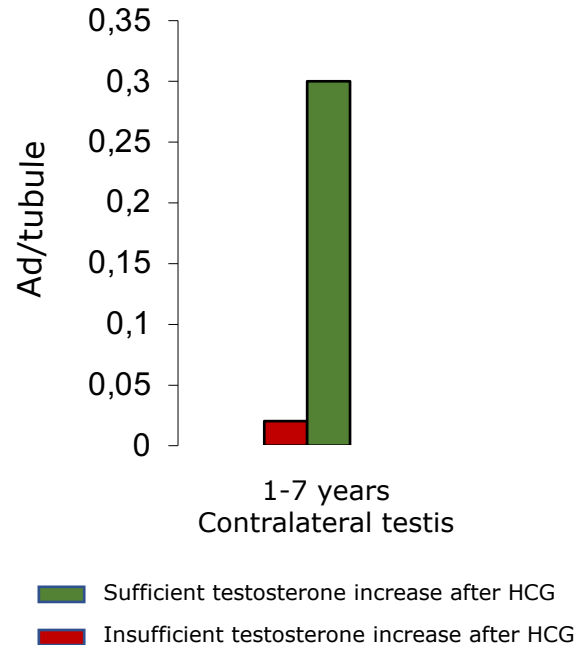


Normal
mini-puberty



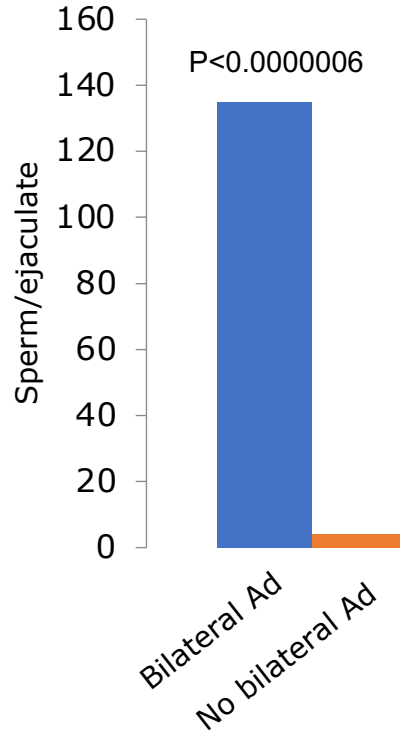
Cryptorchid testis-
defective mini-puberty

Testosterone stimulates the differentiation of Ad spermatogonia

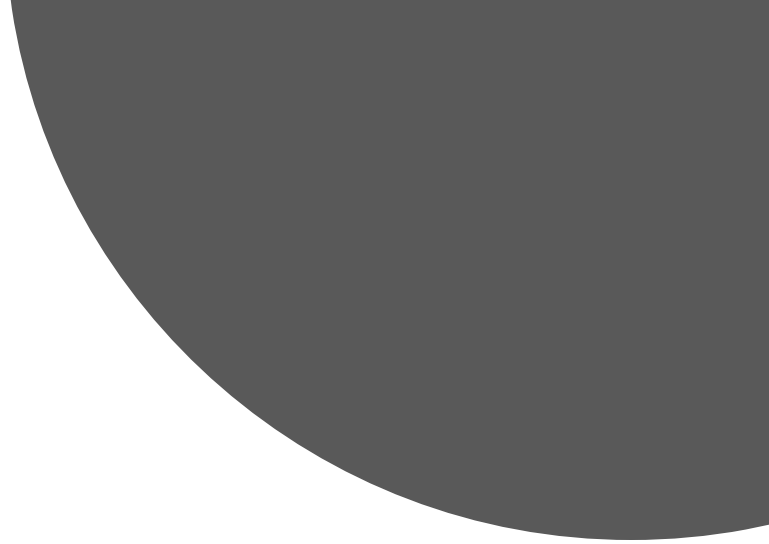
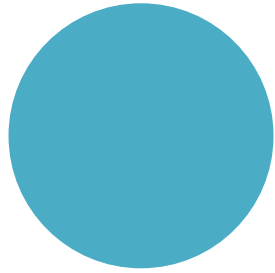


Zivkovic D, Bica DT, Hadziselimovic F. Relationship between adult dark spermatogonia and secretory capacity of Leydig cells in cryptorchidism. BJU Int. 2007 Nov;100(5):1147-9;

Normal or defective mini-puberty



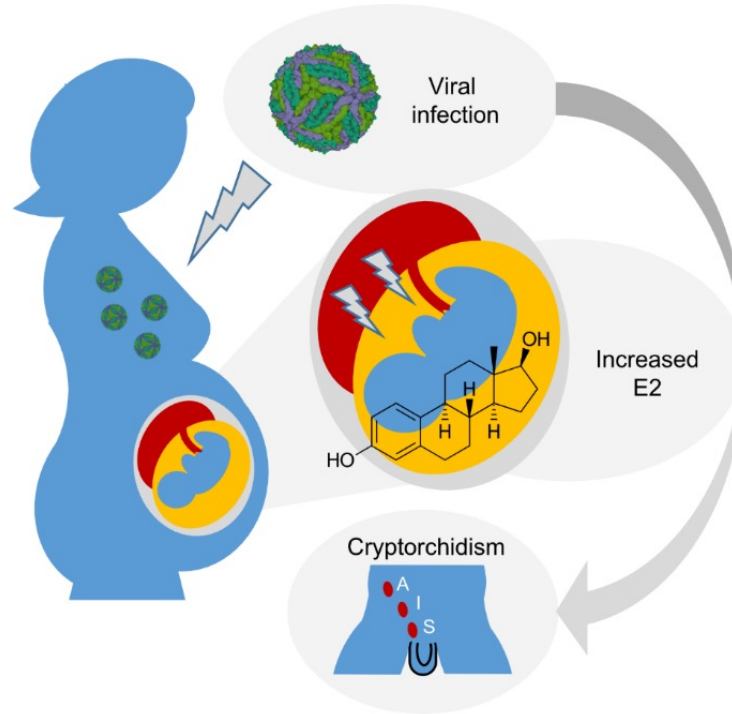
➤ Ad spermatogonia are a discriminating factor for the fertility outcome in cryptorchidism



ETIOLOGY

Hypothesis

Estrogen-Hypothesis



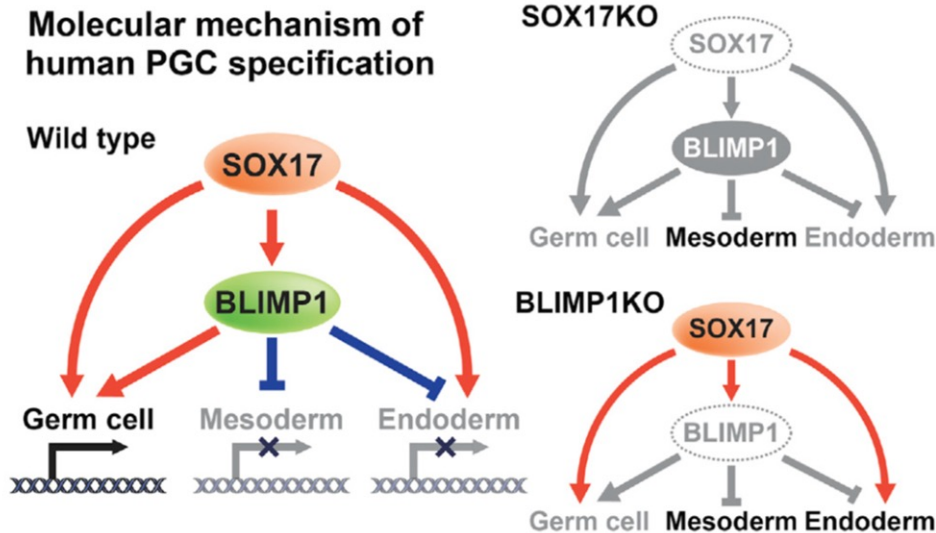
Hadziselimović et al., Elevated placental estradiol: a possible etiological factor of human cryptorchidism. J Urol. 2000

Epigenetics-Hypothesis

- Epigenetic mechanisms are critical for male infertility related to cryptorchidism and its possible transgenerational transmission
- Spermatogonia can pass histone posttranslational modifications from one generation to the next

Hadziselimovic F, Verkauskas G, Stadler MB. Epigenetics, cryptorchidism, and infertility. Basic Clin Androl. 2023

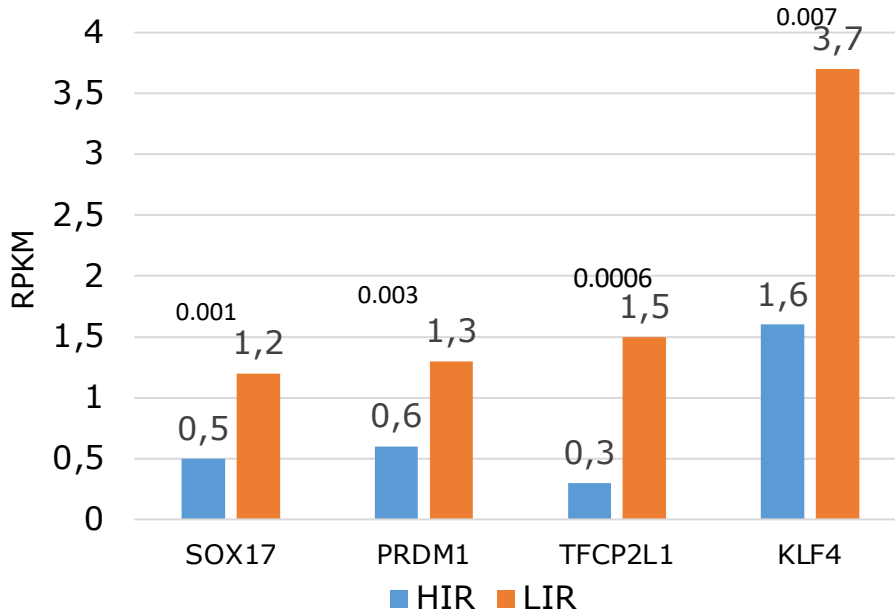
Epigenetic control of primordial germ cell specification



Irie *et al.* Cell 2015

- ✓ Resetting of the epigenome in human primordial germ cells is critical for their development
- ✓ Irie *et al.* showed that SOX17 and PRDM1 (BLIMP1) drive germline DNA demethylation by repressing DNA methylation pathways

Differential expression of epigenetic factors in HIR versus LIR patients



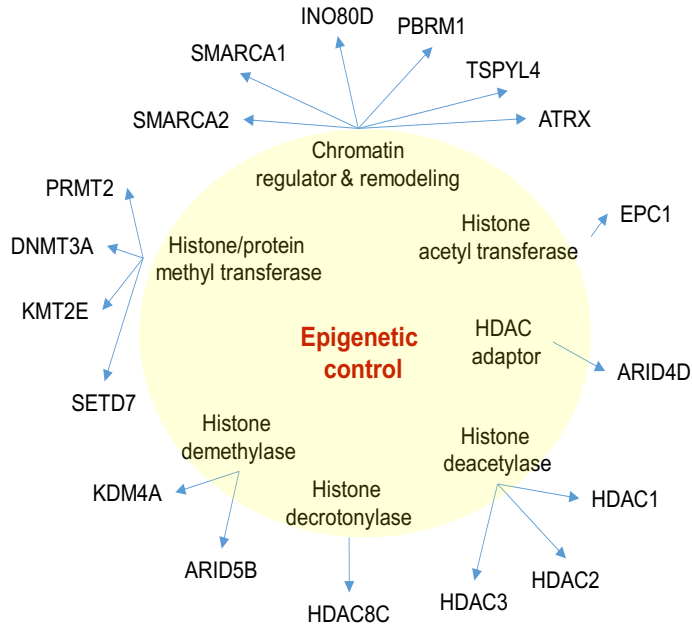
- ✓ Four genes involved in epigenetic process are expressed to lower levels in HIR testes
- This indicates disturbed epigenome resetting and finally abrogated germ cell pluripotency

Roles of epigenetic marks

- ✓ Histone lysine methyltransferases broadly regulate gene expression
- ✓ Thereby these enzymes control cell proliferation, differentiation, migration, and tissue invasion

Hadziselimovic F, Verkauskas G, Stadler MB. Epigenetics, cryptorchidism, and infertility. Basic Clin Androl. 2023 Sep 21;33(1):24.

Hormone treatment affects expression of epigenetic factors



- ✓ HIR samples display altered expression of several genes encoding histone methyltransferases
- ✓ Diminished expression of histone deacetylases
- ✓ Increased expression of HDAC8 deacetylase

- This indicates altered histone code
- Curative GnRHa treatment restores expression of epigenetic regulators

In utero female origin of male infertility

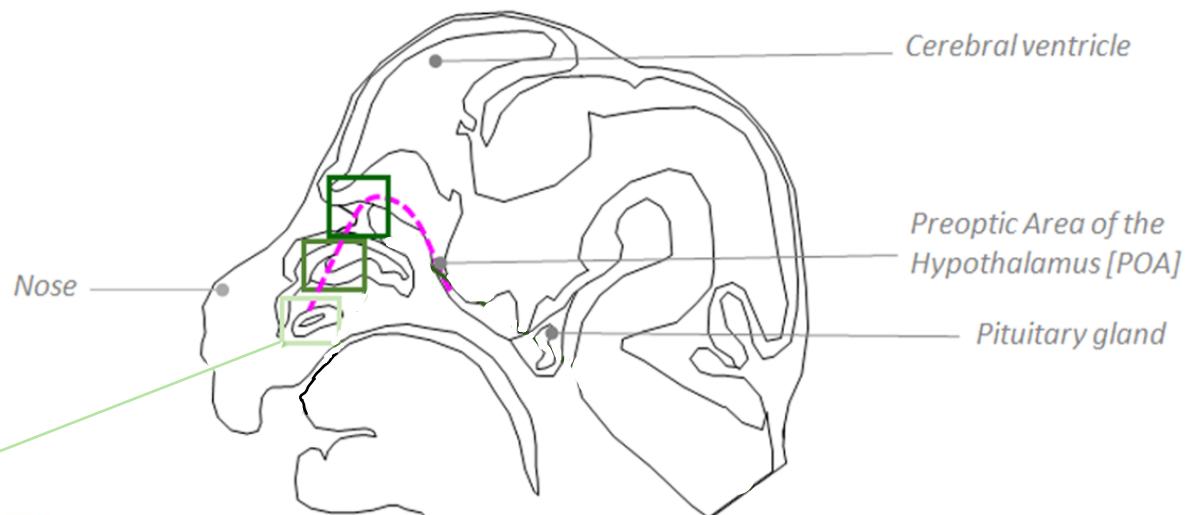
- We have proposed that cryptorchidism and infertility may be a consequence of **elevated fetal estradiol levels during pregnancy**
- ✓ The estrogen metabolite 16 α -hydroxy-estrone negatively regulates the expression of SOX17, a key regulator of primordial germ cells (Sangam et al. Am J Respir Crit Care Med. 2023)
- This is consistent with our finding **that SOX17 and other epigenetic factors are expressed at lower levels in HIR than in LIR testes**
- ✓ 1983 we reported **impaired transformation of primordial germ cells in cryptorchid testes as a possible precursor of seminoma**
- We propose that **intrauterine perturbation of the primordial germ cell epigenome** underlies **adult infertility** and **seminoma development**



ETIOLOGY

LH-deficiency

The brain-testis link



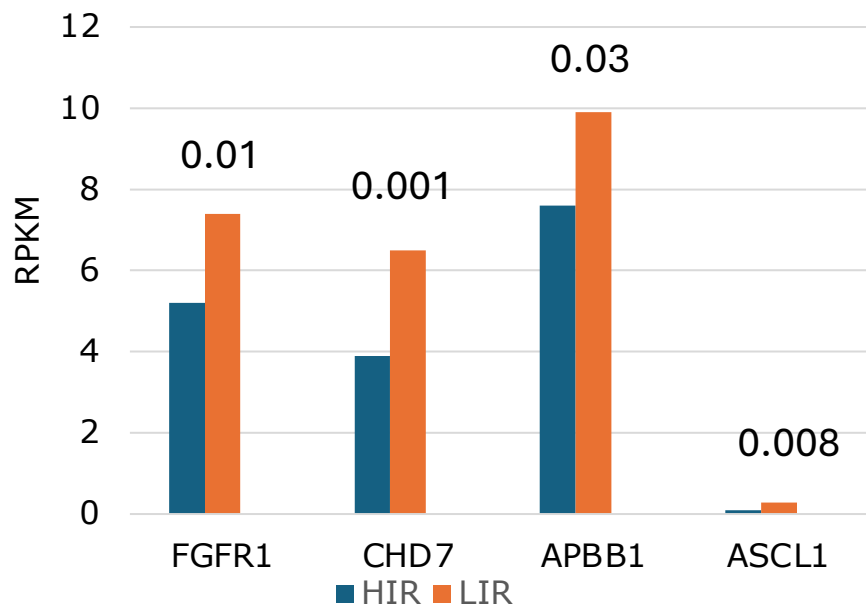
VNO
Vomero Nasal Organ

GnRH neurogenesis

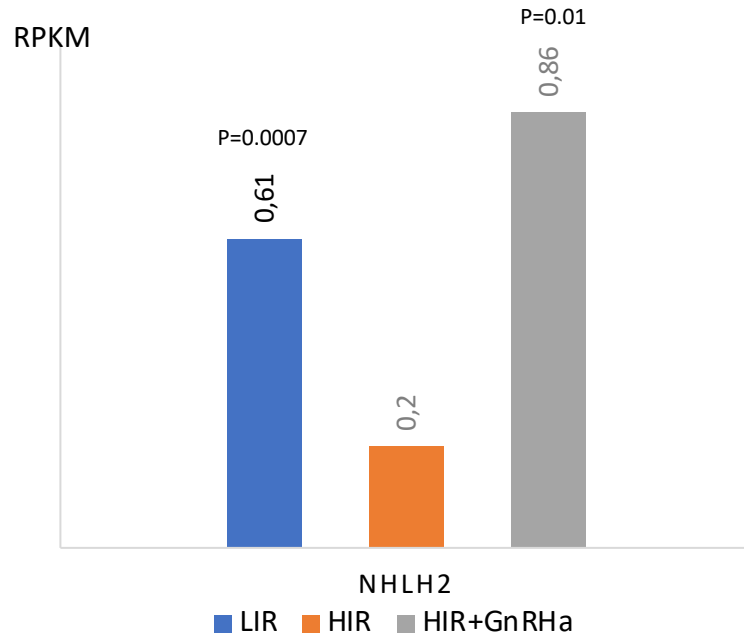
Fgf8/FGFR1*
Chd7
FE65
Nhlh2/Nhlh1
Ascl1



Brain gene expression is reduced in HIR testes



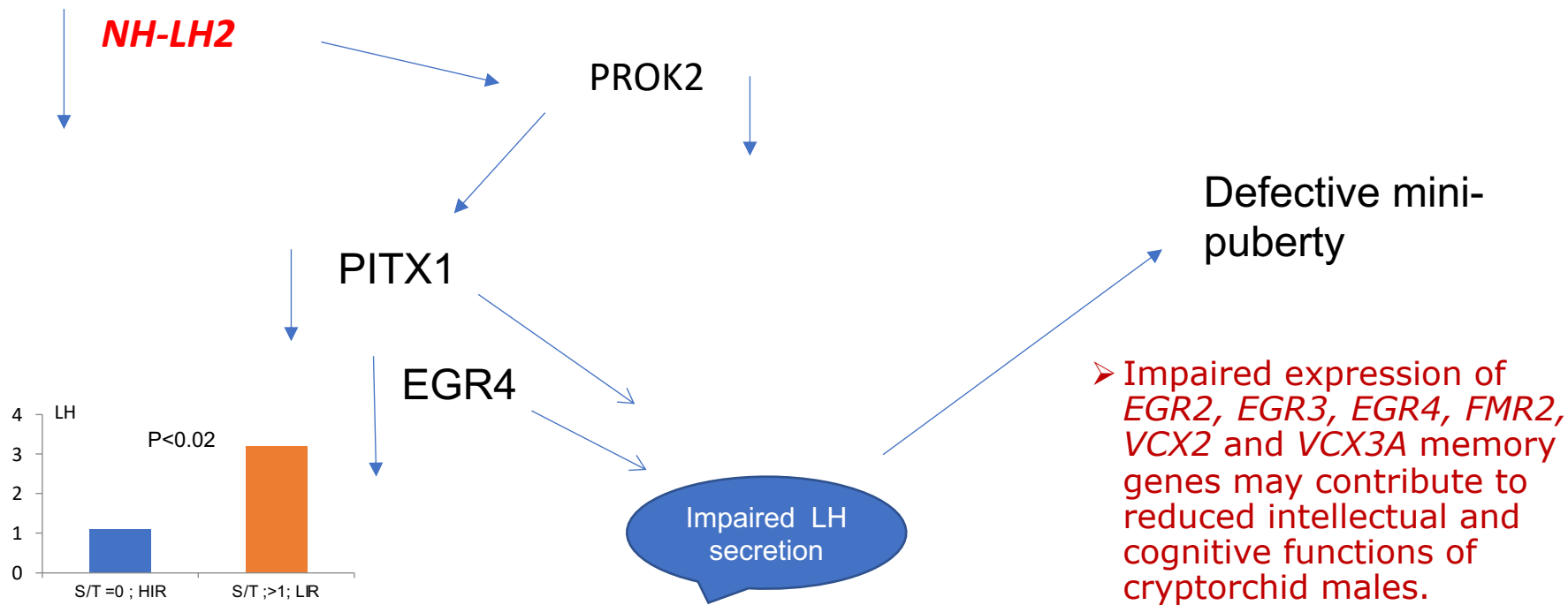
NHLH2 expression is induced in treated HIR testes



✓ Only NHLH2 is affected by Buserelin

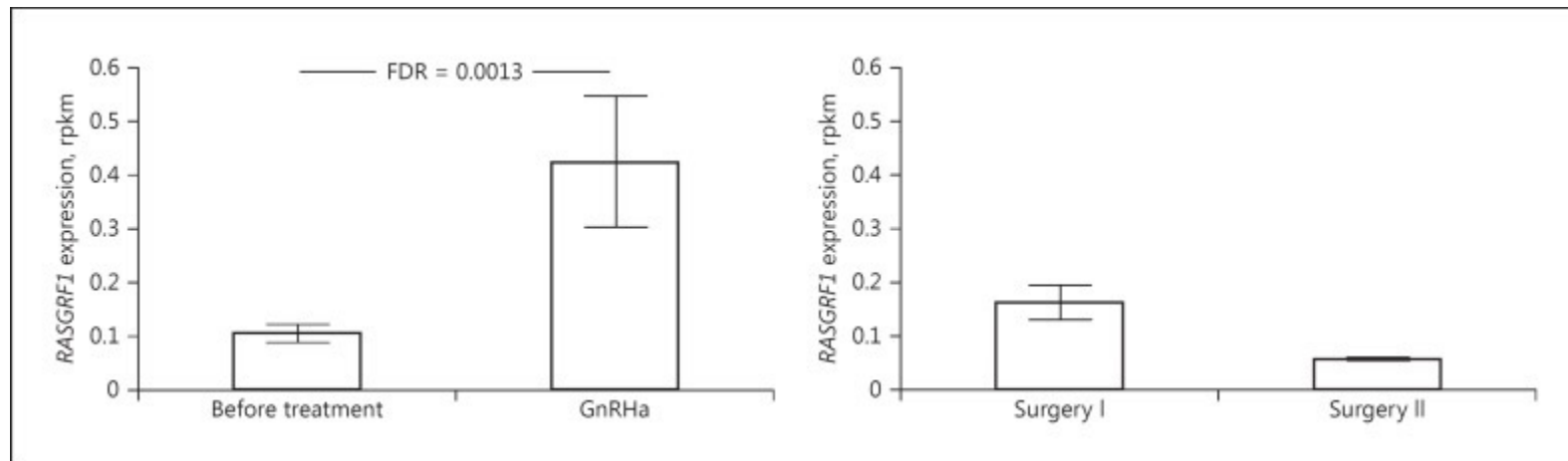
Hadziselimovic F et al., Molecular clues in the regulation of mini-puberty involve neuronal DNA binding transcription factor NHLH2. Basic Clin Androl. 2021

Memory genes



Hadziselimovic F, Girard J. Herzog B:
1976. Stresa

RASGRF1



The brain-testis link

- Our results are consistent with the hypothesis that hypogonadotropic hypogonadism in cryptorchid boys with altered mini-puberty may affect neuronal genes important for memory and learning
- This could help explain the negative correlation between cryptorchidism and intellectual abilities

Hadziselimovic F, Herzog B: Hodenerkrankungen im Kindesalter (Hippokrates Verlag, Stuttgart 1990).

Infertility

Testicular level

Transposons are discrete segments of
DNA that move around



19

RETROTRANSPOSONS

Retrotransposons and fertility

- Impaired expression of *DDX4*, *MAEL*, *MOV10L1*, *PIWIL2*, *PIWIL4*, *TDRD9* and *GTSF1* in HIR testes suggests that **gene instability induced by impaired expression of transposon silencing genes** contributes to the development of infertility.

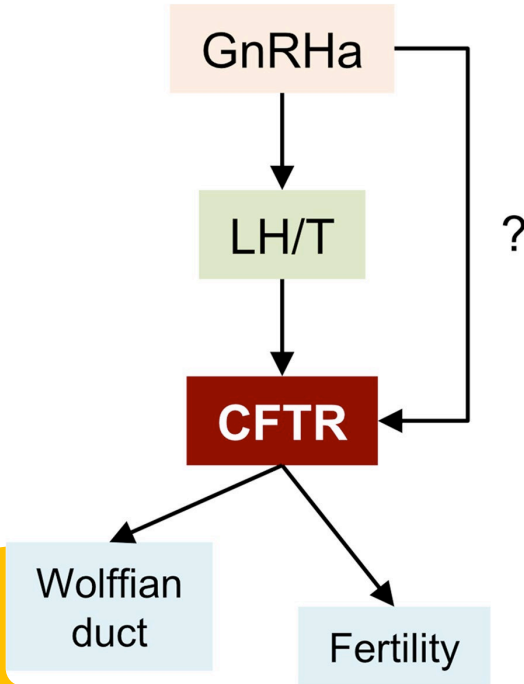
Hadziselimovic F, Hadziselimovic NO, Demougin P, Krey G, Oakeley E. Piwi-pathway alteration induces LINE-1 transposon derepression and infertility development in cryptorchidism. Sex Dev. 2015;9(2):98-104

The role of CFTR

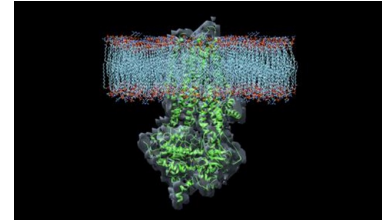
- In HIR group hypogonadotropic hypogonadism induces the reactivation of retrotransposons and downregulation of ASZ1, PIWILs and CFTR
- Curative GnRH stimulates CFTR and PIWIL3 inducing silencing of retrotransposons
- GnRH stimulates expression of androgen sensitive epididymal genes CRISP1, WFDC8, SPINK13, PAX2
- The high incidence of abnormal epididymis in HIR patients is a combination of hypogonadotropic hypogonadism and insufficient CFTR activity.

Hadziselimovic F et al., A novel role for CFTR interaction with LH and FGF in azoospermia and epididymal maldevelopment caused by cryptorchidism. Basic Clin Androl. 2022

Mechanism of fertility development in cryptorchid testis



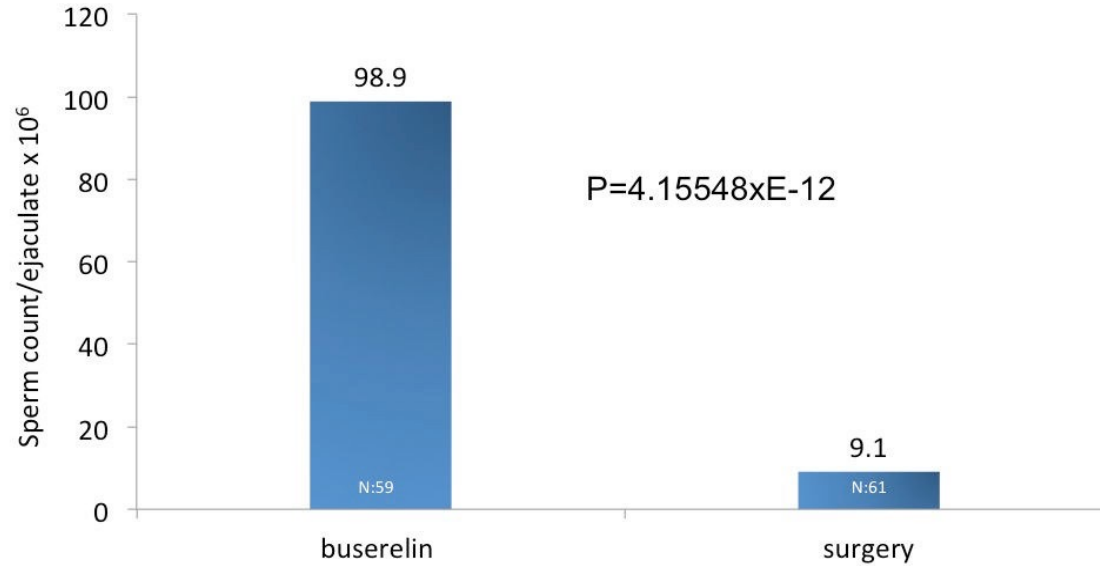
- ✓ Cryptorchid testis from HIR boys with hypogonadotropic hypogonadism show suboptimal expression of ASZ1, PIWIL and CFTR genes
- transposon reactivation
- infertility



Hormone treatment affects expression of regulatory long non-coding RNAs

- ✓ lncRNAs can cooperate with chromatin-modifying enzymes to promote epigenetic regulation of genes
- ✓ Our observations indicate that LINC00261, FENDRR, HOTAIR and FOXA1 participate in an alternative pathway for curative GnRHa treatment to rescue impaired fertility
- GnRHa treatment may act as a surrogate for mini-puberty by triggering the differentiation of Ad spermatogonia via lncRNA-mediated epigenetic effects

Sperm Count in 120 [High Risk Infertility Group] Cryptorchid Males



CONCLUSIONS

- Idiopathic central hypogonadism explains the phenomenon of defective mini-puberty in otherwise healthy cryptorchid boys
- A testicular biopsy should be routinely performed during the orchidopexy because of its substantial prognostic power
- Testicular biopsy is the only diagnostic procedure capable of identifying patients who need to be treated with LH-RHa

Hutson JM. Cryptorchidism and Hypospadias. In Feingold et al.eds . Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2022 Dec 14.

- There is now a consensus that type-A spermatogonia are likely to be the stem cells for future spermatogenesis, and that their appearance between 3 and 12 months of age, as neonatal gonocytes transform, is the key step in postnatal germ cell development (68, 69). **Inappropriate statement**
- Should this be confirmed, it implies that **early surgical intervention should lead to an excellent prognosis**, as long as the subsequent germ cell deficiency is **secondary** to postnatal heat stress of the maldescended testis, and therefore reversible. **No evidence**

68. Hutson J.M., Li R., Southwell B.R., Petersen BL, Thorup J, Cortes D.. Germ cell development in the postnatal testis: the key to prevent malignancy in cryptorchidism? Frontiers in endocrinology. 2012;3A:176.
69. Vikraman J., Hutson J.M., Li R., et al., The undescended testis: clinical management and scientific advances. Semin Pediatr Surg 2016