Focus on Gonadotrophin Signalling

What have we learned about gonadotropin function from gonadotropin subunit and receptor knockout mice?

T Rajendra Kumar

Department of Molecular & Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160, USA

Correspondence should be addressed to T R Kumar; Email: tkumar@kumc.edu

Abstract

A number of biochemical and physiological studies elucidated the roles of pituitary and placental glycoprotein hormones. Advances in the past two decades in manipulating the mouse genome by random or site-specific mutagenesis have heralded a new dimension to our understanding of the biology of gonadotropins. It is now possible to model many human reproductive disorders involving gonadotropins/gonadotropin-signaling in the mouse. Mutant mice selectively lacking either FSH or LH or their cognate receptors have been generated. The gonadotropin ligand and the corresponding receptor knockout mice mostly phenocopy each other. Analyses with these genetic models confirmed earlier physiological studies; in addition they also revealed novel roles for gonadotropins previously unrecognized. While FSH action seems dispensable for male but not female fertility, absence of LH causes infertility in both the sexes. While Sertoli cell number and germ cell carrying capacity of the Sertoli cells is compromised in FSH mutants, both somatic and germ cell lineages are affected in the LH mutants resulting in complete male infertility. FSH mutant females demonstrate a pre-antral stage block in folliculogenesis and FSH alone is not sufficient to promote full folliculogenesis in the absence of LH. Pre-ovulatory stage follicles do not form and most of the follicles undergo apoptosis in the absence of LH. Many extra-gonadal phenotypes have been described for the receptor knockout mice and whether these bear any resemblances to those in patients with similar inactivating mutations in the receptors for FSH and LH remains an open question. Thus the *in vivo* models will continue to have a significant impact in understanding gonadotropin physiology and pathophysiology and serve as novel genetic tools to study signaling mechanisms in the gonads.

Reproduction (2005) **130** 293–302

Introduction

The pituitary and placental gonadotropins, luteinizing hormone (LH), follicle stimulating hormone (FSH), chorionic gonadotropin (CG) belong to an evolutionarily conserved glycoprotein hormone family (Pierce & Parsons 1981). These are non-covalently linked heterodimeric glycoproteins that consist of a common α subunit (α -GSU) and a hormone-specific β subunit. Gonadotropins signal through G-protein coupled transmembrane receptors on the gonads and regulate gonadal growth, differentiation, and steroid production (Bousfield *et al.* 1994). LH and CG are structurally similar; human chorionic gonadotropin β (hCG β) is characterized by the presence of a unique carboxy terminus peptide (CTP). This sequence is known to confer a longer half-life to CG in serum (Bousfield *et al.* 1994). Both hormones bind identical receptors and stimulate cAMP production in target cells. Distinct genes

encode gonadotropin subunits; the subunits are synthesized as precursor proteins, processed, assembled and secreted via distinct mechanisms from pituitary gonadotropes or trophoblast cells (Pierce & Parsons 1981). Gene expression of the gonadotropin subunits is gender-specific and is co-ordinately regulated according to physiological demands by a number of autocrine, paracrine, and endocrine factors. Gonadotropin-releasing hormone (GnRH) is a hypothalamic decapeptide that binds to gonadotrope receptors and activates the pituitary gonadotropin subunit gene expression (Ruf *et al.* 2003). Members of transforming growth factor- β superfamily including activins, inhibins, follistatin and bone morphogenetic proteins primarily regulate FSH homeostasis (Shimasaki *et al.* 2004). Understanding the biology of gonadotropins has many clinical implications with regard to fertility management and designing novel therapeutic strategies for many gonadal cancers.

α -GSU knockout mice

To study the functional role of α -GSU, particularly during early pituitary development, Camper and her colleagues developed mice lacking α -GSU (Kendall *et al.* 1995). Since α -GSU is the common glycoprotein subunit for thyroid stimulating hormone (TSH), LH, and FSH, all three of the glycoprotein hormones were absent in these mutant mice. The mutant mice demonstrated profound hypothyroidism resulting in dwarfism. Embryological studies identified that thyroid development was arrested in late gestation and pituitary thyrotrope hyperplasia owing to a lack of thyroid hormone feedback was evident. Pituitary morphogenesis and GnRH neuron migration appeared normal in the absence of α -GSU. The mutant mice were hypogonadal; however, sexual differentiation and early development were unaffected in the absence of circulating gonadotropins. Homozygous male mice were infertile, had decreased testis size and undetectable serum testosterone levels. The accessory sex glands were atrophied consistent with lack of stimulation by testosterone. Histological analysis of the testes revealed normal fetal stage development, but at 8 weeks of age, smaller tubules were apparent, interstitial cells were rare, and spermatogenesis was blocked at the first meiotic division (Kendall *et al.* 1995). In female mutant mice, there was a failure in the opening of the vaginal orifice. These mice were infertile, demonstrated small ovaries and thin uteri and suppressed serum estradiol levels. Histological analysis of the ovary identified no antral follicles and corpora lutea confirming absence of estrous cycles (Kendall *et al.* 1995). These genetic studies with the α -GSU mice confirmed that early gonadal development is independent of gonadotropin function.

FSH β knockout mice

In the naturally occurring hypogonadal (*hpg*) mutant mouse, both FSH and LH are suppressed (Mason *et al.* 1986a, Mason *et al.* 1986b, 1987). Similarly, α -GSU knockout mice (described above) lack both of these gonadotropins in addition to TSH. These models are therefore disadvantageous to analyze the roles of only LH or FSH. To study the isolated deficiency of only FSH, we deleted most of the coding sequence of FSH β gene in ES cells and subsequently generated FSH β knockout mice from these targeted cells (Kumar *et al.* 1997). Since heterodimeric assembly is essential for *in vivo* biological activity, in the absence of the hormone-specific β subunit, circulating FSH was absent in these mice. FSH β heterozygous mice were normal and fertile and did not demonstrate any overt phenotypes.

FSH β knockout male mice were fertile despite reduced testes size and volume of the seminiferous tubules. Although all stages of spermatogenesis appeared qualitatively normal, the epididymal sperm number and sperm motility were reduced by 75% and 40% respectively

(Kumar *et al.* 1997). These studies confirmed species-specific differences in FSH regulation of spermatogenesis between mice and primates including humans immunized with various FSH-based vaccines (McLachlan *et al.* 2002, Moudgal & Sairam 1998, Moudgal *et al.* 1997, Plant & Marshall 2001). Stereological analysis confirmed ~30% reduction in the Sertoli cell number and the germ cell carrying capacity consistent with reduced testis size (~65%) in the mutant mice (Kumar *et al.* 2001, Wreford *et al.* 2001). The net number of Leydig cells per testis and serum testosterone in the mutants was not affected. Accordingly, the accessory sex glands appeared normal. It was also reported that the FSH-receptor demonstrates low-level constitutive activity in the absence of FSH ligand (Baker *et al.* 2003) and this explains somewhat milder phenotypes in FSH β knockout males compared with those in FSH-receptor knockout mice (see below). In contrast to apparently normal fertility in males, FSH β knockout females are infertile and demonstrated decreased ovary size and thin but mostly variable size uterine horns. Serum estradiol levels were not altered, but progesterone was reduced by 50% and LH levels were 2–3 fold elevated compared with age-matched controls. Ovarian histology indicated a preantral stage block in folliculogenesis with no corpora lutea. Primordial and multilayer preantral follicles appeared normal with normal granulosa, thecal cells and oocytes. Pregnant mare serum gonadotropin (PMSG)/hCG injections pharmacologically rescued FSH β knockout females suggesting the ovulatory competence is unaffected in the absence of FSH (Kumar *et al.* 1997). Further characterization of the adult ovarian mutant phenotype involved an analysis of gene expression in the absence of FSH. Consistent with normal thecal layer recruitment, P450 17 α -hydroxylase and LH-R expression was localized in the mutant ovaries (Burns *et al.* 2001). Expression of many granulosa cell markers including P450 aromatase, serum/glucocorticoid kinase and inhibin/activin subunit mRNAs was reduced but no accumulation of LH-receptor mRNA in granulosa cells was evident. Cyclin D2 a downstream regulator of FSH action was marginally decreased without up-regulation of many cell cycle inhibitor mRNAs typically associated with cell cycle withdrawal at luteinization. Thus in the absence of FSH, although granulosa cells do not proliferate beyond the antral stage, they do not initiate programs of terminal differentiation observed during normal luteinization in wild type ovaries (Burns *et al.* 2001). These observations are consistent with the unaffected ovulatory competence of the FSH β knockout female mice; since the mutant female mice can be pharmacologically as well as genetically rescued resulting in normal luteinization of the follicles.

Hormonal control of somatic cell oocyte interactions during ovarian follicle development is poorly understood. To test the hypothesis that FSH regulates the ability of granulosa cells to make connections with the oocyte, FSH β knockout female mice were analyzed for transzonal projections (TZPs) between granulosa cells and oocytes in the ovaries. In FSH β knockout mice, similar to immature

control mice, granulosa cells exhibit orientation towards the oocyte manifest by the elaboration of TZPs. *In vivo* FSH treatment of FSH β knockout mice results in alterations in somatic cell adhesion to the oocyte, enhances oocyte chromatin remodeling and apical centrosome positioning at sites of granulosa-zona contact (Combelles *et al.* 2004). FSH priming also decreases the density of TZPs and this coincides with changes in oocyte transcriptional activity and meiotic competence (Combelles *et al.* 2004). Thus, these studies have identified a critical role for FSH signaling in mediating the somatic cell oocyte interactions via regulation of TZPs and suggest that signaling from the oocyte acts as a checkpoint for oocyte development until hormone signaling in the somatic cells specifies further progression of the folliculogenesis.

Long-term consequences of the absence of FSH have been evaluated and these studies identified age-related uterine and ovarian hypertrophy pathologies in FSH β knockout females (Abel *et al.* 2003). At 1 year of age, uterine mass is significantly increased (more than 8 fold) in the majority of FSH β knockout females; however, the uteri are non-contractile and do not respond to electrical or pharmacological stimulation (Abel *et al.* 2003). The ovarian size is increased accompanied by hypertrophy of the interstitial tissue, with occasional presence of ovarian cysts and epithelial inclusions. Serum androgen levels are also slightly elevated (1.6 nmol/L compared with 1.0 nmol/L in the controls) in these mice. Ovariectomy resulted in decreased uterine mass and increased serum LH levels (Abel *et al.* 2003). These age-related phenotypes resemble the serous ovarian adenocarcinomas found in humans and suggest possible hormonal imbalance occurring as a result of defects in FSH-mediated signaling.

Genetic rescue of FSH β knockout mice

To confirm that the absence of FSH (as a result of the designed null mutation in the FSH β locus) alone contributed to the mutant phenotypes in FSH β knockout mice and to test whether interspecies glycoprotein hormone hybrids function *in vivo*, genetic rescue experiments were performed in two different ways (Kumar *et al.* 1998). In the first approach (type I rescue), a well characterized 10 kb of hFSH β transgene that contained all the appropriate regulatory elements for gonadotrope-specific expression and hormonal regulation was introduced in to the FSH β null background by a genetic intercross. The assumption was that the hFSH β transgene when expressed in the mouse pituitary environment in the absence of mouse FSH β , would recombine with the endogenous mouse α -GSU subunit and produce an interspecies hybrid FSH. In the second approach (type II rescue), two transgenes including the human α -GSU and hFSH β driven by MT promoter were similarly introduced into FSH β null background. In this line of mice hFSH is ectopically produced in low levels by multiple tissues. In both types of genetic rescue, the expression of hFSH transgenes in the FSH β

null background, male mice were fertile; the testicular size, tubular volume and sperm number and motility were restored to values similar to those observed in wild type control mice. While the type I rescue female mice also were fertile and produced normal number of offspring when mated to wild type mice, only 20% of the type II rescue female mice were fertile and produced fewer pups than normal mice. These genetic rescue studies indicated that hFSH β transgene regulation and function in the mouse pituitary are indistinguishable from the endogenous mouse FSH β gene (Kumar *et al.* 1998).

FSH-receptor knockout mice

FSH binds to seven transmembrane spanning G-protein coupled receptors on the Sertoli cells in the testis and granulosa cells in the ovary (Bousfield *et al.* 1994). Previous biochemical studies indicated this receptor signaling stimulates cAMP production. The gene encoding FSH-receptor from a number of species was cloned (Griswold *et al.* 1995, Heckert & Griswold 2002). The expression of FSH-receptor is correlated to seminiferous tubule stage during spermatogenesis and its regulation is not well understood. To assess the consequences of loss of FSH signaling through FSH-receptor, Sairam and his colleagues generated FSH-receptor knockout (FORKO) mice and later extensively characterized these mutants (Dierich *et al.* 1998, Sairam & Krishnamurthy 2001). Subsequently, Abel and her colleagues also produced and characterized FORKO mice (Abel *et al.* 2000). Although the FORKO model grossly phenocopies FSH β knockout mice, several unique phenotypes were also evident indicating that disruption of a cellular autonomous signaling pathway has more severe phenotypic consequences than loss of the corresponding secreted ligand that binds and stimulates the same receptor.

Male reproductive phenotypes in FORKO mice

FORKO males were fertile, displayed small testes and partial spermatogenic failure with defects in sperm viability and motility (Dierich *et al.* 1998). Although initially reported as fertile, later analyses revealed that FORKO males had a reduced fertility and a delay in puberty based on mating studies (Krishnamurthy *et al.* 2001a). Flow cytometric evaluation of germ cells revealed a significant increase in the percentage of spermatogonia and non-germ cells whereas there was a significant decrease in elongated spermatids. Defects in sperm head shape, chromatin condensation and chromatin remodeling were also observed (Krishnamurthy *et al.* 2000, Xing *et al.* 2003). During the prepubertal period, the expression of transition proteins and protamine-2 was greatly diminished leading to delayed spermatogenesis. Although round spermatids were abundant at this age, by 7 weeks only few tubules contained round spermatids. Mutant males also had decreased levels of testosterone and consequently a

reduction in epididymis and seminal vesicle size (Dierich *et al.* 1998). To further investigate the reasons for low testosterone levels, Leydig cell function was analyzed in FORKO mice. LH-receptor density on Leydig cells and binding of LH to its receptor were normal. However, response to exogenous LH to produce testosterone was greatly reduced in mutants (Krishnamurthy *et al.* 2001*b*). These studies indicated that intercellular communication between Sertoli cells and Leydig cells was altered in the absence of FSH-receptor signaling. This may be an important issue in fertility management therapies aimed at inhibiting signaling through the FSH receptor.

Female reproductive phenotypes in FORKO mice

FORKO females had small ovaries and demonstrated a block in folliculogenesis before antral follicle formation, similar to FSH β knockout mice (Dierich *et al.* 1998). In adult mutant females, serum levels of LH and FSH were elevated and estrogens levels greatly reduced. Around 12 months of age, the majority of the mutant mice developed ovarian cysts and neoplasms including unilateral sex cord-stromal tumors (Danilovich *et al.* 2001). The incidence of these tumors was also observed in heterozygous mice around 15 months. The heterozygous mice initially had reduced fertility and they stopped breeding by 7–9 months. There was an accelerated loss of oocytes as a result of atresia and apoptosis that are typical characteristics of reproductive senescence (Balla *et al.* 2003, Danilovich & Sairam 2002, Yang *et al.* 2003*b*). These phenotypic changes were accompanied by circulating reproductive hormonal imbalance. Further molecular analysis of ovarian marker genes including those involved in oocyte and granulosa cell growth and development confirmed that the oocyte-somatic cell interactions were perturbed (Balla *et al.* 2003, Danilovich & Sairam 2002, Yang *et al.* 2003*b*). During this accelerated aging in heterozygous mice, prominent uterine pathology was also evident. In older heterozygous mice, the uterine weights were increased by 2-fold and occasionally uterine masses were observed in the right uterine horn. Increased angiogenesis, vascular abnormality and adenomyosis were also observed in the right uterine horn that contained pathological mass. Serum estrogen and progesterone were reduced whereas testosterone was elevated. Together, these studies suggest a haploinsufficiency phenotype due to gene dosage effect of the FSH-receptor (Danilovich *et al.* 2002).

Extra-gonadal phenotypes in FORKO mice

In addition to many ovarian defects due to chronic estrogen deficiency resembling those of human menopause, FORKO mice also demonstrated defects outside the ovary. Early loss of estrogen led to obesity and skeletal abnormalities including hunchback appearance (Danilovich *et al.* 2000). Heterozygous mice similarly showed these phenotypes coincident with their accelerated aging.

Despite a marked estrogen insufficiency, the estrogen receptors remained functional since estrogen replacement therapy (by providing estradiol-17 β) reversed the accumulation of adipose tissue (Danilovich *et al.* 2000).

FORKO female mice also demonstrated many symptoms of menopause-associated hypertension in women. These include elevated blood pressure and a blunted response to angiotensin II induced vasoconstriction with significantly increased media-to-lumen ratio. The circulating levels of angiotensin II were decreased in FORKO female mice. Unlike in human hypertension, these studies suggested that although hypertension was observed in female FORKO mice, it was not mediated by angiotensin II in this menopause-associated mouse model (Javeshghani *et al.* 2003).

Estrogen is known to exert pleiotropic effects in the nervous system. The lack of estrogen and an observed estrogen-testosterone imbalance in the FORKO female mice provided a useful model to explore the neurological phenotypes dependent on estrogen function (Danilovich *et al.* 2003*b*). Choline acetyltransferase activity was decreased in many central cholinergic structures including striatum, hippocampus, cortex and dorsal root ganglia. Aberrations were also observed in the enzyme activities of mitogen-activated protein kinases in the hippocampus, dorsal root ganglion and sciatic nerves. Sensory and hippocampal ganglia contained hyperphosphorylated neurofilament proteins that are important structural entities in these ganglia. The ability of neurite outgrowth of explanted ganglia was also decreased with and without estrogen treatment. Further studies identified that the size and amount of immunoreactive glial fibrillary acidic protein was increased in cortex and hippocampus and expression of estrogen α and β receptors in amygdala was reduced in the FORKO mutants (Danilovich *et al.* 2003*a*). These changes were reflected in increased signs of anxiety in these mutant mice. Heterozygous mice showed similar neurological phenotypes of anxiety and degenerative changes in the nervous system (Danilovich *et al.* 2003*a*).

LH β knockout mice

Recently, an LH β knockout mouse model was generated to study the consequences of loss of only LH ligand function independent of FSH, and to better define the critical roles of LH in Leydig cell development and function and ovarian folliculogenesis (Ma *et al.* 2004). The coding sequence of LH β gene was disrupted in ES cells; male chimeras generated from these mutant ES cells initially gave rise to viable and fertile heterozygous mice and subsequently homozygous mice lacking LH β were generated. Pituitary immunostaining using LH β -specific antibodies, western blot analysis of pituitary proteins and serum radioimmunoassay all confirmed that a null mutation at the LH β locus that led to LH β and consequently LH heterodimer deficiency, was engineered (Ma *et al.* 2004).

LH β knockout males were infertile and demonstrated reduced size testes (by 75% compared with controls) and accessory glands, consistent with decreased serum (>90%) and intra-testicular (>95%) testosterone levels, and pituitary serum FSH levels were unaffected. Histological analysis of testes indicated insignificant interstitium containing very few and small size Leydig cells. Gene expression analyses confirmed an increase in fetal Leydig cell marker, thrombospondin-2, and reduction in many of the steroidogenic pathway enzymes and serum assays demonstrated increased levels of the androgen precursor, androstenedione, indicating the presence of mostly fetal and immature Leydig cells in the mutant testes (Ma *et al.* 2004).

To analyze the consequences of severely reduced testosterone on spermatogenesis, the mutant testes were analyzed histologically and expression of spermatogenesis markers was assessed. The mutant testes consist of spermatogonia, spermatocytes (meiotic cells) and round spermatids, but not late stage or elongated spermatids (Ma *et al.* 2004). Expression of histone H1-like linker protein, a late stage spermatid marker was suppressed in the mutant testes. Hence, FSH alone is not sufficient to promote full spermatogenesis in the absence of LH and/or testosterone. Since Sertoli cells are the major targets of androgen action within the testis, expression of Sertoli-specific markers was evaluated in the mutant testes. These studies identified that although expression of some markers (FSH receptor and inhibin α) was not affected, expression of inhibin β A- and β B-subunits and Anti-Müllerian hormone (AMH) was up-regulated (Ma *et al.* 2004). Thus, in LH β mutant mice phenocopy LH-receptor knockout mice (Lei *et al.* 2001, Zhang *et al.* 2001), the loss of LH leads to both somatic and spermatogenic cell defects, similarly seen in other models with a Sertoli cell-selective androgen receptor deletion. LH β mutant mice provide a useful model for further studying somatic-germ cell interactions in the testis.

LH β knockout females, similar to males were hypogonadal and infertile. Histological analysis of the ovaries indicated absence of healthy antral, preovulatory follicles and corpora lutea, confirming impaired estrous cycles. Primary and secondary follicles appeared normal, whereas many antral follicles were abnormal containing degenerating oocytes. Despite these defects in granulosa cells and oocytes, a prominent thecal layer was obvious in follicles at different stages of progression (Ma *et al.* 2004). These observations suggested that differentiation of thecal layer was not impaired in the absence of LH signaling. However, expression of various thecal cell markers including many steroidogenic enzymes was significantly reduced. Consequently, serum progesterone and estradiol levels were decreased by ~70–75% and the mutant uteri were hypoplastic consisting of a thin endometrial layer (Ma *et al.* 2004). These ovarian phenotypes in LH β knockout mice are distinct from those of FSH β knockout mice and provide a genetic evidence for distinct roles of FSH and LH during folliculogenesis.

Pharmacological rescue of both male and female LH β knockout mice was achieved by short-term treatment of hCG. Testes and ovarian markers are up-regulated following hormone replacement of the mutants with hCG indicating that responsiveness to LH is not irreversibly lost in target cells in the absence of LH (Ma *et al.* 2004). Thus, LH β knockout mice serve as a useful model to study LH- and steroid-responsive genes and for identifying to what extent steroids mediate LH effects in target cells.

LH-receptor knockout mice

The LH-receptor, similar to the FSH-receptor is also a seven transmembrane G-protein coupled receptor. Multiple mRNAs are transcribed from the LH-receptor locus and are developmentally regulated (Huhtaniemi 1995). In the male, LH-receptors are expressed on the Leydig cells early during embryogenesis in the testes. In the female, they are expressed on theca and granulosa cells in the ovary; LH receptors are also expressed in luteal cells. Evidence also exists for extra-gonadal expression of LH-receptors, however, their functional significance is not clear (Rao 2001a, 2001b). To study the consequences of loss of LH action, the Huhtaniemi and Rao groups independently developed LH-receptor knockout (LuRKO) mice and reported similar phenotypes in these mice (Lei *et al.* 2001, Zhang *et al.* 2001). While LH signaling was not essential for embryonic development, sex determination and prenatal development of sex organs, many postnatal defects were observed in reproductive tract development resulting in infertility (Lei *et al.* 2001, Zhang *et al.* 2001).

Male reproductive phenotypes in LuRKO mice

LuRKO male mice demonstrated a failure of testicular descent, and reduced size testes, accessory sex organs and external genitalia. The number and size of Leydig cells were also significantly reduced. Serum gonadotropins were elevated and intratesticular and serum testosterone was highly suppressed, whereas serum estradiol levels were moderately elevated (Lei *et al.* 2001, Zhang *et al.* 2001). Spermatogenesis was blocked at the round spermatid stage. Expression of androgen receptors, and FSH-receptors remained unchanged. In contrast, steroidogenic acute regulatory protein (StAR) and estrogen receptor- α were suppressed, and expression of estrogen receptor- β was up-regulated. The expression of many Leydig cell-specific marker genes was gradually decreased from birth until adult stage when many of them were undetectable (Zhang *et al.* 2004). Testosterone replacement therapy partially restored the phenotypes and improved spermatogenesis nevertheless the mutant males remained infertile (Pakarainen *et al.* 2005b, Rao & Lei 2002). Similarly, some but not all of the epididymal phenotypes are restored to those observed in normal mice by androgen replacement therapy. More recent studies from Huhtaniemi group suggest that residual

intra-testicular testosterone is sufficient to restore full spermatogenesis up to elongated spermatids (step 13–16) in LuRKO males at 12 months of age (Zhang *et al.* 2003). Standard regimens of male contraception often include suppression of gonadotropin secretion leading to reduced but detectable levels of testosterone in only 60% of the treated men. Late-onset spermatogenesis recovery in LuRKO mice suggests that only total abolition of testicular androgen action will result in the consistent azoospermia necessary for effective male contraception (Zhang *et al.* 2003).

Reproductive defects in female LuRKO mice

Female LuRKO mice demonstrated small ovaries and thin uteri and the age of vaginal opening was delayed by almost a week compared with wild type controls. Histological analysis of the ovaries revealed antral but no preovulatory follicles or corporal lutea. Serum levels of estrogen and progesterone were suppressed (Lei *et al.* 2001, Zhang *et al.* 2001). Consistent with the size of the uterine horns, the thickness of all uterine layers decreased and only a few glands were present in the endometrium. Estradiol and progesterone replacement therapy of 4 week-old female LuRKO mice for 3 weeks resulted in normal vaginal development but the ovarian morphology did not change (Rao & Lei 2002). Although the uterus became thicker, the number of endometrial glands remained low. Ovarian steroid replacement did not restore fertility in LuRKO mice (Rao & Lei 2002).

From a comparative perspective, there are species-specific differences in gonadotropin regulation of the reproductive physiology. This is particularly striking when species including human, monkey and mice are compared. Unlike the former two, mice do not produce hCG and hence many aspects of early sexual differentiation are different between these species. Nevertheless, mice with null mutations in LH β and LH-R do phenocopy patients with inactivating mutations in the corresponding loci.

Extra-gonadal phenotypes in LuRKO mice

Extra-gonadal effects of LH/hCG are not well understood, despite many reports indicating that their receptors exist outside the reproductive tract. Two of the extra-gonadal phenotypes in LuRKO mice were characterized. These include an obese phenotype and defects in bone structure (Rao & Lei 2002, Yarram *et al.* 2003). The obese phenotype was evident early but became more pronounced around 1 year of age in both homozygous and heterozygous mutants. Histologically, the fat tissue contained hypertrophied adipocytes in these older mice (Rao & Lei 2002).

The bone phenotypes in LuRKO mice were those related to bone mineral density and bone histomorphometry. There was a decrease in bone mineral density in mutant males and all the bone morphometric parameters

were reduced in LuRKO female mice. Although expression of the LH receptors on osteoblasts was confirmed by a variety of molecular techniques, these cells failed to bind 125 I-hCG. In accordance with this, treatment of osteoblasts with hCG also failed to stimulate cAMP production (Yarram *et al.* 2003). Therefore, the defects in bone structure could be secondary to loss of steroids in the absence of LH signaling in LuRKO mice.

Controversy still exists with regard to extra-gonadal actions of LH/hCG. Conflicting evidence that either supports or contradicts such actions in mice has been reported. In one model, steroid replacement therapy failed to restore normal fertility and pregnancies in LuRKO female mice suggesting that extra-gonadal actions, perhaps mediated via the uterine receptors are important (Lei *et al.* 2001, Lin *et al.* 2005). More recent work has demonstrated redundancy of extra-gonadal LH action using another LuRKO model (Pakarainen *et al.* 2005a). In this model, normal fertility was restored in LuRKO mice after orthotopic transplantation of ovaries from wild-type mice indicating that absence of uterine LH receptors does not affect implantation and pregnancy (Pakarainen *et al.* 2005a). It is not clear what is the exact basis for this discrepancy between these two models. However, there are many reports indicating the extra-gonadal actions of LH/hCG in other species including human (Carlson *et al.* 2004, Eblen *et al.* 2001, Lin *et al.* 2003, Mishra *et al.* 2003, Rao *et al.* 2004a, Rao *et al.* 2004b, Rao 2001a, Rao *et al.* 2003, Toth *et al.* 2001, Yang *et al.* 2003a, Zygmunt *et al.* 2002). Whether these species differences truly reflect the fundamental differences in sexual differentiation and female reproductive physiology or due to variations in the experimental settings remains to be established. Future *in vivo* studies may provide more corroborative evidence for the extra-gonadal actions of LH/hCG.

Mouse models for gonadotropin signaling and human reproductive disorders

During the last two decades, manipulation of the mouse genome with regard to gonadotropin function has yielded valuable genetic models that phenocopy many human reproductive disorders (Huhtaniemi 2000b, Kumar & Matzuk 2000, Markkula & Huhtaniemi 1996). Complete loss of FSH function (loss of FSH-receptor signaling) in humans causes ovarian dysgenesis that is genetically traceable in families (Aittomaki *et al.* 1996, Aittomaki *et al.* 1995). Some of the sibling brothers of these female patients are fertile although they have reduced testicular volume and oligospermia (Tapanainen *et al.* 1997). These phenotypes closely resemble those seen in FSH β knockout and FORKO mice. In addition, incomplete loss of FSH action as a result of some inactivating mutations in the FSH receptor cause a partial phenotype and these patients are

responsive to high-dose gonadotropin treatment (Beau *et al.* 1998, Touraine *et al.* 1999). Four different inactivating mutations in the human FSH β coding exon have also been reported (Huhtaniemi *et al.* 1999, Huhtaniemi 2002, Matthews *et al.* 1993). While four of the affected women have similar phenotypes including follicular maturation arrest and infertility, consistent with the phenotypes of FSH β knockout female mice, three men with inactivating FSH β mutations were infertile as a result of azoospermia (Layman *et al.* 2002, Lindstedt *et al.* 1998, Phillip *et al.* 1998). One explanation for this distinct phenotype in men with inactivating mutation of FSH β gene, not observed with the FSH ligand and receptor knockout mice could be that these men might have additional defects resulting in absence of sperm leading to infertility.

LH β knockout and LuRKO mice also phenocopy human patients with inactivating mutations in the LH β subunit or LH-receptor (Huhtaniemi 2000a, Themmen *et al.* 1998, Themmen & Huhtaniemi 2000). Two cases of loss of function mutations in the human LH β gene were also reported (Valdes-Socin *et al.* 2004, Weiss *et al.* 1992). In the first case, only immunoreactive LH was detectable; in both the cases, the mutation results in absence of bioactive LH in serum. Both patients were infertile hypogonadal men; normally masculinized at birth, had descended testes and testicular biopsy revealed absence of full spermatogenesis and mature Leydig cells (Valdes-Socin *et al.* 2004, Weiss *et al.* 1992). Although their circulating testosterone levels were low (<0.5 ng/ml), they were responsive to exogenous hCG stimulation. Long-term hCG treatment resulted in testicular enlargement, normal virilization and spermatogenesis (Valdes-Socin *et al.* 2004, Weiss *et al.* 1992). Phenotypes of women with similar inactivating LH β mutations are not known to date. Phenotypes of patients with partial versus complete inactivating LH receptor mutations vary. In the incomplete form, the phenotype is hypospadias (Misrahi *et al.* 1997) and the complete loss of LH receptor function in men leads to pseudohermaphroditism, i.e., complete sex reversal (Kremer *et al.* 1995). These patients have absent masculinization, and lack of testosterone consistent with absence of Leydig cells (Kremer *et al.* 1995). Interestingly, androgens must be produced from other sources or produced at very low levels from the testis, since accessory glands are present in these patients (Kremer *et al.* 1995). Women with inactivating LH receptor mutations have primary amenorrhea and normal primary and secondary sex characters and do not respond to hCG stimulation (Toledo *et al.* 1996). Ovarian histology closely resembles that of LH β knockout or LuRKO mice and demonstrates all stages of folliculogenesis excepting pre-antral follicles and corpora lutea (Toledo *et al.* 1996). Thus, the available gonadotropin ligand and receptor knockout mouse models closely but not completely resemble human patients with corresponding mutations. These mouse models will allow us to further analyze the roles of gonadotropins in gonad development, delineate

the signaling pathways and identify downstream targets in the gonads of both sexes. These models will also eventually help to more precisely define the molecular basis for both common and the unique phenotypes observed in human patients.

Conclusions and future directions

Gonadotropin research started from classical physiological studies, utilized biochemical methods to characterize the chemical nature of the hormones and provided valuable structure-function information. Modern molecular biology tools helped to understand the importance of glycosylation as well as biosynthesis and secretion of these hormones. The biology of gonadotropins in the context of whole animal physiology has been re-visited but with more powerful genetic approaches enabling controlled gene function. Particularly, mouse manipulation approaches revolutionized the field and offered limitless opportunities to regulate the synthesis and secretion of gonadotropins *in vivo*. These approaches are vital to model many human reproductive disorders in mice.

Where are we heading in gonadotropin research? Our knowledge of GnRH signal relay and integration resulting in co-ordinately regulated expression of gonadotropins and their secretion from the pituitary is very limited. The availability of various existing cell lines (Kumar 2001, Mellon *et al.* 1991) and the ability to generate additional novel gonadotrope cell lines will be important for understanding this process. The ability to spatio-temporally control gene expression in mice may offer clues to roles of gonadotropins in distinct phases of gonad development (Gossen & Bujard 2002). More detailed analysis of the available mutant mouse models with enhanced or impaired gonadotropin action will provide answers regarding the signal transduction pathways downstream of LH and FSH in gonads of both sexes. The mutant mice also provide novel *in vivo* resources to analyze large-scale gene and protein expression profiles in the absence or over expression of gonadotropins. More recently, small interference RNA (siRNA) technology has become useful for abrogating gene function in various cell lines (Hannon & Rossi 2004, Karagiannis & El-Osta 2005, Paddison & Hannon 2002, Silva *et al.* 2004). In this approach, a desired mRNA encoding a protein of interest is eliminated by binding of a specific small RNA sequence that contains features such as stems and loops which selectively hybridizes and 'knockdown' the target mRNA (Hannon & Rossi 2004, Karagiannis & El-Osta 2005, Paddison & Hannon 2002, Silva *et al.* 2004). Although more widely used for various *in vitro* studies, the application of the siRNA approach to temporally block RNA expression/function *in vivo* has not yet been routinely successful. It is anticipated that in the near future the siRNA approach will be used to identify various temporal events in gonadotropin action both *in vitro* and *in vivo*. Meanwhile other

in vitro approaches continue to provide valuable structural information on gonadotropins that can be translated into explaining the *in vivo* physiology. For example, a tetradomain glycoprotein hormone analog consisting of TSH β , FSH β , hCG β and α -subunits tandemly linked was expressed *in vitro*. This analog elicited multiple hormone activities *in vivo* and pharmacologically rescued FSH β knockout mice (Garcia-Campayo *et al.* 2002, Garcia-Campayo *et al.* 2005). It may prove useful for studying thyroid regulation of the fertility status *in vivo* during normal and abnormal reproductive cycles. Similarly, the *in vitro* characterized yoked hCG-receptor single chain protein was expressed in gonads of transgenic mice to create a familial precocious puberty mouse model (Meehan *et al.* 2005). More recently, LuRKO mice have been used as recipients for transplantation of Leydig cell progenitors and it was demonstrated that these transplanted cells produced testosterone *de novo* in the mutant testis (Lo *et al.* 2004). This study exemplifies the use of mouse models for formulating therapies for human male infertility cases in the future.

Acknowledgements

I thank Dr Irving Boime for critically reading the manuscript, many useful discussions and encouragement. Work done in the author's laboratory has been supported in part by The Moran Foundation, Department of Pathology at Baylor College of Medicine, Houston, TX, USA, Department of Molecular & Integrative Physiology, University of Kansas Medical Center and National Institutes of Child Health and Human Development. The author declares that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

- Abel MH, Huhtaniemi I, Pakarinen P, Kumar TR & Charlton HM 2003 Age-related uterine and ovarian hypertrophy in FSH receptor knockout and FSH β subunit knockout mice. *Reproduction* **125** 165–173.
- Abel MH, Wootton AN, Wilkins V, Huhtaniemi I, Knight PG & Charlton HM 2000 The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction. *Endocrinology* **141** 1795–1803.
- Aittomaki K, Herva R, Stenman UH, Juntunen K, Ylostalo P, Hovatta O & de la Chapelle A 1996 Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. *Journal of Clinical Endocrinology and Metabolism* **81** 3722–3726.
- Aittomaki K, Lucena JL, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, Kaskikari R, Sankila EM, Lehtvaslaiho H & Engel AR *et al.* 1995 Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell* **82** 959–968.
- Baker PJ, Pakarinen P, Huhtaniemi IT, Abel MH, Charlton HM, Kumar TR & O'Shaughnessy PJ 2003 Failure of normal Leydig cell development in follicle-stimulating hormone (FSH) receptor-deficient mice, but not FSH β -deficient mice: role for constitutive FSH receptor activity. *Endocrinology* **144** 138–145.
- Balla A, Danilovich N, Yang Y & Sairam MR 2003 Dynamics of ovarian development in the FORKO immature mouse: structural and functional implications for ovarian reserve. *Biology of Reproduction* **69** 1281–1293.
- Beau I, Touraine P, Meduri G, Gougeon A, Desroches A, Matuchansky C, Milgrom E, Kuttann F & Misrahi M 1998 A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. *Journal of Clinical Investigation* **102** 1352–1359.
- Bousfield GR, Perry WM & Ward DN 1994 Gonadotropins: chemistry and biosynthesis. In *The Physiology of Reproduction*, pp 1749–1792. Eds E Knobil & JD Neill. New York: Raven Press.
- Burns KH, Yan C, Kumar TR & Matzuk MM 2001 Analysis of ovarian gene expression in follicle-stimulating hormone beta knockout mice. *Endocrinology* **142** 2742–2751.
- Carlson HE, Kane P, Lei ZM, Li X & Rao CV 2004 Presence of luteinizing hormone/human chorionic gonadotropin receptors in male breast tissues. *Journal of Clinical Endocrinology and Metabolism* **89** 4119–4123.
- Combelles CM, Carabatsos MJ, Kumar TR, Matzuk MM & Albertini DF 2004 Hormonal control of somatic cell oocyte interactions during ovarian follicle development. *Molecular Reproduction and Development* **69** 347–355.
- Danilovich N & Sairam MR 2002 Haploinsufficiency of the follicle-stimulating hormone receptor accelerates oocyte loss inducing early reproductive senescence and biological aging in mice. *Biology of Reproduction* **67** 361–369.
- Danilovich N, Roy I & Sairam MR 2001 Ovarian pathology and high incidence of sex cord tumors in follitropin receptor knockout (FORKO) mice. *Endocrinology* **142** 3673–3684.
- Danilovich N, Roy I & Sairam MR 2002 Emergence of uterine pathology during accelerated biological aging in FSH receptor-haploinsufficient mice. *Endocrinology* **143** 3618–3627.
- Danilovich N, Sairam MR & Maysinger D 2003a The menopausal mouse: a new neural paradigm of a distressing human condition. *Neuroreport* **14** 1617–1622.
- Danilovich N, Harada N, Sairam MR & Maysinger D 2003b Age-related neurodegenerative changes in the central nervous system of estrogen-deficient follitropin receptor knockout mice. *Experimental Neurology* **183** 559–572.
- Danilovich N, Babu PS, Xing W, Gerdes M, Krishnamurthy H & Sairam MR 2000 Estrogen deficiency, obesity, and skeletal abnormalities in follicle-stimulating hormone receptor knockout (FORKO) female mice. *Endocrinology* **141** 4295–4308.
- Dierich A, Sairam MR, Monaco L, Fimia GM, Gansmuller A, LeMeur M & Sassone-Corsi P 1998 Impairing follicle-stimulating hormone (FSH) signaling *in vivo*: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. *PNAS* **95** 13612–13617.
- Eblen A, Bao S, Lei ZM, Nakajima ST & Rao CV 2001 The presence of functional luteinizing hormone/chorionic gonadotropin receptors in human sperm. *Journal of Clinical Endocrinology and Metabolism* **86** 2643–2648.
- Garcia-Campayo V, Kumar TR & Boime I 2002 Thyrotropin, follitropin, and chorionic gonadotropin expressed as a single multifunctional unit reveal remarkable permissiveness in receptor-ligand interactions. *Endocrinology* **143** 3773–3778.
- Garcia-Campayo V, Boime I, Ma X, Daphna-Iken D & Kumar TR 2005 A single-chain tetradomain glycoprotein hormone analog elicits multiple hormone activities *in vivo*. *Biology of Reproduction* **72** 301–308.
- Gossen M & Bujard H 2002 Studying gene function in eukaryotes by conditional gene inactivation. *Annual Reviews in Genetics* **36** 153–173.
- Griswold MD, Heckert L & Linder C 1995 The molecular biology of the FSH receptor. *Journal of Steroid Biochemistry and Molecular Biology* **53** 215–218.
- Hannon GJ & Rossi JJ 2004 Unlocking the potential of the human genome with RNA interference. *Nature* **431** 371–378.
- Heckert LL & Griswold MD 2002 The expression of the follicle-stimulating hormone receptor in spermatogenesis. *Recent Progress in Hormone Research* **57** 129–148.

- Huhtaniemi I** 1995 Molecular aspects of the ontogeny of the pituitary-gonadal axis. *Reproduction, Fertility and Development* **7** 1025–1035.
- Huhtaniemi I** 2000a Activating and inactivating hormone receptor mutations. *Hormonal Research* **53** 9–16.
- Huhtaniemi I** 2000b The Parkes lecture. Mutations of gonadotrophin and gonadotrophin receptor genes: what do they teach us about reproductive physiology. *Journal of Reproduction and Fertility* **119** 173–186.
- Huhtaniemi I, Jiang M, Nilsson C & Pettersson K** 1999 Mutations and polymorphisms in gonadotropin genes. *Molecular and Cellular Endocrinology* **151** 89–94.
- Huhtaniemi IT** 2002 The role of mutations affecting gonadotrophin secretion and action in disorders of pubertal development. *Best Practice & Research. Clinical Endocrinology & Metabolism* **16** 123–138.
- Javeshghani D, Touyz RM, Sairam MR, Virdis A, Neves MF & Schiffrin EL** 2003 Attenuated responses to angiotensin II in follitropin receptor knockout mice, a model of menopause-associated hypertension. *Hypertension* **42** 761–767.
- Karagiannis TC & El-Osta A** 2005 RNA interference and potential therapeutic applications of short interfering RNAs. *Cancer Gene Therapy*.
- Kendall SK, Samuelson LC, Saunders TL, Wood RI & Camper SA** 1995 Targeted disruption of the pituitary glycoprotein hormone alpha-subunit produces hypogonadal and hypothyroid mice. *Genes & Development* **9** 2007–2019.
- Kremer H, Kraaij R, Toledo SP, Post M, Fridman JB, Hayashida CY, van Reen M, Milgrom E, Ropers HH & Mariman E et al.** 1995 Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nature Genetics* **9** 160–164.
- Krishnamurthy H, Danilovich N, Morales CR & Sairam MR** 2000 Qualitative and quantitative decline in spermatogenesis of the follicle-stimulating hormone receptor knockout (FORKO) mouse. *Biology of Reproduction* **62** 1146–1159.
- Krishnamurthy H, Babu PS, Morales CR & Sairam MR** 2001a Delay in sexual maturity of the follicle-stimulating hormone receptor knockout male mouse. *Biology of Reproduction* **65** 522–531.
- Krishnamurthy H, Kats R, Danilovich N, Javeshghani D & Sairam MR** 2001b Intercellular communication between Sertoli cells and Leydig cells in the absence of follicle-stimulating hormone-receptor signaling. *Biology of Reproduction* **65** 1201–1207.
- Kumar TR** 2001 Neuroendocrine cell-specific immortalization using transgenic approaches. In *Transgenics in Endocrinology*, pp 425–466. Eds MM Matzuk, CE Brown, TR Kumar, Totowa: Humana Press.
- Kumar TR & Matzuk MM** 2000 Gene knockout models to study the hypothalamus-pituitary-gonadal axis. In *Gene engineering and molecular models in endocrinology*, Ed. MA Shupnik. Totowa: Humana Press.
- Kumar TR, Low MJ & Matzuk MM** 1998 Genetic rescue of follicle-stimulating hormone beta-deficient mice. *Endocrinology* **139** 3289–3295.
- Kumar TR, Wang Y, Lu N & Matzuk MM** 1997 Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nature Genetics* **15** 201–204.
- Kumar TR, Varani S, Wreford NG, Telfer NM, de Kretser DM & Matzuk MM** 2001 Male reproductive phenotypes in double mutant mice lacking both FSHbeta and activin receptor IIA. *Endocrinology* **142** 3512–3518.
- Layman LC, Porto AL, Xie J, da Motta LA, da Motta LD, Weiser W & Sluss PM** 2002 FSH beta gene mutations in a female with partial breast development and a male sibling with normal puberty and azoospermia. *Journal of Clinical Endocrinology and Metabolism* **87** 3702–3707.
- Lei ZM, Mishra S, Zou W, Xu B, Foltz M, Li X & Rao CV** 2001 Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. *Molecular Endocrinology* **15** 184–200.
- Lin DX, Lei ZM, Li X & Rao Ch V** 2005 Targeted disruption of LH receptor gene revealed the importance of uterine LH signaling. *Molecular and Cellular Endocrinology* **234** 105–116.
- Lin PC, Li X, Lei ZM & Rao Ch V** 2003 Human cervix contains functional luteinizing hormone/human chorionic gonadotropin receptors. *Journal of Clinical Endocrinology and Metabolism* **88** 3409–3414.
- Lindstedt G, Nystrom E, Matthews C, Ernest I, Janson PO & Chatterjee K** 1998 Follitropin (FSH) deficiency in an infertile male due to FSHbeta gene mutation. A syndrome of normal puberty and virilization but underdeveloped testicles with azoospermia, low FSH but high luteinizing hormone and normal serum testosterone concentrations. *Clinical Chemistry and Laboratory Medicine* **36** 663–665.
- Lo KC, Lei Z, Rao Ch V, Beck J & Lamb DJ** 2004 De novo testosterone production in luteinizing hormone receptor knockout mice after transplantation of leydig stem cells. *Endocrinology* **145** 4011–4015.
- Ma X, Dong Y, Matzuk MM & Kumar TR** 2004 Targeted disruption of luteinizing hormone beta-subunit leads to hypogonadism, defects in gonadal steroidogenesis, and infertility. *PNAS* **101** 17294–17299.
- Markkula M & Huhtaniemi I** 1996 Transgenic animals and gonadotrophins. *Reviews of Reproduction* **1** 97106.
- Mason AJ, Hayflick JS, Zoeller RT, Young WS 3rd, Phillips HS, Nikolics K & Seeburg PH** 1986a A deletion truncating the gonadotropin-releasing hormone gene is responsible for hypogonadism in the hpg mouse. *Science* **234** 1366–1371.
- Mason AJ, Pitts SL, Nikolics K, Szonyi E, Wilcox JN, Seeburg PH & Stewart TA** 1986b The hypogonadal mouse: reproductive functions restored by gene therapy. *Science* **234** 1372–1378.
- Mason AJ, Pitts SL, Nikolics K, Szonyi E, Wilcox JN, Seeburg PH & Stewart TA** 1987 Gonadal development and gametogenesis in the hypogonadal mouse are restored by gene transfer. *Annals of the New York Academy of Sciences* **513** 16–26.
- Matthews CH, Borgato S, Beck-Peccoz P, Adams M, Tone Y, Gambino G, Casagrande S, Tedeschini G, Benedetti A & Chatterjee VK** 1993 Primary amenorrhoea and infertility due to a mutation in the beta-subunit of follicle-stimulating hormone. *Nature Genetics* **5** 83–86.
- McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, de Kretser DM, Pratis K & Robertson DM** 2002 Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. *Recent Progress in Hormone Research* **57** 149–179.
- Meehan TP, Harmon BG, Overcast ME, Yu KK, Camper SA, Puett D & Narayan P** 2005 Gonadal defects and hormonal alterations in transgenic mice expressing a single chain human chorionic gonadotropin-luteinizing hormone complex. *Journal of Molecular Endocrinology* **34** 489–503.
- Mellon PL, Windle JJ & Weiner RI** 1991 Immortalization of neuroendocrine cells by targeted oncogenesis. *Recent Progress in Hormone Research* **47** 69–93; 93–66 discussion.
- Mishra S, Lei ZM & Rao Ch V** 2003 A novel role of luteinizing hormone in the embryo development in cocultures. *Biology of Reproduction* **68** 1455–1462.
- Misrahi M, Meduri G, Pissard S, Bouvattier C, Beau I, Loosfelt H, Jolivet A, Rappaport R, Milgrom E & Bougneres P** 1997 Comparison of immunocytochemical and molecular features with the phenotype in a case of incomplete male pseudohermaphroditism associated with a mutation of the luteinizing hormone receptor. *Journal of Clinical Endocrinology and Metabolism* **82** 2159–2165.
- Moudgal NR & Sairam MR** 1998 Is there a true requirement for follicle stimulating hormone in promoting spermatogenesis and fertility in primates? *Human Reproduction* **13** 916–919.
- Moudgal NR, Jeyakumar M, Krishnamurthy HN, Sridhar S, Krishnamurthy H & Martin F** 1997 Development of male contraceptive vaccine—a perspective. *Human Reproduction Update* **3** 335–346.
- Paddison PJ & Hannon GJ** 2002 RNA interference: the new somatic cell genetics? *Cancer Cell* **2** 17–23.

- Pakarainen T, Zhang FP, Poutanen M & Huhtaniemi I 2005a Fertility in luteinizing hormone receptor-knockout mice after wild-type ovary transplantation demonstrates redundancy of extragonadal luteinizing hormone action. *Journal of Clinical Investigation* **115** 1862–1868.
- Pakarainen T, Zhang FP, Makela S, Poutanen M & Huhtaniemi I 2005b Testosterone replacement therapy induces spermatogenesis and partially restores fertility in luteinizing hormone receptor knockout mice. *Endocrinology* **146** 596–606.
- Phillip M, Arbelle JE, Segev Y & Parvari R 1998 Male hypogonadism due to a mutation in the gene for the beta-subunit of follicle-stimulating hormone. *New England Journal of Medicine* **338** 1729–1732.
- Pierce JG & Parsons TF 1981 Glycoprotein hormones: structure and function. *Annual Reviews of Biochemistry* **50** 465–495.
- Plant TM & Marshall GR 2001 The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. *Endocrine Reviews* **22** 764–786.
- Rao CV, Zhou XL & Lei ZM 2004a Functional luteinizing hormone/chorionic gonadotropin receptors in human adrenal cortical H295R cells. *Biology of Reproduction* **71** 579–587.
- Rao CV, Li X, Manna SK, Lei ZM & Aggarwal BB 2004b Human chorionic gonadotropin decreases proliferation and invasion of breast cancer MCF-7 cells by inhibiting NF-kappaB and AP-1 activation. *Journal of Biological Chemistry* **279** 25503–25510.
- Rao CV 2001a An overview of the past, present, and future of non-gonadal LH/hCG actions in reproductive biology and medicine. *Seminars in Reproductive Medicine* **19** 7–17.
- Rao CV 2001b Multiple novel roles of luteinizing hormone. *Fertility and Sterility* **76** 1097–1100.
- Rao CV & Lei ZM 2002 Consequences of targeted inactivation of LH receptors. *Molecular and Cellular Endocrinology* **187** 57–67.
- Rao SC, Li X, Rao Ch V & Magnuson DS 2003 Human chorionic gonadotropin/luteinizing hormone receptor expression in the adult rat spinal cord. *Neuroscience Letters* **336** 135–138.
- Ruf F, Fink MY & Sealton SC 2003 Structure of the GnRH receptor-stimulated signaling network: insights from genomics. *Frontiers in Neuroendocrinology* **24** 181–199.
- Sairam MR & Krishnamurthy H 2001 The role of follicle-stimulating hormone in spermatogenesis: lessons from knockout animal models. *Archives of Medical Research* **32** 601–608.
- Shimasaki S, Moore RK, Otsuka F & Erickson GF 2004 The bone morphogenetic protein system in mammalian reproduction. *Endocrine Reviews* **25** 72–101.
- Silva J, Chang K, Hannon GJ & Rivas FV 2004 RNA-interference-based functional genomics in mammalian cells: reverse genetics coming of age. *Oncogene* **23** 8401–8409.
- Tapanainen JS, Aittomaki K, Min J, Vaskivuo T & Huhtaniemi IT 1997 Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nature Genetics* **15** 205–206.
- Themmen AP, Martens JW & Brunner HG 1998 Activating and inactivating mutations in LH receptors. *Molecular and Cellular Endocrinology* **145** 137–142.
- Themmen APN & Huhtaniemi IT 2000 Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. *Endocrine Reviews* **21** 551–583.
- Toledo SP, Brunner HG, Kraaij R, Post M, Dahia PL, Hayashida CY & Kremer HTAP 1996 An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. *Journal of Clinical Endocrinology and Metabolism* **81** 3850–3854.
- Toth P, Lukacs H, Gimes G, Sebestyen A, Pasztor N, Paulin F & Rao CV 2001 Clinical importance of vascular LH/hCG receptors—a review. *Reproductive Biology* **1** 5–11.
- Touraine P, Beau I, Gougeon A, Meduri G, Desroches A, Pichard C, Detoef M, Paniel B, Prieur M & Zorn JR *et al.* 1999 New natural inactivating mutations of the follicle-stimulating hormone receptor: correlations between receptor function and phenotype. *Molecular Endocrinology* **13** 1844–1854.
- Valdes-Socin H, Salvi R, Daly AF, Gaillard RC, Quatresooz P, Tebeu PM, Pralong FP & Beckers A 2004 Hypogonadism in a patient with a mutation in the luteinizing hormone beta-subunit gene. *New England Journal of Medicine* **351** 2619–2625.
- Weiss J, Axelrod L, Whitcomb RW, Harris PE, Crowley WF & Jameson JL 1992 Hypogonadism caused by a single amino acid substitution in the beta subunit of luteinizing hormone. *New England Journal of Medicine* **326** 179–183.
- Wreford NG, Kumar TR, Matzuk MM & de Kretser DM 2001 Analysis of the testicular phenotype of the follicle-stimulating hormone beta-subunit knockout and the activin type II receptor knockout mice by stereological analysis. *Endocrinology* **142** 2916–2920.
- Xing W, Krishnamurthy H & Sairam MR 2003 Role of follitropin receptor signaling in nuclear protein transitions and chromatin condensation during spermatogenesis. *Biochemical and Biophysical Research Communications* **312** 697–701.
- Yang M, Lei ZM & Rao Ch V 2003a The central role of human chorionic gonadotropin in the formation of human placental syncytium. *Endocrinology* **144** 1108–1120.
- Yang Y, Balla A, Danilovich N & Sairam MR 2003b Developmental and molecular aberrations associated with deterioration of oogenesis during complete or partial follicle-stimulating hormone receptor deficiency in mice. *Biology of Reproduction* **69** 1294–1302.
- Yarram SJ, Perry MJ, Christopher TJ, Westby K, Brown NL, Lamminen T, Rulli SB, Zhang FP, Huhtaniemi I & Sandy JR *et al.* 2003 Luteinizing hormone receptor knockout (LuRKO) mice and transgenic human chorionic gonadotropin (hCG)-overexpressing mice (hCG alphabeta+) have bone phenotypes. *Endocrinology* **144** 3555–3564.
- Zhang FP, Poutanen M, Wilbertz J & Huhtaniemi I 2001 Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. *Molecular Endocrinology* **15** 172–183.
- Zhang FP, Pakarainen T, Poutanen M, Toppari J & Huhtaniemi I 2003 The low gonadotropin-independent constitutive production of testicular testosterone is sufficient to maintain spermatogenesis. *PNAS* **100** 13692–13697.
- Zhang FP, Pakarainen T, Zhu F, Poutanen M & Huhtaniemi I 2004 Molecular characterization of postnatal development of testicular steroidogenesis in luteinizing hormone receptor knockout mice. *Endocrinology* **145** 1453–1463.
- Zygmunt M, Herr F, Keller-Schoenwetter S, Kunzi-Rapp K, Munstedt K, Rao CV, Lang U & Preissner KT 2002 Characterization of human chorionic gonadotropin as a novel angiogenic factor. *Journal of Clinical Endocrinology and Metabolism* **87** 5290–5296.

Received 12 May 2005

First decision 27 May 2005

Revised manuscript received 12 July 2005

Accepted 12 July 2005