*The Turkish Journal of Pediatrics 2004; 46 (supplement): 28-34*

# Transgenic and knockout mouse models for aberrant pituitary-testicular function: relevance to the pathogenesis of cryptorchidism

Ilpo Huhtaniemi[1,2], Matti Poutanen[1]

[1]*Department of Physiology, University of Turku, 20520 Turku, Finland, and* [2]*Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Campus, London W12 ONN, UK*
*ilpo.huhtahiemi@imperial.ac.uk*

**SUMMARY: Huhtaniemi I, Poutanen M. Transgenic and knockout mouse models for aberrant pituitary-testicular function: relevance to the pathogenesis of cryptorchidism. Turk J Pediatr 2004; 46 (supplement): 28-34.**

**In recent years novel information about the hormonal regulation of testicular descent has emerged from genetically modified mice and other experimental animal models with cryptorchid phenotypes. We have studied transgenic (TG) mice overexpressing P450 aromatase (AROM+) and insulin-like 3 (INSL3+), and knockout (KO) mice for the genes of LH receptor (R) (LuRKO) and the thyroid-specific enhancer/binding protein T/ebp/Nkx2.1. LuRKO and AROM+ males are cryptorchid, while the INSL3 overexpressing females present with transabdominal descent of the ovaries. The T/ebp/Nkx2.1 KO mice die at birth, but the transabdominal phase of their testicular descent is normal despite the lack of pituitary gland. Detailed analysis of these mouse models brings novel information about the hormonal requirements, and their spatio-temporal aspects, in testicular descent, and how this process is affected by too low or too high hormone levels, or by imbalance in concentrations of critically important hormones.**

***Key words: testicular descent, cryptorchidism, gonadotropins, gonadotropin receptors, luteinizing hormone, luteinizing hormone receptor, androgen, estrogen, aromatase, insulin-like 3, T/ebp/Nkx2.1, transgenic, knockout, mouse.***

## Introduction

Although there is a significant genetic component in the pathogenesis of cryptorchidism, most of the familial cases are still classified as idiopathic, because the common causative mutations have so far remained unidentified. In many cases cryptorchidism occurs as a "side effect" of a serious developmental disturbance in the fetal and perinatal period. In these cases, either the physical event of testicular descent, or the regulation of testicular endocrine function are compromised at a proximal level, and cryptorchidism is only one of multiple developmental disturbances. Hypogonadotropic hypogonadism, due to defective hormone synthesis and/or secretion at the hypothalamic-pituitary level, is an example of such a pathophysiological alteration. Besides the mutations causing more general inhibition of sexual differentiation and maturation, a few genes are known to cause isolated cryptorchidism, representing apparently a more distal disturbance in the regulatory cascade of testicular descent. Several knockout (KO) mouse models, such as those for insulin-like 3 (INSL3), INSL3 receptor, Hoxa10, Hoxa11 and Desrt, exist today, causing specific inhibition of testicular descent[1-6]. However, human cryptorchidism has so far only exceptionally been linked to similar mutations[7-9]. It is therefore of importance to develop and characterize new genetically modified mouse models, to unravel further details of the regulatory mechanisms of normal and pathological testicular descent.

The developing undifferentiated gonad has both caudal and cranial connections to other abdominal structures. The gubernaculum develops below the gonad in the inguinal region of the abdomen, and the cranial suspensory ligament (CSL) develops between the gonad and the dorsal abdominal wall[10]. Analyses of mutant

mouse models together with experimental models with rats have provided novel information about the hormones and factors involved in the sequence of events governing the formation of these two ligaments determining the differential positions of the male and female gonads. These data indicate an essential role for androgens and INSL3 in this process, while estrogens are likely to suppress testicular descent. Regression of the CSL in the male fetus is androgen-dependent, since the CSL has been shown to persist in completely androgen insensitive TFM male mouse fetuses[11,12], and in male rats prenatally exposed to anti-androgens[13,14]. However, the presence of the CSL does not markedly affect the transabdominal descent of the testes. The second phase of testicular descent, i.e. the inguino-scrotal phase, is strongly androgen-dependent[11]. Similar to mice lacking androgen action, those devoid of INSL3 have been observed to be bilaterally cryptorchid[3,15]. The data has indicated that the reason for cryptorchidism in the INSL3 -deficient mice is the undeveloped gubernacula. Because INSL3 -deficient mice have androgens, the CSL regresses, and the testes are only loosely suspended within the abdominal cavity.

Our laboratory has been developing genetically modified mouse models, both transgenic (TG) and KO, for the study of gonadal endocrine and paracrine regulation. Although our primary goal has not been to address the molecular pathogenesis of cryptorchidism, the phenotypes of some of our mouse models include disturbances of testicular descent. Therefore, these mice corroborate a better understanding of the molecular mechanisms involved in normal testicular descent and its disturbances. The purpose of this review is to present from these mice the information that is relevant to normal testicular descent and the molecular pathogenesis of cryptorchidism.

**P450 aromatase overexpressing (AROM+) mouse**

One of the mouse models we have developed is the P450 aromatase overexpressing (AROM+) TG mouse[16]. In these mice aromatase is expressed under the human ubiquitin C promoter that is prenatally activated in a variety of tissues. Accordingly, our analyses demonstrated a low level of transgene expression both in gonadal and extragonadal tissues of the AROM+ males[16], resulting in marked imbalance of the estrogen/androgen ratio. At the time of birth, no marked difference in testicular testosterone (T) was observed between the TG and control mice[17]. However, the marked induction of mammary gland growth postnatally also indicated a reduced androgen action in fetal life, as androgens are known to suppress the induction of the mammary gland anlage at around E15 in male fetuses[18]. At four months of age, both intratesticular and serum T concentrations were markedly reduced, while estradiol ($E_2$) concentrations were elevated[16,17]. At the same time, serum follicle-stimulating hormone (FSH) concentrations were found to be slightly reduced, while no significant differences occurred in the mean luteinizing hormone (LH) concentrations[16]. The highly elevated $E_2$ exposure is also indicated by increased pituitary size and lactotroph adenomas. As a consequence, serum prolactin was strikingly elevated, and together with sex steroid action it resulted in severe gynecomastia in AROM+ males[18]. Both in experimental animal models and in humans, cryptorchidism has been associated with elevated estrogen concentrations during pregnancy. It has been reported that mothers of cryptorchid children have higher levels of $E_2$ during the first trimester of pregnancy compared with mothers whose offspring had normally descended testes[20]. Furthermore, increased levels of $E_2$ have been reported in placentas of newborn boys with cryptorchidism[21,22]. Animal studies support the correlation found in humans, even though, e.g. in AROM+ mice, the phenotype may be partly due to suppressed androgens, and only partially to increased estrogen/androgen ratio. However, several of the disturbances detected in the urogenital organs of adult AROM+ males are identical to those observed in mice upon perinatal exposure to estrogen. These include Leydig cell hyperplasia and hypertrophy, spermatogenic disturbances, inguinal hernias and cryptorchidism, with the testes located in the abdominal cavity at the inlet of the inguinal canal.

Consistent with the cryptorchidism observed in AROM+ mice, fetal exposure to high concentrations of estrogens causes cryptorchidism in rodents[23-25], associated with down-

regulation of INSL3 expression[25]. However, the data suggest that the INSL3 -dependent intra-abdominal descent had occurred normally in AROM+ males, indicating that the estrogen exposure during fetal life was not strong enough to block INSL3 action. Hence, it is likely that the lack of improper postnatal and pubertal androgen production is the main cause of their cryptorchidism. One of the interesting features of the cryptorchidism in AROM+ mice was that the cryptorchid testes descended into the scrotum with 100% penetrance 4 to 15 days (median 12 days) after starting a P450arom inhibitor (finrozole) treatment[19]. The testicular descent was associated with full recovery of androgen production and other aspects of testicular function, including spermatogenesis. The fully descended testes after finrozole treatment, with normalized testicular androgen production, give rise to a hypothesis that P450arom inhibitors could be used to treat cryptorchidism in humans, as the abnormally high estrogen action is still suggested as one of the possible causes for this pathology[26].

**T/ebp/Nkx.2.1 KO mouse**

The thyroid-specific enhancer-binding protein T/ebp, also called Nkx2.1 or thyroid transcription factor 1, is known to control thyroid- and lung-specific gene transcription. It is also essential for organogenesis of the thyroid, lung, ventral forebrain and pituitary, as has been demonstrated with T/ebp/Nkx.2.1 KO mice[27]. Because of disturbed development of the pituitary gland we surmised that the T/ebp/Nkx.2.1 KO mouse would elucidate the indirect role of the pituitary gland in regulating testicular descent in the fetal period[28]. The mice die at birth because of failure of lung development, and among other anomalies, the entire pituitary gland is missing in the KO mice. This mouse model allowed us to study to what extent fetal testicular endocrine activity was dependent on pituitary regulation. The testes of the mice were on day E18.5 were similarly located, laterally of the urinary bladder, in KO and wild-type (WT) mice, and their internal and external genitals demonstrated a similar degree of masculinization, as well as regression of müllerian ducts. Hence the testicular production of androgens, INSL3 and anti-müllerian hormone (AMH) in the mouse fetus is independent of pituitary stimulation. Because the intratesticular T levels in the KO mice were only about 5% of the WT levels, there must be a considerable safety margin in the production of fetal testicular hormones regulating masculinization, including testicular descent.

The testes and genitals of the KO male mice appeared normally developed and indistinguishable from WT controls when studied on day E18.5. The testes had undergone the transabdominal descent to the lower part of the abdominal cavity, and their ano-genital distance was as long as in control males. The testes displayed similar histology as WT controls, although the Leydig cells were clearly smaller at ultrastructural level. Likewise the intratesticular T level was dramatically reduced, to 5-10% of controls. These observations allowed the following conclusions: 1) The lack of pituitary development documented in T/ebp/Nkx.2.1 -/- mice does not affect male fetal genital differentiation, including the transabdominal phase of testicular descent, 2) the production of the three fetal testicular hormones, T, AMH and INSL3, is sufficient in the absence of pituitary gonadotropin stimulation, 3) fetal Leydig cell steroidogenic capacity in the KO animals is decreased in the absence of pituitary hormones, most likely of gonadotropins, 4) despite absent gonadotopic stimulation, fetal Leydig cells maintain their ultrastructural features of steroidogenic cells, and 5) there appears to be a large safety margin in the need for fetal Leydig cell T production for masculinization because the drastically suppressed T levels in KO males were able to induce normal masculinization. Although the levels of AMH and INSL3 were not monitored, their production was apparently sufficient, because the müllerian ducts were regressed and the transabdominal passage of the testes had occurred normally.

Hence, this study shows that in the mouse, gonadotropin stimulation of fetal testes is not needed for masculinization, including testicular descent. The situation in the human is different, because men with completely inactivating mutations of luteinizing hormone receptor (LHR) are pseudohermaphrodites with total lack of masculinization[29]. The finding is similar to the mouse in the sense that pituitary LH is needed for testicular stimulation, because men with inactivating mutation of the LH β-subunit are normally masculinized at birth[30,31]. In these

cases, human chorionic gonadotropin (hCG) offers the backup for missing LH, and it may in fact be the important LH in fetal life. The relevance of the current study to the human is that disturbance in the hormonal regulation of T or INSL3 production is an unlikely cause of their insufficient production or action. A more likely site of action is a blockage of their action at the receptor level.

**LH receptor KO (LuRKO) mouse**

A mouse model representing more specific inactivation of the pituitary control of fetal testicular function is the KO for the LH receptor[32]. As the T/ebp/Nkx.2.1 KO mice, are devoid of pituitary gland, these mice are normally masculinized at birth[33], which shows that LH action in utero is not necessary in this species to stimulate sufficient Leydig cell androgen and INSL3 production to induce fetal masculinization and the transabdominal phase of testicular descent. In this respect the mouse differs from the human, where complete LHR inactivation causes pseudohermaphroditism. The safety mechanism to maintain fetal Leydig cell function in the absence of LH/LHR stimulation is provided in the mouse by a network of paracrine influences (see e.g.)[34,35]. In the human, in contrast, LHR function is compulsory, but missing pituitary LH secretion can be compensated for by placental hCG[29]. The postnatal sexual maturation of these mice does not occur in the absence of LH-stimulated testicular T production. The testes of the mice remain small and cryptorchid. However, the crucial role of T for the final stage of testicular descent and attainment of spermatogenesis is shown by T treatment, which brings about testicular descent and initiates apparently normal spermatogenesis (Pakarainen et al. unpublished observations). However, for a reason still unknown the mice remain subfertile despite apparently normal spermatogenesis and sexual behavior.

**Insulin like 3 overexpressing mouse (INSL3+)**

The last model relevant to testicular descent is the TG mouse expressing INSL3 under the ubiquitin-C promoter [36]. Similar to the AROM+ mice, the transgene is expressed in various adult tissues both in male and female fetuses from day E15 onwards. The male animals were indistinguishable from WT controls, but the females showed a distinct phenotype. Ovaries of INSL3+ females had descended to the bottom of the abdominal cavity. They were suspended by a long CSL and a caudal structure resembling male guber-naculum. Normally, in fetal mice INSL3 expression shows sexual dimorphism, being expressed only in males[37]. Hence, this TG model is able to demonstrate specific effects of INSL3 in the absence of the other fetal testicular hormones, T and AMH, which are not produced by the fetal ovary. The data observed are in line with the hypothesis that INSL3 action is independent of the other male hormones, and is alone mainly responsible for the development of the gubernaculum, while the CSL involutes due to T action. However, studies on rat gubernacula in vitro suggest that for full gubernaculum development in males, both INSL3 and androgens are needed[38], alluding to the possibility that androgens also have a role in the later stages of gubernaculum devel-opment. Interestingly, the mechanism of CSL regression in females is similar to males. While prenatal androgen treatment in rats causes regression of the CSL[39,40], this leads only to a minor degree of ovarian descent. This, together with the presence of CSL in INSL3+ females with descended ovaries[36] indicates that gubernaculum formation is a stronger force than the persistence of CSL, as regards gonadal location. As shown by us and others[36,41], female mice respond to INSL3 by forming guber-naculum cords structurally very similar to those developing normally in males. Curiously, this resulted in full trans-abdominal descent of the ovaries. This phenomenon, together with the minor role of the CSL in determining the gonadal position, indicates that the lack of fetal expression of INSL3 in females is essential for the location of the ovaries lateral to the kidneys. The INSL3 gene occurs widely in different mammalian species, including humans, and hence it is likely that the sexually dimorphic availability of INSL3 is a common determinant for differential gonadal positioning between the sexes. However, unlike in males, the gonadal position in females does not seem essential for fertility, at least in the mouse, because the INSL3+ females with descended ovaries were fertile[36]. Recent data have revealed that a G protein-coupled receptor, LGR8[42-44], serves as the receptor for INSL3. Interestingly, LGR8 is also widely expressed in the brain, kidney,

muscle, testis, thyroid, uterus, peripheral blood cells and bone marrow, while no obvious phenotype was detected in these tissues in INSL3+ mice. The data with the INSL3+ mice, therefore, indicate that gubernaculum formation is the most sensitive biological response to INSL3, while the other roles of this hormone remain to be discovered.

The regulatory mechanisms involved in INSL3 expression in fetal and adult testis remain to be explored in detail. However, our data and those of others indicate a central role for steroidogenic factor-1 (SF-1) in the transcriptional activation of the INSL3 promoter[15,45]. Accordingly, the INSL3 promoter contains at least four potential recognition sequences for SF-1 that could sustain the spatio-temporal expression of the gene. Of them three are located at the 188 bp 5'-flanking region, with full transcriptional activity in vitro. Our data further indicated that the SF-1 binding site located at position -114 to -107 relative to the translation initiation codon has the strongest transactivation function[45]. Furthermore, we demonstrated that of the three SF-1 binding sites analyzed, the most distal binding motif (-144) was found to have the highest affinity for SF-1 but did not result in highest transcriptional activation. This indicates that the location of the SF-1 binding motif is critical in order to properly recruit other factors for transcriptional initiation. However, the cell-specific expression of INSL3 cannot be determined by the expression of SF-1 only, and accordingly, it has been shown that SF-1, can interact with numerous other coregulators and transcription factors[46]. In the context of cryptorchidism, it is interesting to note that SF-1 can also interact with estrogen receptors α and β (ERα and β), and ERα and estrogen receptor-related receptors α and β (ERRα and β) can bind to SF-1 binding sites. Hence, the possibility exists for a direct action of estrogens and related compounds on INSL3 gene transcription. However, of these putative co-regulators of the INSL3 gene, direct evidence exists only for the inhibitory effects of DAX-1[45], while the interaction of the other factors with INSL3 promoter remains to be explored.

## Conclusions

The four genetically modified mouse models, AROM+, T/ebp/Nkx.2.1 KO, LuRKO and INSL3+, were originally developed for studies on the endocrine regulation of sexual development. In some of the models, despite clearcut endocrine disturbances, the fetal phase of transabdominal descent of the testes was fully normal. These studies demonstrated that, at least in the mouse, the fetal testicular hormone production needed for the transabdominal phase of testicular descent is independent of regulation by pituitary hormones. It is either autonomous or regulated by intratesticular paracrine and autocrine mechanisms. In contrast, the postnatal phase is very sensitive to hypogonadotropism, or decreased ratio of androgens/estrogens. If these disturbances in hormone balance are corrected, the testicular descent and fertility can be fully recovered, which speaks against the importance of early perinatal imprinting of male fertility through testicular hormones.

### REFERENCE

1. Satokata I, Benson G, Maas R. Sexually dimorphic sterility phenotypes in Hoxa 10-deficient mice. Nature 1995;374:460-463.
2. Rijli FM, Matyas R, Pellegrini M, et al. Cryptorchidism and homeotic transformations of spinal nerves and vertebrae in Hoxa-10 mutant mice. Proc Natl Acad Sci USA 1995; 92: 8185-8189.
3. Nef S, Parada LF. Cryptorchidism in mice mutant for Insl3. Nat Genet 1999; 22: 295-299.
4. Bradford WW, Benson GV, Ma I, et al. Characterization of Hoxa-10/Hoxa-11 transheterozygotes reveals functional redundancy and regulatory interactions. Dev Biol 2000; 224: 373-387.
5. Lahoud MH, Ristevski S, Venter DJ, et al. Gene targeting of Desrt, a novel ARID class DNA-binding protein, causes growth retardation and abnormal development of reproductive organs. Genome Res 2001; 11: 1327-1334.
6. Tomiyama H, Hutson JM, Truong A, Agoulnik AI. Transabdominal testicular descent is disrupted in mice with deletion of insulinlike factor 3 receptor. J Pediatr Surg 2003; 38: 1793-1798.
7. Gorlov IP, Kamat A, Bogatcheva NV, et al. Mutation of the GREAT gene causes cryptorchidism. Hum Mol Genet 2002; 11: 2309-2318.
8. Canto P, Escudero I, Soderlund D, et al. A novel mutation of the insulin-like 3 gene in patients with cryptorchidism. J Hum Genet 2003; 48: 86-90.
9. Ferlin A, Simonato M, Bartoloni L, et al. The INSL3-LGR8/GREAT ligand-receptor pair in human cryptorchidism. J Clin Endocrinol Metab 2003; 88: 4273-4279.
10. Hutson JM, Hasthorpe S, Heyns CF. Anatomical and functional aspects of testicular descent and cryptorchidism. Endocr Rev 1997; 18: 259-280.

11. Hutson JM, Donahoe PK. The hormonal control of testicular descent. Endocr Rev 1986; 7; 270-283.

12. Barthold JS, Mahler HR, Newton BW. Lack of feminization of the cremaster nucleus in cryptorchid androgen insensitive rats. J Urol 1994; 152: 2280-2286.

13. Shono T, Ramm-Anderson S, Goh DW, Hutson JM. The effect of flutamide on testicular descent in rats examined by scanning electron microscopy. J Pediatr Surg 1994; 29: 839-844.

14. van der Schoot P, Elger W. Androgen-induced prevention of the outgrowth of cranial gonadal suspensory ligaments in fetal rats. J Androl 1992; 13: 534-542.

15. Zimmermann S, Schwarzler A, Buth S, Engel W, Adham IM. Transcription of the Leydig insulin-like gene is mediated by steroidogenic factor-1. Mol Endocrinol 1998; 12: 706-713.

16. Li X, Nokkala E, Yan W, et al. Altered structure and function of reproductive organs in transgenic mice overexpressing human aromatase. Endocrinology 2001; 142: 2435-2442.

17. Li X, Strauss L, Mäkelä S, et al. Multiple structural and functional abnormalities in the P450 aromatase expressing transgenic male mice are ameliorated by a P450 aromatase inhibitor. Am J Pathol 2004; 164: 1039-1048.

18. Robinson GW, Karpf AB, Kratochwil K. Regulation of mammary gland development by tissue interaction. J Mammary Gland Biol Neoplasia 1999; 4: 9-19.

19. Li X, Wärri A, Ahonen T, et al. Mammary gland development in transgenic male mice expressing human P450 aromatase. Endocrinology 2002; 143: 4074-4083.

20. Bernstein L, Pike MC, Depue RH, Ross RK, Moore JW, Henderson BE. Maternal hormone levels in early gestation of cryptorchid males: a case-control study. Br J Cancer 1988; 58: 379-381.

21. Husmann DA. Excess fetal estrogen as an etiological cause of human cryptorchidism. J Urol 2000; 64: 1696.

22. Hadziselimovic F, Geneto R, Emmons LR. Elevated placental estradiol: a possible etiological factor of human cryptorchidism. J Urol 2000; 164: 1694-1695.

23. Gill W, Schumacher G, Bibbo M, Straus F, Schoenberg H. Association of diethylstilbestrol exposure in utero with cryptorchidism, testicular hypoplasia and semen abnormalities. J Urol 1979; 122: 36-39.

24. McLachlan J, Newbold R, Bullock B. Reproductive tract lesions in male mice exposed prenatally to diethylstilbestrol. Science 1975; 190: 991-992.

25. Nef S, Shipman T, Parada LF. A molecular basis for estrogen-induced cryptorchidism. Dev Biol 2000; 224: 354-361.

26. Sharpe R. The 'oestrogen hypothesis'- where do we stand now? Int J Androl 2003; 26: 2-15.

27. Kimura S, Hara Y, Pineau T, et al. The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. Genes Dev 1996; 10: 60-69.

28. Pakarinen P, Kimura S, El-Gehani F, Pelliniemi LJ, Huhtaniemi I. Pituitary hormones are not required for sexual differentiation of male mice: phenotype of the T/ebp/Nkx2.1 null mutant mice. Endocrinology 2002; 143: 4477-4482.

29. Themmen APN, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr Rev 2000; 21: 551-583.

30. Weiss J, Axelrod L, Whitcomb RW, Crowley WF Jr, Jameson JL. Hypogonadism caused by a single amino acid substitution in the beta subunit of luteinizing hormone. N Engl J Med 1992; 326: 179-183.

31. Valdes-Socin H, Salvi R, Pralong F, Gaillard R, Beckers A. Male hypogonadism caused by isolated LH deficiency: from pathology to gene. Eighth International Pituitary Congress, June 2003, New York, Abstract P31.

32. Zhang FP, Poutanen M, Wilbertz J, Huhtaniemi I. Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. Mol Endocrinol 2001; 15: 182-193.

33. Zhang FP, Pakarainen T, Zhu F, Poutanen M, Huhtaniemi I. Molecular characterization of postnatal development of testicular steroidogenesis in luteinizing hormone receptor knockout mice. Endocrinology 2004; 145; 1453-1463.

34. El-Gehani F, Zhang FP, Pakarinen P, Rannikko A, Huhtaniemi I. Gonadotropin-independent regulation of steroidogenesis in the rat fetal testis. Biol Reprod 1998; 58: 116-123.

35. El-Gehani F, Tena-Sempere M, Huhtaniemi I. Vasoactive intestinal peptide (VIP) is an important endocrine regulatory factor of fetal rat testicular steroidogenesis. Endocrinology 1998; 139: 1474-1480.

36. Koskimies P, Suvanto M, Nokkala E, et al. Female mice carrying a ubiquiting promoter-Insl3 transgene have descended ovaries and inguinal hernias but normal fertility. Mol Cell Endocrinol 2003; 206; 159-166.

37. Zimmermann S, Schottler P, Engel W, Adham IM. Mouse Leydig insulin-like (Ley I-L) gene: structure and expression during testis and ovary development. Mol Reprod Dev 1997; 47: 30-38.

38. Emmen JM, McLuskey A, Adham IM, Engel W, Grootegoed JA, Brinkmann AO. Hormonal control of gubernaculum development during testis descent: gubernaculum outgrowth in vitro requires both insulin-like factor and androgen. Endocrinology 2000; 141: 4720-4727.

39. Emmen JM, McLuskey A, Grootegoed JA, Brinkmann AO. Androgen action during male sex differentiation includes suppression of cranial suspensory ligament development. Hum Reprod 1998; 13: 1272-1280.

40. Lee SM, Hutson JM. Effect of androgens on the cranial suspensory ligament and ovarian position. Anat Rec 1999; 255: 306-315.

41. Adham IM, Steding G, Thamm T, et al. The overexpression of the insl3 in female mice causes descent of the ovaries. Mol Endocrinol 2002; 16: 244-252.

42. Hsu SY, Nakabayashi K, Nishi S, et al. Activation of orphan receptors by the hormone relaxin. Science 2002; 295: 671-674.

43. Overbeek PA, Gorlov IP, Sutherland RW, et al. A transgenic insertion causing cryptorchidism in mice. Genesis 2001; 30: 26-35.

44. Bogatcheva NV, Truong A, Feng S, Engel W, Adham IM, Agoulnik AI. GREAT/LGR8 is the only receptor for insulin-like 3 peptide. Mol Endocrinol 2003; 7: 2639-2646.

45. Koskimies P, Levallet J, Sipilä P, Huhtaniemi I, Poutanen M. Murine relaxin-like factor (RLF) promoter: functional characterization and regulation by transcription factors SF-1 and DAX-1. Endocrinology 2002; 143: 909-919.

46. Val P, Lefrancois-Martinez AM, Veyssiere G, Martinez A. SF-1 a key player in the development and differentiation of steroidogenic tissues. Nucl Recept 2003; 1: 8-18.