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Gonadotropin Gene Targeting and Biological Implications

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Pituitary gonadotropins FSH and LH are heterodimeric glycoproteins consisting of a common alpha and a hormone-specific beta subunit that are non-covalently linked. These hormones orchestrate gonadal growth, differentiation, and function by regulating both steroidogenesis and gametogenesis. Advances in the past two decades in manipulating the mouse genome by site-specific mutagenesis have heralded a new dimension to our understanding of the biology of gonadotropins. Using these gene-targeting approaches, knockout mice lacking the hormone-specific gonadotropin subunits, and hence the functional dimeric hormones, have been generated. These individual gonadotropin-deficient mice are useful to delineate the distinct in vivo biological roles of FSH and LH. These mice also serve as valuable genetic tools to study the signaling mechanisms within the gonads and help a better understanding of some forms of human infertility.

Key Words: Knockout mice; pituitary; LH; FSH; hCG; testis; ovary.

Introduction

The pituitary and placental gonadotropins, luteinizing hormone (LH), follicle stimulating hormone (FSH), chorionic gonadotropin (CG), belong to an evolutionarily conserved glycoprotein hormone family *(1)*. These are non-covalently linked heterodimers 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 *(2)*. LH and CG are structurally similar; hCGβ is characterized by the presence of a unique carboxy terminus peptide (CTP). This sequence is known to confer apical polarity for hCG secretion from placenta and a longer half-life to CG in serum *(2)*. Both hormones bind identical receptors and stimulate cAMP production in target cells. Distinct genes encode gonadotropin subunits; the subunits are synthesized as precursors, and are processed, assembled, and secreted via distinct mechanisms from pituitary gonadotropes or trophoblast cells *(1)*. Gene expression and synthesis of the gonadotropin subunits is gender-specific and coordinately regulated according to physiological demands and is tightly regulated by a number of autocrine, paracrine, and endocrine factors. Gonadotropin-releasing hormone (GnRH) is a hypothalamic decapeptide that binds to gonadotrope GnRH receptors and activates the pituitary gonadotropin subunit genes *(3)*. Members of transforming growth factor-β superfamily including activins, inhibins, follistatin, and bone morphogenetic proteins primarily regulate FSH homeostasis *(4)*. Understanding the biology of gonadotropins has many clinical implications with regard to fertility management and designing novel therapeutic strategies for many gonadal cancers. In this review, I will focus on two loss-of-function mouse models to study the in vivo physiology of FSH and LH and discuss how genetic approaches have been used to test the previously postulated physiological roles of these hormones.

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Gene Knockout Approaches

Loss-of-function genetic approach is useful to define and test gene function in vivo. The basic principle of this approach is selectively to engineer a mutation in a locus of interest to abolish the encoded protein function in vivo *(5–15)*. The desired mutations are engineered by a homologous recombination strategy in pluripotent mouse embryonic stem (ES) cells that are first enriched by appropriate drug selection. These mutant ES cells are later injected into host blastocysts and transferred to foster mice to achieve germline transmission of the engineered mutation. Heterozygous mutant mice thus obtained are subsequently intercrossed to produce homozygous mutants. The ability to manipulate the mouse genome has revolutionized practically every field of modern biology *(5–15)*. Engineering mice with mutations as small as a single base pair change to large megabase-range chromosomal alterations is now possible. Tissue/cell-specific gene deletions and/or temporally controlled inactivation of gene function are also possible to engineer in mice *(5–15)*.

α-GSU Knockout Mice

To study the functional role of α-GSU, particularly during early pituitary development, Camper and her colleagues

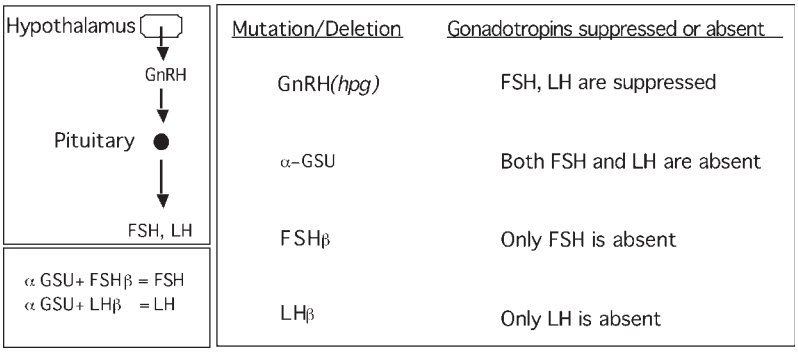

Fig. 1. Pituitary glycoprotein hormones and mutations in various genes. Gonadotropin-releasing hormone (GnRH) elicited from hypothalamus activates pituitary LH and FSH production (left panel, top). Both these gonadotropins are heterodimeric glycoproteins; they share a common α subunit (α-GSU) that is non-covalently linked to a hormone-specific β subunit i.e., FSHβ or LHβ (left panel, bottom). Right panel: A deletion in GnRH encoding gene causes suppressed levels of both FSH and LH in the naturally occurring mouse mutant, *hpg*. Similarly targeted deletion of the common α-GSU subunit leads to absence of LH, FSH, and also TSH. In contrast, targeted mutations in FSHβ or LHβ subunit genes lead to selective absence of either FSH or LH in mice. In all these mutant mouse models, the responsiveness to gonadotropins is retained in target cells of both sexes.

developed mice lacking α-GSU *(16)*. Because α-GSU is the common glycoprotein subunit for TSH, LH, and FSH, all three 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 wk of age, smaller tubules were apparent, interstitial cells were rare, and spermatogenesis was blocked at the first meiotic division *(16)*. 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 *(16)*. These genetic studies with the α-GSU mice confirmed that early gonadal development is independent of gonadotropin function.

FSHβ Knockout Mice

In the naturally occurring hypogonadal (*hpg*) mutant mouse, both FSH and LH are suppressed *(17–19)*. Similarly, α-GSU knockout mice (described above) lack both of these gonadotropins in addition to TSH (Fig. 1). 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 FSHβ gene in ES cells and subsequently generated FSHβ knockout mice from these targeted cells *(20)*. 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. FSHβ heterozygous mice were normal and fertile and did not demonstrate any overt phenotypes.

FSHβ 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 *(20)*. Stereological analysis confirmed a reduction in the Sertoli cell number and the germ cell carrying capacity consistent with reduced testis size in the mutant mice *(21,22)*. The net number of Leydig cells per testis and serum LH and testosterone levels were not affected. Accordingly, the accessory sex glands appeared normal. The normal Leydig cell phenotypes despite reduced Sertoli cell number in FSHβ knockout mice were attributed to low-level constitutive activity of the FSH receptor, in the absence of agonist stimulation *(23)*.

Although phenotypes of the FSHβ knockout male mice are consistent with those reported earlier in the male rat experimentally treated with GnRH antagonists or immunoneutralization of circulating FSH *(24)*, they identified important species-specific differences with regard to FSH action in the testis of other mammalian species *(24–28)*. For example, a clear need for post-pubertal FSH action has been established in various aspects of spermatogenesis including the quality of sperm produced in both monkeys and men actively immunized with various FSH-based vaccines *(24–28)*. One way to directly address this variability in FSH action in males of different species could involve developing a temporal gene inactivation strategy *(29–31)* in mice

in which the FSHβ gene is ablated selectively during postpubertal period and compare the testicular phenotypes to those in existing FSHβ knockout mouse model.

In contrast to apparently normal fertility in males, FSHβ 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 and LH levels were slightly elevated. 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. PMSG/hCG injections pharmacologically rescued FSHβ knockout females suggesting that the ovulatory competence is unaffected in the absence of FSH *(20)*. These observations suggest that the ability of FSHβ knockout females to respond to gonadotropins and ovulate in response to an exogenous bolus of LH is not affected in the absence of endogenous FSH.

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 17β-hydroxylase and LH-R expression was localized in the mutant ovaries *(32)*. 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 mouse ovaries *(32)*.

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, FSHβ knockout female mice were analyzed for transzonal projections (TZPs) between granulosa cells and oocytes in the ovaries. In FSHβ 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 FSHβ 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 *(33)*. FSH priming also decreases the density of TZPs, and this coincides with changes in oocyte transcriptional activity and meiotic competence *(33)*. 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 FSHβ knockout females *(34)*. At 1 yr of age, uterine mass is significantly increased in the majority of FSHβ knockout females; however, the uteri are non-contractile and do not respond to electrical or pharmacological stimulation *(34)*. 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 elevated in these mice. Ovariectomy resulted in decreased uterine mass and increased serum LH levels *(34)*. 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 FSHβ Knockout Mice

To confirm that the absence of FSH (as a result of the designed null mutation in the FSHβ locus) alone contributed to the mutant phenotypes in FSHβ knockout mice and to test whether interspecies glycoprotein hormone hybrids function in vivo, genetic rescue experiments were performed in two different ways *(35)*. In the first approach (type I rescue), a well-characterized 10 kb of human FSHβ transgene that contained all the appropriate regulatory elements for gonadotrope-specific expression and hormonal regulation was introduced into the FSHβ null background by a genetic intercross. The assumption was that the hFSHβ transgene, when expressed in the mouse pituitary environment in the absence of mouse FSHβ, would recombine with the endogenous mouse α-GSU subunit and produces an interspecies hybrid FSH. In the second approach (type II rescue), two transgenes including the human α-GSU and hFSHβ driven by MT promoter were similarly introduced into FSHβ 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 FSHβ 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 male 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 hFSHβ transgene regulation and function in the mouse pituitary are indistinguishable from the endogenous mouse FSHβ gene *(35)*.

Double Mutant Mice to Study the Physiology of FSH

Prior to the production and characterization of FSHβ knockout mice, functional analysis of other gene knockout models defined the physiological regulators of FSH homeostasis. Important among them are mouse models for some

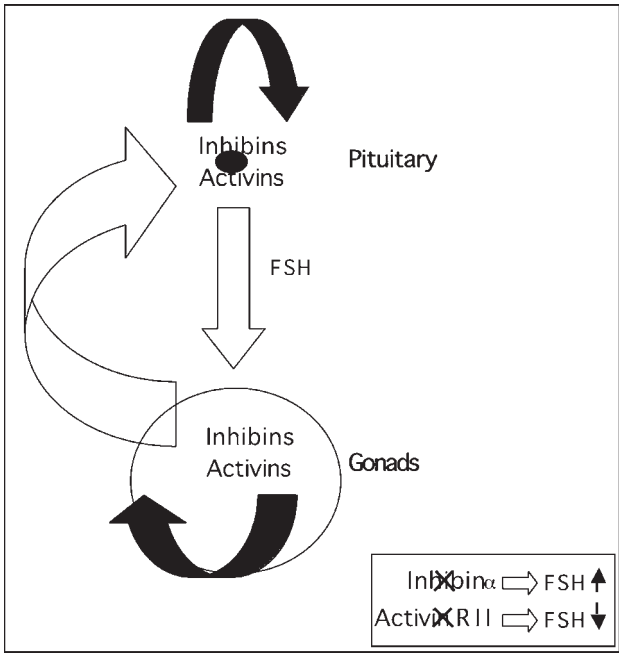

Fig. 2. Inhibins and activins regulate FSH homeostasis. Inhibins and activins are produced in multiple tissues including the somatic cells in the gonads and the anterior pituitary. They act as both autocrine and endocrine regulators of FSH homeostasis. Mutations in mice that abolish inhibin and activin signaling lead to increased or decreased FSH levels, respectively. Either individual or combinations of mutations in inhibin or activin pathway were introduced into FSHβ null background to further understand the in vivo regulation of FSH by these peptides.

of the TGF-β superfamily members and their receptors including inhibin and activin receptor IIA (Fig. 2).

Inhibin/FSHβ Double Knockout Mice

Inhibin α knockout mice developed hemorrhagic gonadal sex-cord stromal tumors and demonstrated elevated serum FSH levels *(36)*. As the tumors progressed with age, they secreted higher levels of activins that caused liver apoptosis and the mice exhibited a cancer-like cachexia (or wasting syndrome), which ultimately resulted in death of these mice *(37)*. Because FSH normally stimulates gonadal activin production, and inhibin mutant mice had higher serum levels of FSH, the role of FSH in gonadal tumor progression in the absence of inhibin was tested. This goal was achieved in two ways: first, double mutants lacking inhibin α and GnRH (both FSH and LH are suppressed) were produced by a genetic intercross using inhibin and *hpg* double heterozygous mice *(38)*. These double mutant mice survived significantly longer than mice lacking inhibin α alone and did not demonstrate gonadal tumors or cachexia symptoms suggesting that gonadotropins (both FSH and LH) are important modulators of gonadal tumor progression in inhibin α knockout mice *(38)*.

Subsequently, with the availability of FSHβ knockout mice, double mutants lacking both inhibin and FSH were generated to directly address the role of FSH alone in tumor development in inhibin α knockout mice *(39)*. The majority of the inhibin α/FSHβ double mutant males lived up to 1 yr without any sign of testicular tumors and did not demonstrate a wasting syndrome or liver apoptosis. In contrast, double mutant females initially demonstrated normal ovarian histology. However, they eventually developed hemorrhagic ovarian tumors, but lived significantly longer than mice lacking inhibin α alone *(39)*. These phenotypes in double mutants lacking both inhibin and FSH were functionally correlated to low levels of serum estradiol, activins, and aromatase. Thus, genetic analyses identified FSH as an important modifier factor for gonadal tumor development in the absence of inhibin *(39)*.

Activin Receptor Type II/FSHβ Double Knockout Mice

Activins were originally discovered in gonadal extracts for their ability to promote FSH production from pituitary gonadotropes *(40)*. They signal through type II and type I serine/threonine kinase receptors that regulate phosphorylation of the signal transducers, the SMAD proteins *(41–43)*. The type II activin receptor (*Acvr2*) is expressed in multiple cell types during mouse embryogenesis and in the adult all along the reproductive axis *(44,45)*. The majority of the *Acvr2* knockout mice were viable, had suppressed pituitary and serum FSH levels, and demonstrated reproductive defects *(46)*. Female mutant mice were infertile due to enhanced follicular atresia, and no corpora lutea were observed in the ovaries. Mutant males had decreased testes size and Sertoli cell number, and are subfertile or occasionally infertile *(46)*. Serum LH, testosterone and hypothalamic GnRH levels were comparable to those in wild-type control males *(47)*. Thus, it appears that at least the majority of the male phenotypes were secondary to the suppressed FSH levels. Since ACVR2 is also expressed in the testis and activins signal through this receptor, double mutant mice lacking both FSHβ and ACVR2 were generated and their male phenotypes characterized to delineate the local effects of ACVR2 signaling, independent of those in the pituitary to regulate FSH *(22)*. Double mutants were fertile, demonstrated no significant reduction in testes size, and produced small litters compared with mice lacking either FSHβ or ACVR2 alone. Although histological analyses revealed normal spermatogenesis, the epididymal sperm number was significantly reduced compared with individual mutants. Analysis of testicular marker gene expression did not reveal any major differences in the mutants. In particular, expression of ACVR2B, the other activin type 2 receptor, is not upregulated in the mutant testes as a compensatory mechanism. Despite these normal phenotypes, stereological analysis revealed a reduction in type A and I spermatogonia compared to that in individual mutants *(22)*. These results provide genetic evidence to confirm that ACVR2 signaling plays an important local role within the testis independent of its actions via FSH homeostasis in the pituitary.

Inhibin α/Acvr2 *Double Knockout Mice*

Because the gonadal tumors in inhibin α knockout mice produce high levels of activins that stimulate gonadal cells, it was genetically tested whether abolishing activin signaling would affect the tumor development. To address this, double mutants lacking *inhibin* α and *Acvr2* were generated and characterized. These double mutants continued to develop gonadal tumors but did not demonstrate wasting syndrome or liver apoptosis *(48)*. These data indicated that the absence of inhibin signaling is the major cause for gonadal tumor development and activins signaling through ACVR2 receptor caused the liver apoptosis in inhibin α knockout mice.

Although activins and inhibins were originally identified as gonadal peptides, later studies identified more widespread expression of these peptides in a variety of tissues where they can act locally in an autocrine and paracrine manner. For example, inhibins and activins are expressed in pituitary gonadotropes and act locally in addition to their endocrine effects *(44)*. To directly address the local roles of activins and inhibins in FSH homeostasis in the pituitary, *inhibin* α */Acvr2* double mutants and individual mutants lacking either *inhibin* α or *Acvr2* were castrated to remove the feedback effects from the gonad. Two weeks later, FSHβ mRNA, serum FSH, and pituitary FSH content were analyzed using these castrated mutant mice. These studies demonstrated that activins primarily regulate the transcription of FSHβ, whereas inhibins primarily regulate biosynthesis and secretion of FSH *(47)*. Hence, within the pituitary, locally produced inhibins and activins exert their actions at distinct phases of FSH homeostasis.

FSH β/Anti-Müllerian Hormone Double Knockout Mice

Anti-Müllerian hormone (AMH) is also a member of the TGF-β superfamily similar to inhibins and activins *(49–53)*. In the mouse ovary AMH inhibits the initiation of primordial follicle growth and suppresses FSH actions on granulosa cells in vitro. Because factors affecting the sensitivity of ovarian follicles to FSH are important for follicle growth, mutant mice lacking both *FSH*β and *Amh* were generated to test the effects on ovarian follicle development *(54)*. Functional analyses with these mutant mice indicated that more follicles begin to grow under the influence of exogenous FSH in *Amh* only knockout mice. Furthermore, loss of FSH expression had no impact on the number of primordial and preantral follicles, whereas the loss of inhibitory action of AMH on the recruitment of primordial follicles in *Amh* knockout mice is increased in the absence of FSH. Thus, AMH is one of the factors determining the sensitivity of ovarian follicles for FSH and that AMH is a dominant regulator of early follicular growth *(54)*.

LHβ Knockout Mice

Recently, an LHβ knockout mouse model 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, was generated *(55)*. The coding sequence of LHβ 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 LHβ were generated. Pituitary immunostaining using LHβ-specific antibodies, Western blot analysis of pituitary proteins, and serum radioimmunoassay all confirmed that a null mutation at the LHβ locus that led to LHβ and, consequently, LH heterodimer deficiency, was engineered *(55)*.

LHβ knockout males were infertile and demonstrated reduced size testes and accessory glands, consistent with decreased serum and intratesticular 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 *(55)*.

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 *(55)*. 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. Because 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 βA- and βB-subunits and AMH was upregulated *(55)*. Thus, LHβ mutant mice phenocopy LH-receptor knockout mice *(56,57)*; 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 *(58–60)*. LHβ mutant mice provide a useful model for further studying somatic-germ cell interactions in the testis.

LHβ knockout females, similar to males, were hypogonadal and infertile *(55)*. 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 *(55)*. Despite these defects in granulosa cells and oocytes, a prominent thecal layer was obvious in follicles at different stages of progression. These observations sug-

gested that differentiation of thecal layer was not impaired in the absence of LH signaling *(55)*. However, expression of various thecal cell markers including many steroidogenic enzymes was significantly reduced. Consequently, serum progesterone and estradiol levels were decreased and the mutant uteri were hypoplastic consisting of a thin endometrial layer. These ovarian phenotypes in LHβ knockout mice are distinct from those of FSHβ knockout mice *(20)* and provide genetic evidence for distinct roles of FSH and LH during folliculogenesis.

Pharmacological rescue of both male and female LHβ 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 *(55)*.

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 *(61–63)*. Loss of FSH function (loss of FSH-receptor signaling) in humans causes ovarian dysgenesis that is genetically traceable in families, and these patients present symptoms seen in FSHβ knockout mice *(64, 65)*. Some of the sibling brothers of these female patients are fertile, although they have reduced testicular volume and oligospermia *(66)*. Mutations in the human FSHβ coding exon have also been reported, and these women presented a similar spectrum of phenotypes seen in the mouse models *(67–69)*. One case of a loss-of-function mutation in the human LHβ gene was also reported with typical features seen in LHβ KO mice *(70)*. Hence, loss-of-function mouse models for both FSH and LH are now available to understand the isolated roles of pituitary gonadotropins in normal physiology and various pathological conditions.

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 *(1,2)*. The biology of gonadotropins in the context of whole animal physiology has been re-visited with powerful genetic approaches enabling controlled gene function *(62)*. In particular, 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 coordinately regulated expression of gonadotropins and their secretion from the pituitary is very limited. The availability of various existing cell lines and the ability to generate additional novel gonadotrope cell lines will be important for understanding this process *(71,72)*. The ability to spatiotemporally control gene expression in mice *(29–31)* may offer clues to roles of gonadotropins in distinct phases of gonad development. 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 overexpression of gonadotropins. The in vitro approach continues 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 TSHβ, FSHβ, hCGβ and α-subunits tandemly linked was expressed in vitro. This analog elicited multiple hormone activities in vivo and pharmacologically rescued FSHβ knockout mice *(73,74)*. It may prove useful for studying thyroid regulation of the fertility status in vivo during normal and abnormal reproductive physiological conditions. Similarly, the in vitro characterized yoked hCG-receptor single-chain protein was expressed in gonads of transgenic mice to create an animal model for familial precocious puberty *(75)*. There are several benefits of gonadotropin research; we can develop contraceptive strategies to prevent alarmingly increasing global population outbreak as well as enhance fertility to offer opportunities to many infertile couples throughout the world to have babies.

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