REVIEW

Molecular regulation of follicle-stimulating hormone synthesis, secretion and action

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Abstract

Follicle-stimulating hormone (FSH) plays fundamental roles in male and female fertility. FSH is a heterodimeric glycoprotein expressed by gonadotrophs in the anterior pituitary. The hormone-specific FSHβ-subunit is non-covalently associated with the common α-subunit that is also present in the luteinizing hormone (LH), another gonadotrophic hormone secreted by gonadotrophs and thyroid-stimulating hormone (TSH) secreted by thyrotrophs. Several decades of research led to the purification, structural characterization and physiological regulation of FSH in a variety of species including humans. With the advent of molecular tools, availability of immortalized gonadotroph cell lines and genetically modified mouse models, our knowledge on molecular mechanisms of FSH regulation has tremendously expanded. Several key players that regulate FSH synthesis, sorting, secretion and action in gonads and extragonadal tissues have been identified in a physiological setting. Novel post-transcriptional and post-translational regulatory mechanisms have also been identified that provide additional layers of regulation mediating FSH homeostasis. Recombinant human FSH analogs hold promise for a variety of clinical applications, whereas blocking antibodies against FSH may prove efficacious for preventing age-dependent bone loss and adiposity. It is anticipated that several exciting new discoveries uncovering all aspects of FSH biology will soon be forthcoming.

Key Words

- pituitary
- FSH
- LH
- testis
- ovary
- extragonadal
- reproduction
- miRNA
- transgenic mice

Introduction

Follicle-stimulating hormone (FSH) is a critical regulator of reproductive physiology. It is a heterodimeric glycoprotein that consists of two distinct subunits, α and β. The α-subunit is common to all pituitary and placental glycoprotein hormones, whereas the β-subunit is hormone-specific and the heterodimer confers biological activity. The overall regulation of FSH involves control at the level of subunit gene transcription, translation, dimer assembly, formation of different isoforms that influence synthesis and release and physiological functions (Pierce & Parsons 1981, Bousfield et al. 2006, Ulloa-Aguirre et al. 2017, Narayan et al. 2018). While earlier research has established FSH as an indispensable player in proper functioning of the reproductive axis, recent findings indicate its much widespread actions. In this review, we provide the molecular mechanisms driving each phase of FSH regulation from the initiation of its synthesis to ultimate physiological effects, with a focus on identifying the interlink between the encompassing effector pathways.
Transcriptional regulation of α-glycoprotein hormone subunit-encoding gene

The α-subunit is expressed in trophoblast, gonadotroph and thyrotroph cell types, and its expression is differentially regulated depending on the specific pituitary or placental cell type.

An earlier study showed that a short 18-bp sequence serves as a cAMP response element that is independent of other regulatory elements around the 5′ flanking region of the human CGA promoter (Silver et al. 1987). In fact, proteins that bind to this cAMP response element positively regulate the alpha-subunit gene by cooperative interaction (Nilson et al. 1989). Another study suggested that placenta-specific expression of human CGA may be evolutionary outcome of a functional cAMP response element, whereas a different cis-acting element might be responsible for its expression in the pituitary (Bokar et al. 1989). It is also known that gonadotropin-releasing hormone (GnRH), produced by the hypothalamus, is a key regulator of Cga mRNA expression (Burrin & Jameson 1989). Transcription of Cga in cultured rat pituitary cells was found to be altered upon pulsatile GnRH administration (Shupnik 1990). Later, two DNA elements were identified that were involved in GnRH-mediated expression of the mouse Cga (Schoderbek et al. 1993). Moreover, the co-localization of GnRH and phorbol myristate acetate (an activator of protein kinase C) responsiveness indicates that the effect of GnRH on transcriptional regulation of mouse Cga is most likely through the PKC pathway.

Various groups also investigated signaling pathways in addition to deciphering DNA elements and transcription factors to better understand their regulatory mechanisms. Maurer and coworkers identified two unrelated DNA elements and designated them as GnRH response element (GnRH-RE), an element that was adequate to allow GnRH response, and a pituitary glycoprotein hormone basal element (PGBE), that enhanced basal expression of the α-subunit (Maurer et al. 1999). GnRH-RE was found to encompass a consensus binding region for the E26 transformation-specific (Ets) family of transcription factors and demonstrated that GnRH-induced activation of MAPK pathway, mediated by GnRH-RE, is required and also sufficient enough for the transcriptional activation of Cga gene. Recently, it was established that GnRH-mediated activation of Cga expression requires stress-activated protein kinase 1 (MSK1) and mitogen through an epigenetically regulated mechanism involving histone modifications (Haj et al. 2017).

Other than GnRH-associated response elements, two upstream regions of the Cga gene were identified that distinctly regulate its basal promoter transcription and PMA-stimulated promoter activity independently in mature gonadotroph cells. Steroidogenic factor 1 (SF-1) is involved in mediating these activities through its binding site in the promoter (Fowkes et al. 2002). Fowkes and coworkers have also shown that GATA factors may be related to ERK activation of transcription, and PKC-induced transcription relies partially on ERK interaction on elements downstream of −244 bp of the promoter. Another study from this group reported that SF-1 also significantly increases forskolin-stimulated Cga transcription and a phosphorylation site at the Serine 203 residue seems to be necessary for the activity (Fowkes & Burrin 2003). A recent study has identified the Msh homeobox 1 (Msx1) as a negative regulator that represses the gonadotroph-specific Cga expression. A decline in Msx1 expression in a temporal manner relieves this repression allowing production of the gonadotropic hormones (Xie et al. 2013). Another study validated the role of Msx1 as a transcription repressor of Cga gene and demonstrated that the mechanism by which Msx1 regulates inhibition of Cga gene expression involves its interaction with the TATA-binding protein in thyrotrhops (Park et al. 2015). Other important factors of Cga gene regulation includes Sine oculis-related homeobox 3 (SIX3) homeodomain transcription factors that represses transcription of the alpha-subunit in immature gonadotroph cell lines (Xie et al. 2015).

Transcriptional regulation of FSHβ subunit-encoding gene

The β-subunit of FSH confers the specific biological activity of FSH dimer. Hence, synthesis of the FSHβ subunit is a rate-limiting step for the production of the biologically active FSH (Papavasiliou et al. 1986). The production of FSHβ subunit is under tight regulation, and one of the most important points of control is at the level of FSHβ-encoding gene transcription. Its expression is also under a critical temporal regulation as FSHβ-encoding mRNA level was observed to increase about 4- to 5-fold during afternoon of proestrus while the increase was about 3-fold during estrus in rodents (Ortolano et al. 1988, Halvorson et al. 1994). Among many regulatory factors, GnRH and activins are key players in controlling FSHβ expression and both these act through independent as well as related pathways.
Regulation of FSHβ-encoding gene expression by GnRH

GnRH is released in a pulsatile manner from neurons in the hypothalamus and reaches pituitary via the hypophyseal-portal system. GnRH receptor is specifically expressed by the gonadotroph cells (Tutsumi et al. 1992, Stojilkovic et al. 1994, Kaiser et al. 1997). GnRH pulse frequency determines the rate of FSHβ production as many studies have shown that a decrease in GnRH pulse frequency favors FSHβ production over that of LHβ (Haisenleder et al. 1988, Dalkin et al. 1989, 2001). FSH levels in serum are reduced by 60–90% in mice without GnRH (Mason et al. 1986) and administration of GnRH to rats increased Fshb mRNA expression by fourfold (Dalkin et al. 2001), indicating that GnRH regulation of FSHβ takes place at the level of transcription.

Of the several proposed effector pathways by which GnRH regulates mouse FSHβ expression, the major ones include PKC and MAPK signaling pathways (Bonfil et al. 2004, Coss et al. 2004, Liu et al. 2005). The GnRH ligand binding activates GnRH receptors (GnRHR), which increase the activities of calcium/calmodulin kinase II and protein kinase C (PKC) (Liu et al. 2002, Haisenleder et al. 2003) via activation of Gq and G11 family of G-proteins (Stanislaus et al. 1997). GnRHR activation also initiates induction of distinct mitogen-activated protein kinase (MAPK) pathways, namely ERK1/2, p38 and JNK (Roberson et al. 1995, Sundaresan et al. 1996, Liu et al. 2002). Activation of the MAPK pathway is achieved via PKC (Naor 2009) or through GnRHR association with Raf and calmodulin, which play a key role in mediating Ca²⁺ action on ERK activation, independent of the phospholipase C activity (Roberson et al. 2005).

GnRH also regulates FSHβ expression via induction of immediate early genes (IEGs), which includes Jun, Fos, Atf3 and Egr1. One such IEG product is activator protein-1 (AP-1), which consists of various Fos and Jun dimeric isoforms (c-Fos, Fra-1, Fra-2, FosB, c-Jun, JunB and JunD) that are induced by GnRH (Wurmbach et al. 2001, Karak et al. 2003). Regulation of Jun and Atf3 by GnRH requires calcium, calcineurin and nuclear factor of activated T cells (NFAT) that confers responsiveness to various genes responsible for FSH synthesis (Binder et al. 2012). Strahl and coworkers identified two AP-1-binding sites within the ovine Fshb promoter sequence that are sufficient enough to stimulate its expression independently (Strahl et al. 1997, 1998). MAPK-mediated GnRH induction of murine Fshb takes place via AP-1-interactive sites, where c-Jun and FosB bind to induce the promoter (Liu et al. 2002, Coss et al. 2004). AP-1 proteins have also been shown to regulate human Fshb promoter activity via two specific response elements (Wang et al. 2008). Integrated GnRH response also requires factors that are associated with basal Fshb expression, like nuclear transcription factor Y (NF-Y) in mouse and upstream stimulatory factor 1 (USF1) in rat, and the mechanism involves interactions of these factors with AP-1 (Coss et al. 2004, Ciccone et al. 2008). c-Fos-knockout mice demonstrate small ovaries and atretic follicles (Johnson et al. 1992), similar to phenotypes of Fshb-knockout mice (Kumar et al. 1997) indicating a possible key role of this proto-oncogene in regulation of FSHβ. GnRH is also reported to increase c-Fos half-life, thus in turn resulting in increased FSHβ expression (Reddy et al. 2013). In addition to these factors, the CREB transcription factor was found to be involved in GnRH-regulated Fshb expression in rats through interaction at a homologous CRE/AP-1 site (Ciccone et al. 2008), although mice deficient in CREB exhibit unaltered FSH levels suggesting a species-specific role of this factor. It is further demonstrated that inducible cAMP early repressors (ICER) antagonize the CREB stimulatory action to attenuate Fshb transcription at high GnRH pulse frequencies (Ciccone et al. 2010). It has been recently reported that ICER influenced GnRH control of Fshb expression is mediated via ERK1/2 regulatory pathway (Thompson et al. 2016).

GnRH stimulation was recently shown to increase intracellular reactive oxygen species (ROS) via NOX/DUOX-mediated activity, suggesting a new concept of ROS involvement as a signaling intermediate in response to GnRH induction (Kim & Lawson 2015). This finding further opens up the possibility that ROS generated by other processes may also influence GnRH stimulation of FSH expression. GnRH induces β-catenin, a classical intermediate of the WNT signaling pathway, which is shown to influence FSHβ production in a murine model (Boerboom et al. 2015). Another proposed factor is phosphoprotein-enriched in astrocytes 15 (PEA-15) an ERK scaffolding protein present in cytosol. It was suggested to mediate convergence of PKC and MAPK/ERK pathways induced by GnRH (Choi et al. 2011). GABA alpha4beta3delta receptor agonist DS1 was recently reported to stimulate FSHβ expression in association with the ERK signaling pathway through the activation of Gnrhr promoter (Mijiddorj et al. 2015). In an alternate mechanism, it was suggested that GnRH regulation of Homer protein homolog 1 (Homer 1) splicing may contribute to FSH expression (Wang et al. 2014b).
Regulation of FSHβ expression by activin-follistatin-inhibin loop

Activin is a dimeric peptide composed of two identical beta subunits that can have two major isoforms, βA and βB. Activins were identified as gonadal peptides, which stimulate the production of FSH (Ling et al. 1986, Vale et al. 1986). They bind to the type II receptor, resulting in phosphorylation of the type I receptor (Attisano & Wrana 2002), which then consequently phosphorylates the intracellular proteins Smad2 and Smad3 initiating the signal response cascade. The phosphorylated proteins bind to Smad4 and forms the ‘activating complex’, which is then translocated to the nucleus where it binds DNA through a direct interaction of Smad-binding elements (SBE) with specific domains on Smad3 and Smad4 (Massague 1998, Shi et al. 1998) to induce transcription of the FSHβ-encoding gene (Bernard & Tran 2013). It was previously reported that activin induces increased FSH release from the pituitary (Ling et al. 1986) and FSHβ expression in gonadotropes (Weiss et al. 1995). Three prominent activin-response elements have been identified in the FSHβ-encoding gene promoter region till date. A classical Smad-binding consensus sequence at −267, consisting a palindrome GTCTAGAC, was first proposed to be an important response element in rodents (Bernard 2004, Gregory et al. 2005, Suszko et al. 2005), but was later on found to have no significant contribution in activin induction of Fshb (Coss et al. 2007, McGillivray et al. 2007). This specific site is not present in human FSHβ promoter. Bailey and coworkers identified three separate elements associated with FSHβ-encoding gene promoter, which were essential for activin induction, and these proximal sites are found to be present in all species examined (Bailey et al. 2004). SMAD-associated factors, like Pre-B-cell leukemia transcription factor 1 (Pbx1) and PBX/knotted 1 homeobox 1 (Prep1) proteins were also identified that seem to bind the response elements and aid in tethering of Smads to the promoter following activation (Suszko et al. 2003, Bailey et al. 2004). In an alternate mechanism, paired-like homeodomain transcription factors 1 and 2 (PITX1 and 2) regulate SMAD 2/3/4-stimulated Fshb transcription through a conserved cis-element (Lamba et al. 2008). It was recently established that SMAD4 and forkhead box L2 (FOXL2) are essential factors for in vivo transcription of Fshb (Fortin et al. 2014). Other than the mostly studied SMAD-mediated activation, TAK1 signaling pathway, a member of the MAPKK family, was recognized as a potent player in the induction of FSHβ-encoding gene transcription by activin (Safwat et al. 2005). But, a recent study contradicted the idea by demonstrating that activin A signaling is independent of the TAK1 (MAP3K7)/p38 MAPK pathway and depends on SMAD proteins (Wang & Bernard 2012). Other factors that can stimulate activin induction include morphogenetic proteins (BMPs) (Otsuka & Shimasaki 2002, Lee et al. 2007, Nicol et al. 2008), but their mechanism of action and associated signaling pathways are not clear.

Inhibin and follistatin are two potent antagonists of activin, regulating its inductive effects via two distinct inhibitory loops. Inhibin is a heterodimer that consists of one subunit identical to activin and another unique alpha-subunit (Nakamura et al. 1998). The production of inhibin from ovarian granulosa cells is stimulated by FSH, which in turn downregulates FSH production. The proposed mechanism of inhibin actions includes competitive inhibition at activin receptors or interaction at inhibin-specific-binding sites that alters active activin-binding process (Gregory & Kaiser 2004, Robertson et al. 2004). On the other hand, follistatin, a glycoprotein having nearly ubiquitous expression in various tissues, inhibits activin actions by directly binding to it (Shimasaki et al. 1988). Taken together, functions of activin, inhibin and follistatin forms a complex regulatory loop that tightly controls FSH production at the level of transcription. A considerable inter-species variation of FSH regulation at the level of transcription was observed as evidenced by the identification of different TATA box and other regulatory elements across species by in silico analysis (Kutteyil et al. 2017).

Regulation of FSHβ-encoding gene expression by steroid hormones

The expression of FSHβ-encoding gene is under feedback regulation by estrogen and progesterone, both acting at the level of hypothalamus and anterior pituitary. But in rodents, estrogen does not directly modulate Fshb gene expression and seems to have only a partial effect in FSH negative feedback, as post-ovariectomy estrogen regulation by estrogen and progesterone, both acting at the level of hypothalamus and anterior pituitary. But in rodents, estrogen does not directly modulate Fshb gene expression and seems to have only a partial effect in FSH negative feedback, as post-ovariectomy estrogen treatment does not show total suppression (Shupnik et al. 1988). It was also reported that the expression of Fshb mRNA was not altered in overiectomized rats (Shupnik et al. 1989, Dalkin et al. 1993) or in rat pituitary tissue (Shupnik & Fallest 1994). An in vitro study with murine Fshb promoter also supported these results showing that Fshb expression was not stimulated by estrogen (Thackray et al. 2006). Hence, it could be postulated that estrogen indirectly modulates the expression of
the FSHβ-encoding gene by regulating the actions of GnRH, activin or other effectors. It was, in fact, reported that estrogen receptor alpha (ERα)-knockout mouse had higher expression of activin B in pituitary (Couse et al. 2003).

Progesterone (P4), unlike estrogen, imparts both positive and negative feedback on FSHβ-encoding gene expression. It acts indirectly at the level of hypothalamus by regulating GnRH production and directly in the anterior pituitary (Levine et al. 2001). Many studies have hypothesized that FSHβ-encoding gene expression in the anterior pituitary is induced by progestins (reviewed in detail in Burger et al. 2004). Fshb mRNA expression and secretion were blocked by P4 antagonists during preovulatory (Ringstrom et al. 1997) and secondary FSH surge (Knox & Schwartz 1992) in rats. Murine Fshb promoter was also observed to be induced by P4 in vitro (Thackray et al. 2006). A region of the DNA from −500 to −95 of the proximal promoter, which was previously shown to consist of six response elements in both ovine and mouse genes, was identified to be responsible for progesterone responsiveness, thus explaining the direct involvement of P4 in Fshb gene expression.

Androgens can also directly upregulate FSHβ-encoding mRNA levels in the pituitary. Various studies have reported that testosterone administration on GnRH antagonist-treated rodents showed increased Fshb mRNA production (Paul et al. 1990, Wierman & Wang 1990, Burger et al. 2004). In vitro studies have established that only gonadotrope cells are enough for the induction of FSHβ-encoding mRNA expression, suggesting that the androgen effect takes place uniquely at the pituitary, and the androgen-driven activation of ovine and murine Fshb promoters involved the direct binding of androgen receptors to specific hormone response elements (Spady et al. 2004, Thackray et al. 2006). However, unlike the previous reports, testosterone-regulated Fshb transcription is mediated through the activin signaling pathway in both rat model and cell line studies (Burger et al. 2007).

Other than estrogen, progesterone and androgen, glucocorticoids are also known to regulate FSHβ production in the anterior pituitary. Fshb expression was shown to selectively increase upon glucocorticoid administration in both rats and primary pituitary cultures (Ringstrom et al. 1991, McAndrews et al. 1994, Kilen et al. 1996, Leal et al. 2003). A summary of major players in transcriptional regulation of FSH subunits is shown in Fig. 1.

Other regulators of FSHβ-encoding gene expression

Other than previously discussed major regulatory pathways, many novel mechanisms were identified to influence FSHβ-encoding gene expression in recent years. Unsaturated long-chain fatty acids were shown to suppress the transcriptional activity of the Fshb gene in rats, and their effect is mediated via a −2824 to −2343 bp region upstream of the promoter sequence (Moriyama et al. 2016). Bone morphogenetic protein 2 (BMP-2) is another factor that affects FSH expression via the induction of SMAD2/3 signaling through BMP type 1A receptor activation. It was suggested BMP2 action may involve similar signaling pathway as activins (Wang et al. 2014c).

Post-transcriptional regulation of FSHβ-encoding gene expression

Whereas translational regulation of the FSHβ-encoding gene and its associated pathways have been extensively studied for many years, the effects of post-transcriptional regulation and its implication to the overall FSH synthesis was not clarified until recent years. Recently, microRNAs (miRNAs) have emerged as key players in crosstalk between different gonadal signaling pathways and their activities can effectively inhibit or upregulate a process. They are now considered as important regulators of the hypothalamic–pituitary–gonadal axis. MiRNAs primarily act post-transcriptionally by destabilizing or degrading mRNA in the cytoplasm. They bestow tight control to the overall gene regulation in addition to maintaining mRNA cell specificity (Farh et al. 2005, Cui et al. 2006, Sood et al. 2006, Tsang et al. 2007, Lin et al. 2013). FSH regulates the production of a wide range of miRNAs that target genes encompassing diverse signaling pathways (Yao et al. 2009, 2010), but not much is known about how miRNAs affect the expression of FSH itself. Most of the studies investigating post-transcriptional regulation of FSH were done in stable cell lines or established primary cultures. To investigate miRNA-regulated production of the specific FSHβ subunit in vivo, Wang and coworkers developed a Dicer-knockout mouse model and established that Dicer-dependent miRNAs are indispensable for FSHβ production (Wang et al. 2015).

One important control point of post-transcriptional regulation of FSHβ-encoding mRNA by miRNAs is exerted at the level of GnRH regulation as shown by a targeted pathway analysis approach in porcine pituitary cell
cultures (Ye et al. 2013). Besides identifying miRNAs that regulate GnRH induction of FSHβ-encoding gene, this study identified 3 downregulated and 10 upregulated miRNAs that most likely are direct targets to the 3′-UTR region of FSHβ-encoding mRNA. Recently, they demonstrated that miR-361-3p directly targets FSHβ-encoding mRNA and negatively regulates its synthesis, providing further functional evidence that miRNA-mediated post-transcriptional modifications are involved in FSH production (Ye et al. 2017).

Another study identified that miR-132/212 are required for GnRH-stimulated expression of FSHβ-encoding mRNA, and this induction involves the SIRT1-FOXO1 pathway. Mir-132/212 is also directly involved in post-transcriptional decrease of the sirtuin 1 (SIRT1) deacetylase (Lannes et al. 2015). Together, a regulatory loop that maintains high levels of miR-125b and low levels of miR-132 desensitizes the gonadotrope cells to GnRH stimuli, thereby disrupting the overall GnRH induction pathway (Lannes et al. 2016). Although the importance

Figure 1
Transcriptional regulation of FSH subunit-encoding genes. The three major players that regulate FSH subunit gene transcription include GnRH, activin–inhibin–follistatin and steroids. GnRH binds to GnRH receptors expressed on gonadotropes. MAPK/ERK phosphorylation is one of the major downstream pathways activated by GnRH. These signals are further transmitted by translocation and recruitment of key transcription factors EGR1, ATF3, SF-1 and MAPK pathway-activated recruitment of c-Jun-c-Fos and AP-1 onto α-GSU and FSHβ-encoding gene promoters. Estrogen, progesterone and testosterone can indirectly regulate FSH subunit-encoding gene transcription by suppressing GnRH. Testosterone and glucocorticoids can also directly regulate FSHβ subunit-encoding gene transcription. Estrogen and testosterone can also regulate activin regulation of FSHβ subunit-encoding gene transcription. Activins bind to activin receptor type II and phosphorylate ALK4/7 type 1 receptors. This heterotrimeric complex via a TAK1 pathway, phosphorylates receptor-specific SMAD 2/3 transducers, which complex with the phosphorylated common SMAD4 and bind to SMAD-binding elements on FSHβ-encoding gene promoter. SMAD7 is an inhibitory SMAD that negatively regulates activin action. SMADs cooperatively bind with other co-factors including a key transcription factor, FOXL2 (not shown in the figure). Follistatin and inhibin bind activin and negatively regulate FSHβ subunit-encoding gene transcription.
of post-transcriptional regulation of FSH synthesis by miRNAs has been realized, the mechanisms that control the process needs further investigation.

In terms of FSH action, a genome-wide study investigating miRNA signatures in human granulosa cells demonstrated that miRNA-mediated post-transcriptional regulation might play a significant role in gonadotropin signaling and found a novel miRNA that had an intronic origin from the FSH receptor gene (Velthut-Meikas et al. 2013).

Post-translational regulation of FSH

Enzymatic and covalent modifications of the hormone-specific FSHβ subunit polypeptide after its translation are critical steps in the assembly, synthesis and release of the biologically active FSH. The specific structural attributes and their molecular characteristics due to such modifications have been extensively investigated and reviewed (Combarnous 1992, Stockell Hartree & Renwick 1992, Stanton et al. 1996, Sood et al. 2006, Butnev et al. 2015). The major post-translational modifications of FSHβ subunit resulting in the formation of different isoforms are N-glycosylation and sialylation, while the contribution of sulfation has no major biological effect on FSH secretion and or function (Bousfield & Dias 2011).

Regulation of FSHβ N-glycosylation

The process of N-glycosylation is mediated by a multi-subunit oligosaccharyl transferase (OST) enzyme complex. The OST complex transfers a previously formed oligosaccharide from a dolichol pyrophosphate molecule to the newly formed FSHβ chain in the endoplasmic reticulum and Golgi compartments (Baenziger & Green 1988, Butnev et al. 1996). N-acetyl-d-glucosamine precursors are co-translationally added to the conserved Asn24 and/or Asn7 residues and are consequently converted to complex glycan chains by branching through addition of various sugar units. There could be one, two or no N-glycans attached onto FSHβ, resulting in the formation of hypoglycosylated (FSH21/18, missing one glycan chain on an Asn residue) or fully glycosylated (FSH24, having branched glycans on both Asn residues) or de-glycosylated (FSH15, having no glycans on both Asn residues) FSH isoforms. Earlier studies have investigated the alterations in FSH biological activity due to differential glycosylation mainly through in vitro bioassays and radioimmunoassays. It was suggested that different glycosylation variants have the ability to induce changes in receptor stability or conformation. Thus, activation or inhibition of a specific signal transduction pathway in target tissues could be a FSH glycosylation-dependent feature in FSH signaling (Zambrano et al. 1999). However, the overall conformational integrity of the glycans is not essential for effective receptor binding, but the rate of activity is altered following deglycosylation (Manjunath et al. 1982).

The state of FSH glycosylation is affected by various physiological conditions. The process of FSH glycosylation and glycan composition changes dynamically throughout the normal menstrual cycle (Wide & Eriksson 2013). Galactose-1-phosphate uridylytransferase (GALT), an enzyme known to modulate production of different FSH glycoforms and its biological activity, was observed to have higher expression during the proestrous and estrous phases in rats (Daude et al. 1996), indicating a distinct role of estrous cycle in determining the formation of glycosylated species.

Calvo and coworkers indicated that deglycosylated forms might be less biologically active than their native fully glycosylated forms in granulosa cells (Calvo et al. 1986). Contrary to this finding, Bousfield and coworkers showed that hypoglycosylated FSH (hFSH21/18) was up to 26 times more active than the fully glycosylated hFSH (hFSH24). The enhanced activity of hFSH21/18 was suggested as a result of availability of more binding sites for hFSH21/18 than that for hFSH24 in the FSH receptor (Bousfield et al. 2014). Further support came from an in vitro study that confirmed that hFSH21/18 was more effective in inducing cAMP production, CREB phosphorylation and PKA activity (Jiang et al. 2015). The first in vivo study by Wang and coworkers investigated bioactivities of the different FSH glycoforms, using Fshb-null mice in a pharmacological rescue approach (Wang et al. 2016a). This study identified that recombinant hFSH21/18 and hFSH24 glycoforms have identical bioactivities when injected into immature Fshb-null mice (Wang et al. 2016a). They also showed that N-glycosylation of FSHβ is needed for its assembly with the α-subunit to form the functional FSH heterodimer in mouse pituitaries, and the FSHβ glycans are determinants of FSH secretion and biological activity in vivo (Wang et al. 2016b). Previous in vitro studies have also shown that N-linked glycan structures on FSH affect gene expression, thus regulating production of growth factors, proteins and hormones that are required for ovarian granulosa cell functions (Loreti et al. 2013).
The differential glycosylation of hFSHβ was proposed to be due to selective inhibition of the oligosaccharyl transferase enzyme activity (Walton et al. 2001, Bousfield et al. 2007). But how the selective glycosylation of FSHβ subunit is controlled by OST activity, while the α-subunit undergoes full glycosylation in the same cellular compartment is yet to be understood. It may be that different OST isoforms may be involved in the process. OST isoforms in mammals differ in the characteristics of their catalytic subunit (Kelleher et al. 2003), and it is observed that OST containing the STT3A subunit is involved in the cotranslational process of N-glycosylation, while the STT3B subunit is associated with the post-translational modifications (Ruiz-Canada et al. 2009).

After the highly conserved N-glycosylation in the ER, the folded proteins are translocated to Golgi where diversity of the glycan forms are generated, and the process of glycan branching is a crucial contributor of this diversity. Glycan branching is mediated by a family of N-acetylglucosamine (GlcNAc) transferases. A single gene encodes GlcNAc transferases I, II, and III in human and mouse, while the two isoforms of both GlcNAc transferases IV and V are encoded by separate genes (Bousfield & Dias 2011). Loss of GlcNAc transferase activity was found to be either embryonic lethal (Ioffe & Stanley 1994) or contributes to various reproductive disorders in mice (Wang et al. 2001, Williams & Stanley 2009). The high molecular weight tri- and tetra-antennary glycans are synthesized mainly by GlcNAc transferases IV and V.

Regulation of FSHβ sialylation

FSHβ sialylation involves post-translational modification resulting in the addition of sialic acid units to the end of its oligosaccharide chain. FSH contains predominantly sialylated oligosaccharides, unlike LH which has more sulfated oligosaccharides. It was speculated that FSH-bound sialic acid residues may aid in targeted translocation to separate secretory granules, thereby providing a regulatory mechanism distinct from other gonadotrophins (Baenziger & Green 1988). In vitro bioassay analysis had previously indicated important role of sialic acid in biological activities of equine FSH (Aggarwal & Papkoff 1981). Since number of sialic acid contributes to the overall acidic nature of the FSHβ subunit, sialylation is thought to be responsible for the regulation of the rate of molecular interactions that requires charge specificity. The less acidic or sialylated FSH isoforms may contribute to different or unique hormonal effects at the target cell level (Timossi et al. 2000). Less sialylated FSH-induced increased cAMP release, tissue-type plasminogen activator (tPA) enzyme activity, estrogen production, along with upregulating cytochrome P450 aromatase and tPA mRNA expression (Barrios-De-Tomasi et al. 2002). On the other hand, more sialylated glycoforms stimulated an increased expression of alpha-inhibin subunit mRNA, indicating a possible post-translationally controlled feedback inhibitory mechanism mediated by the extent of FSH charge variation. More acidic mixtures of FSH isoforms, which have slower clearance rate than a less acidic mixture, were shown to facilitate follicular maturation in the ovary and stimulate estrogen production in sheep (West et al. 2002).

Other than enzyme-mediated post-translational modification of FSH glycans, GnRH and gonadal steroids can also affect the synthesis and release of different FSH isoforms. GnRH is known to induce glycosylation, whereas estradiol aids in the GnRH-induced glycosylation process, suggesting an indirect GnRH-driven FSH regulation at the post-translational level. Synthesis of FSH isoforms with differentially linked sialic acids is hormonally regulated in male rats (Ambao et al. 2009). In addition, testosterone also increases sialylation (Wilson et al. 1990). Progesterone is known to influence pituitary glycosylation, consequently altering the relative proportions of FSH isoforms in cattle (Perera-Marin et al. 2008). After ovarian failure that normally occurs as a function of aging, more acidic FSH becomes dominant and significantly detectable in older premenopausal women (Thomas et al. 2009). A change in FSH isoform abundance, toward less acidic molecules was also observed in cows with ovulatory follicles compared to those with atretic follicles, and this shift in FSH isoforms is suggested to decide the capability for producing a preovulatory estradiol rise (Butler et al. 2008). Factors like age, sex and reproductive state can also influence the formation of the type of the FSH isoforms in sheep pituitary (Moore et al. 2000).

Regulation of FSH secretion

FSH is constitutively secreted from the gonadotrope cells via a complex multilayered regulatory process. Moreover, longer half-life in circulation and molecular heterogeneity makes detection of FSH secretory patterns more difficult through peripheral hormone measurements. The regulation of FSH secretion appears to be dominated by...
factors controlling inhibition of both its synthesis and release. This process includes three major aspects: GnRH signaling, and activin–inhibin–follistatin pathways and control by gonadal steroids (Fig. 2).

**GnRH-mediated regulation of FSH secretion**

Only low levels of GnRH are sufficient for the stimulation and maintenance of FSH secretion. FSH release from gonadotrophs, unlike that of LH, is tightly associated with its rate of synthesis (McNeilly 1988). Various regulatory neuropeptides and hypothalamic factors are thought to be responsible for GnRH-mediated FSH secretion. Orexin A, synthesized by a small population of cells in the hypothalamus, was found to significantly inhibit GnRH-stimulated FSH release from rat pituitary cells. This neuropeptide was shown to modify GnRH sensitivity of gonadotrophic cells, and its effect may be age and estrogen dependent (Martynska et al. 2014). Pituitary adenylate cyclase-activating polypeptide (PACAP) is known to facilitate GnRH-mediated secretion of FSH from sheep pituitary gonadotrophs, but the increase in release is only seen at concentrations higher than normal physiological limit (Sawangjaroen et al. 1997). GnRH itself does not directly regulate FSH at the level of secretion, but it regulates mostly at the level of FSH synthesis (McNeilly 1988).

**Activin–inhibin–follistatin-mediated regulation of FSH secretion**

Ling and coworkers first identified activin as a FSH-releasing factor and coined the name to signify its opposing biological activity compared to inhibin (Ling et al. 1986). Inhibin has structural organization homologous to that of transforming growth factor-beta (TGF-β). Earlier investigations on activin–inhibin-mediated secretion of FSH were mainly performed in rodent primary pituitary cultures. Gonadotrophic secretion of activin B was suggested to provide an autocrine signal that selectively modulated FSH secretion in rat pituitary cells cultures, while FSH inhibitory actions of inhibin and follistatin was attributed to their interference with endogenous activin B or its activity (Corrigan et al. 1991). It was also previously reported that follistatin suppresses FSH secretion in a highly specific manner since it shows no demonstrable effect on the release of other pituitary hormones (Ying et al. 1987). Moreover, follistatin-treated pituitary cells showed significantly less depletion of intracellular FSH content than those treated with inhibin. This suggests unlike inhibin, follistatin acts primarily by suppressing FSH secretion. The mode of follistatin action consists of binding and neutralization of both activin A and B forms (Schneyer et al. 2003), and this specific binding to activin is decisive in determining follistatin’s differential biological activity (Sidis et al. 2002). It was later demonstrated that inhibit immunization with anti-inhibin serum in...
diestrous rats also significantly increased FSH secretion in vivo (Gordon et al. 2010).

**Regulation of FSH secretion by gonadal steroids**

Mechanisms of gonadal steroid hormone regulated FSH secretion are mostly interwoven with both GnRH-mediated and activin–inhibin-regulated pathways, indicating the complexity and selective interdependency of these processes. In rodents, estrous cycle plays an important role in dictating this steroid hormone regulated FSH secretion. It was noted that anti-progestins RU486 and ZK98299 affected levels of serum FSH and FSHβ mRNA in a similar manner during proestrus, while they showed divergent patterns on estrus indicating that the active functional state of progesterone receptor/transcriptional activation complex is different during the two cyclic phases (Ringstrom et al. 1997). A study also indicated that estrogen and inhibin both differentially modulate the estrus stage-dependent increased secretion of FSH in bovine pituitary cells (Lane et al. 2005).

It was also shown that the suppression of both basal and activin-mediated FSH secretion takes place in an estrogen-dependent process (Szabo et al. 1998). Progesterone receptors, upon induction by estradiol, respond to activin-induced signal transduction to regulate FSH release. A study in ovine pituitary cells reported that differential effects of estradiol on FSH secretion in ovine pituitary cells may be indirectly mediated by activin (Baratta et al. 2001). An in vivo study on rats confirmed the regulation of FSH secretion by estrogen and inhibins and identified estradiol-17 beta as the major candidate mediating the mechanism (Herath et al. 2001). The negative estrogen feedback was associated with increased levels of both inhibin isoforms in plasma, while inhibin A seems to be the dominant species during the initiation of the process. The mechanism of estrogen suppression of FSH release in vivo was investigated in pre-pubertal mice in a recent study, which indicated that the process takes place via the activation of estrogen receptor-α in kisspeptin neurons directly affecting GnRH release (Dubois et al. 2016). Other than estrogen, progesterone and corticosterone, testosterone is also known to stimulate the release and maintain intracellular levels of FSH by the activation of the androgen receptor (directly or as dihydrotestosterone) and conversion to estradiol or activation of estrogen receptors (Hiipakka & Liao 1998, McPhaul & Young 2001). The steroid hormone actions are mediated by activin, which involves a tightly regulated activin/follistatin autocrine–paracrine loop (Bohsack et al. 2000).

**Other factors regulating FSH secretion**

Several other molecular factors, independently or in association with the above described pathways, contribute to the complex regulation of FSH secretion. One of them is corticosterone, which stimulates selectively FSH release (Kilen et al. 1996). Bone morphogenetic protein-4 (BMP-4) exerts inhibitory effects on FSH release, via the induction of Smad1 phosphorylation and activation of the BMP signaling pathway (Faure et al. 2005). BMP-4 blocks activin stimulation of FSH release in addition to increasing the 17β-estradiol-mediated suppression of FSH secretion. Adiponectin, a protein involved in glucose regulation and fatty acid catabolism, is another factor that increases FSH release from the pituitary gonadotrophs (Kiezun et al. 2014). This establishes a new crosstalk between the glucose-fatty acid metabolic pathways and the reproductive axis.

Roper and coworkers recently identified synaptotagmin 9 (syt-9), a Ca²⁺ sensor protein associated with exocytosis in neuroendocrine cells, as a regulator of FSH secretion in vivo (Roper et al. 2015). Interestingly, they found that syt-9 and FSH were co-localized in pituitaries of female but not male mice. Accordingly, loss of syt-9 reduced FSH secretion only in females, indicating a sex-specific regulation of FSH. A population study on human subjects reported that adrenocorticotropic hormone (ACTH) regulates FSH release. This study identified a novel pathway of gonadotropin secretion mediated by the adrenal cortex (Aleknaviciute et al. 2016). Ghrelin, a neuropeptide involved in the regulation of physiological energy balance, was also found to influence FSH secretion in pre-pubertal sheep (Wojcik-Gladysz et al. 2016). The identification of novel factors from such diverse physiological pathways indicates the complexity of FSH regulation. It further substantiates the idea that various signaling networks, related or unrelated to the direct regulation of the reproductive axis, act in parallel to maintain FSH levels.

**Regulation of FSH action**

The actions of FSH in regulating the reproductive axis have been extensively investigated over the years (Loraine & Schmidt-Elmendorff 1963, Simoni et al. 1999, Howles
2000, Zafeiriou et al. 2000, Sairam & Krishnamurthy 2001, Smitz et al. 2016). FSH binds and activates, a 7 transmembrane (7TMR) domain containing FSH receptor (FSHR) expressed in granulosa cells in ovaries and Sertoli cells in testes (Simoni et al. 1997). Inactivating either Fshb or Fshr leads to consistent reproductive defects (Matthews et al. 1993, Layman et al. 1997, Huhtaniemi et al. 2006) and based on its physiological target, FSH controls distinct biological responses like cell proliferation, differentiation, steroidogenesis, metabolism and apoptosis (Dias et al. 2010) (Fig. 3).

Regulation of FSH functions mediated by FSHR

For several decades, the canonical Gs/cAMP/PKA pathway has been considered as the major mechanism by which FSH exerts its actions within target cells (Dattatreyamurty et al. 1987). However, in recent years, it has been recognized that FSH binding to its receptor also induces several other signaling pathways, adding to the growing complexity (Gloaguen et al. 2011). Activation of protein kinase B (PKB/Akt) was identified as an alternative response of FSH-FSHR binding (Zeleznik et al. 2003), which was reported
to be a cAMP-dependent and PKA-independent pathway (Gonzalez-Robayna et al. 2000, Meroni et al. 2002). This involves an exchange protein (EPAC) directly activated by cAMP. Wayne and coworkers later demonstrated the significance of EPACs in mediating biological activities of FSH (Wayne et al. 2007). A more recent study suggests that FSH-stimulated ERK activation in granulosa cells involves PKA-dependent inactivation of MAP kinase phosphatase (3MKP3) (Donaubauer et al. 2016). FSH-responsive genes are also regulated by forkhead box O1 (FOXO1) protein in ovarian granulosa cells via the phosphatidylinositol-3 kinase/AKT (Herndon et al. 2016). Insulin-like growth factor-2 (IGF-2) expression is also regulated by FSH through the AKT-dependent pathway (Baumgarten et al. 2001). Further, IGF-1 and FSH signaling pathways are shown to interact in granulosa cells both in vitro and in vivo (Zhou et al. 2013, Stocco et al. 2017).

FSHR can couple with other G protein subtypes. FSHR was shown to activate pertussis toxin-sensitive pathways upon binding certain hormone variants or depending upon the specific developmental stage of target cells (Arey et al. 1997, Crepieux et al. 2001). FSHR also activates the inositol trisphosphate (IP3) signaling pathway (Quintana et al. 1994) and interacts directly with Gαq subunit in granulosa cells (Escamilla-Hernandez et al. 2008). In an alternate signaling mechanism observed in Sertoli cells, the FSH-induced IP3 response was explained via functional coupling of FSHR and tissue transglutaminase (Gah), which results in PLCβ activation and IP3 accumulation (Lin et al. 2006).

Other than the well-known heterotrimeric G-proteins, G protein-coupled receptor kinases (GRKs) and β-arrestins are two other classes of proteins shown to interact specifically with FSHRs upon FSH induction. GRKs and β-arrestins regulate FSHR stimulation by controlling selective sensitization, internalization and recycling of FSHR (Nakamura et al. 1998, Troispoux et al. 1999, Reiter et al. 2001, Kishi et al. 2002, Marion et al. 2002, 2006, Krishnamurthy et al. 2003, Piketty et al. 2006). But over last few years, the spectrum of functions performed by β-arrestins has expanded. They also act as signal transducers in a G protein-independent manner at different 7TMRs (Lefkowitz & Shenoy 2005, Reiter & Lefkowitz 2006). Although only FSHR-mediated β-arrestin-dependent and G protein-independent activation of ERK and rpS6 has so far been identified as the effector mechanism, it could be predicted that β-arrestins are associated with the G protein-independent activation of a wide array of differential signaling pathways downstream of the FSHR.

β-Arrestins are known to function as multifunctional scaffolds interacting with several proteins, thereby facilitating the phosphorylation of numerous intracellular targets at other transmembrane receptors (Xiao et al. 2007, Whalen et al. 2011). The adaptor protein phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1) was reported to directly bind FSHR, consequently triggering downstream signaling cascades. APPL1 was originally suggested to interact with the first intracellular FSHR loop and APPL1 induces FSH-dependent PI3K signaling (Nechamen et al. 2004). A recent study showed that FSH-stimulated activation of the inositol–phosphate calcium pathway requires APPL1–FSHR interaction (Thomas et al. 2011). PI3K pathway is also induced by the members of the Src family of kinases in granulosa cells (Wayne et al. 2007). FSH-induced activation of ERK pathway is mediated by Src proteins in granulosa and Sertoli cells (Crepieux et al. 2001, Cottom et al. 2003). FSH also induced epidermal growth factor receptor (EGFR) autophosphorylation in granulosa cells via Src activation (Cottom et al. 2003, Wayne et al. 2007). This suggests an important role of EGFR transactivation in relaying the FSH signals in the target cells. In addition, EGRF inhibition reduced the ability of FSH to stimulate CDK4 activation and ERK/Akt phosphorylation in different model systems (Cottom et al. 2003, Andric & Ascoli 2006, Shimada et al. 2006, Yang & Roy 2006, Wayne et al. 2007). FSH also regulates the initiation of germ cell mitosis/meiosis in embryonic chicken through the involvement of progesterone and upregulation of miR181a. This miRNA inhibits meiotic initiation by suppressing the nuclear receptor subfamily 6 group A member 1 (NR6A1) transcript (He et al. 2013).

Mouse models for studying FSH actions in vivo

The development of transgenic mouse models contributed to major advancements in the field of reproduction research. Several mouse models were generated that largely facilitated our understanding of how FSH acts in normal reproductive physiology and under pathological conditions. The models belong to two categories. In the gain-of-function, reporter gene (e.g., transgenes encoding subunits of a protein or CRE recombinase enzyme) expression is driven by specific promoter sequences. In the loss-of-function model (knockout, KO), mutations at a target locus are first produced in embryonic stem cells (ES) followed by propagation of the resulting mutant allele through germline (Kumar et al. 2009). Multiple
KO mutations could be combined and an intercross of KO model with a gain-of-function model results in a genetic rescue model. Cell- or tissue-specific deletion of a gene is of interest, since such a technology allows us to investigate molecular mechanisms in a tissue of interest selectively, when global null mutations result in undesired or even lethal phenotypes. LoxP-flanked genes of interest combined with CRE-expressing transgenic lines as drivers are used to achieve such tissue-, cell-specific deletions (Schmidt-Supprian & Rajewsky 2007, Bouabe & Okkenhaug 2013, Deng 2014).

A transgenic mouse model overexpressing the human CGA gene was generated, which did not show any major reproductive abnormalities (Fox & Solter 1988). Kumar and coworkers developed the first transgenic mouse model for FSH. They demonstrated gonadotroph-specific expression of a 10kb human FSHB transgene in mouse pituitaries (Kumar et al. 1992). Overexpression of the human FSH subunit did not result in any overt phenotypes in female transgenic mice. Transgenic males, however, showed a marginal increase in testis weight without any major male reproductive consequences (Kumar et al. 1992). This mouse model was further used to demonstrate differences in steroid hormone regulation between mouse Fshb and human FSHB gene expression (Kumar & Low 1993, 1995) and GnRH regulation (Kumar & Low 1995). This model also served as a genetic platform to further identify and narrow down regulatory elements controlling human FSHB expression in vivo (Kumar et al. 2006).

A bi-transgenic human FSH-expressing model was developed by a genetic intercross between two independent transgenic lines. One line expressed a human CGA mini-gene and another the FSHB gene. Both the transgenes were expressed from a mouse metallothionein-1 promoter. This ectopic expression of human FSH dimer was achieved at low and very high levels (Kumar et al. 1999). The low level human FSH-expressing mice developed normally, do not display any abnormalities and are fertile. The very high level human FSH-expressing male mice were infertile, displayed hyperandrogenemia and consequently enlarged seminal vesicles but spermatogenesis looked qualitatively normal in testes of these mice (Kumar et al. 1999). The transgenic female mice expressing very high levels of human FSH were infertile, their ovarian histology was normal initially up to 2 weeks, and several abnormalities were noticed by 3 weeks and beyond. In transgenic mice at 6 weeks of age, the ovaries contained many hemorrhagic follicles with totally disrupted folliculogenesis. At this age, serum levels of estrogen, progesterone, testosterone and activins were elevated. Very few (~5%) transgenic female mice survived and died by 13 weeks of age presumably secondary to urinary bladder and kidney defects (Kumar et al. 1999). Thus, female mice ectopically expressing high levels of human FSH demonstrate at least some features of polycystic ovarian syndrome in women.

Allan and coworkers developed another ectopic human FSH dimer expressing line of mice, in which the human FSH subunit-encoding cDNA transgenes were engineered in tandem and targeted to liver using a rat insulin II promoter (Allan et al. 2001). These transgenic mice were later intercrossed with hpg mice and maintained on the hpg genetic background (hpg; hFSH±). Because of a naturally occurring mutation in the Gnrh gene, hpg mice do no produce GnRH and hence gonadotropins and ovarian steroids and are infertile (Allan et al. 2001). Thus, this genetic approach allowed the investigators to test the effects of ectopically expressed human FSH in the absence of LH and gonadal steroids. Male transgenic mice on hpg background, with human FSH at levels >1 IU/L showed partial rescue of the testis and spermatogenesis phenotypes. Testis size increased and histological analysis revealed that spermatogenesis progressed until early post-meiosis (Allan et al. 2001, Haywood et al. 2003), similar to the phenotypes observed in Lhb-null mice (Ma et al. 2004). When supplemented with testosterone, spermatogenesis was fully restored in these hpg; hFSH+ mice. Female hpg; hFSH+ mice also showed rescue; the ovarian follicle reserve increased and ovarian histology demonstrated many antral follicles and serum inhibin levels elevated. Surprisingly, hFSH+ transgenic mice alone (not on hpg background) by ~6 months of age showed accelerated aging leading to premature ovarian failure (Allan et al. 2001).

Because hpg mice lack both FSH and LH as a result of loss of functional GnRH, to study the in vivo roles of exclusively FSH, a loss-of-function mouse model for FSH action was generated using embryonic stem cell technology (Kumar et al. 1997). Fshb-null mice developed normally and demonstrated reproductive phenotypes. Fshb-null males were fertile despite displaying reduced testis size as a result of reduced Sertoli cell- and germ cell-carrying capacity (Wreford et al. 2001), reduced sperm number and motility (Kumar et al. 1997). Fshb-null females displayed hypoplastic ovaries and uterine horns. Ovarian histology showed a preantral stage block in folliculogenesis, and no evidence of corpora lutea indicating that these null mice were anovulatory (Kumar et al. 1997). However, in a superovulation protocol, when injected with exogenous hormones, these null female mice responded and released comparable number of oocytes similar to control mice (Kumar et al. 1997). Thus, FSH responsiveness is retained...
in these null female mice in the absence of FSH ligand from birth. Subsequent studies with Fshb-null females showed that FSH regulates transzonal projection-mediated communication between granulosa cells and oocytes (Combelles et al. 2004), gap junction proteins between granulosa cells (El-Hayek & Clarke 2015, Clarke 2018) and epidermal growth factor receptor expression in granulosa cells to prime the follicles for ovulation prior to LH action (El-Hayek et al. 2014).

Knockout mice lacking Fshr gene display defects in reproductive function in both male and female mice, similar to those seen in mice lacking FSH (Kumar et al. 1997, Dierich et al. 1998, Abel et al. 2000). Fshb genetic rescue, activin, inhibin and follistatin knockouts, FSH glycosylation mutants and FSH rerouted transgenic models were used in various studies to investigate the entire spectrum of FSH actions in vivo (Matzuk et al. 1992, 1995, Guo et al. 1998, Kumar et al. 1998, Jorgez et al. 2004, Sandoval-Guzman et al. 2012, Abel et al. 2014, Wang et al. 2014c). Recently, these models were reviewed in detail (Kumar 2016) and summarized in Table 1. Inactivating mutations in human FSHB and FSHR are although rare,

### Table 1 Mouse models for FSH synthesis, secretion and action.

<table>
<thead>
<tr>
<th>Model</th>
<th>Phenotypes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human FSHB transgenic</td>
<td>Human FSHβ-overexpression in pituitary gonadotropes. Increased testis weight in males. No overt phenotypes in females</td>
<td>Kumar et al. (1992)</td>
</tr>
<tr>
<td>MT-CGA/MT-FSHB bi-transgenic</td>
<td>Multiple reproductive defects including hemorrhagic and cystic ovaries in females. Males infertile with enlarged seminal vesicles and elevated serum testosterone</td>
<td>Kumar et al. (1999)</td>
</tr>
<tr>
<td>Fshb knockout</td>
<td>Males fertile with decreased testis size. Females infertile with a per-antral stage block in folliculogenesis. Increased bone density</td>
<td>Kumar et al. (1997)</td>
</tr>
<tr>
<td>Fshb genetic rescue</td>
<td>Testis size, sperm number, and motility defects in Fshb-null mice rescued. Folliculogenesis resumed normally in females</td>
<td>Kumar et al. (1998)</td>
</tr>
<tr>
<td>FSHB rerouted transgenic</td>
<td>Fshb-null mice rescued by rerouted FSH. Enhanced ovarian follicle survival, increase in ovulation number, and prolonged reproductive lifespan in females</td>
<td>Wang et al. (2014a)</td>
</tr>
<tr>
<td>FSHB glycosylation mutant transgenic rescue</td>
<td>The double N-glycosylation mutant (Asn⁷A Asn²⁴⁴) transgene fails to rescue Fshb-null mice</td>
<td>Wang et al. (2016a)</td>
</tr>
<tr>
<td>Human FSHβ/hpg transgenic rescue</td>
<td>Males showed increased testis weight up to 5-fold. Females exhibited enlarged ovaries and a strong FSH dose-dependent increase in serum inhibin B levels</td>
<td>Allan et al. (2001)</td>
</tr>
<tr>
<td>Fshr knockout</td>
<td>Phenocopy Fshb-null mice. Males fertile but with reduced testes size. Females infertile</td>
<td>Dierich et al. (1998), Abel et al. (2000)</td>
</tr>
<tr>
<td>Activating Fshr transgenic (D580H)</td>
<td>Hemorrhagic cysts, accelerated loss of small follicles, increased gonadotropins, prolactin and elevated estrogen</td>
<td>Peltoketo et al. (2010)</td>
</tr>
<tr>
<td>Inha knockout</td>
<td>Development of gonadal stromal tumors, elevated levels of serum FSH, activins and estradiol</td>
<td>Matzuk et al. (1992)</td>
</tr>
<tr>
<td>Inha/Fshb double knockout</td>
<td>Slow-growing and less hemorrhagic testicular tumors in males and ovarian tumors in females, and with minimal cachexia</td>
<td>Kumar et al. (1999)</td>
</tr>
<tr>
<td>Acvr2a knockout</td>
<td>Suppressed FSH production and defects in reproductive performance. Males subfertile or infertile and exhibit male sexual behavior defects; females with parturition defects</td>
<td>Matzuk et al. (1995), Ma et al. (2005)</td>
</tr>
<tr>
<td>MT-Follistatin transgenic</td>
<td>Multiple reproductive defects. Marginally elevated FSH in on line with widespread expression of the FS transgene</td>
<td>Guo et al. (1998)</td>
</tr>
<tr>
<td>Smad3/Smad4 gonadotroph-specific knockout</td>
<td>Phenocopy of Fshb-null mice</td>
<td>Li et al. (2017)</td>
</tr>
<tr>
<td>Foxl2 knockout</td>
<td>Impaired FSH synthesis and secretion, ovarian function</td>
<td>Schmidt et al. (2004), Uda et al. (2004), Justice et al. (2011)</td>
</tr>
<tr>
<td>Foxl2 gonadotrope-specific knockout</td>
<td>Impaired FSH synthesis and fertility</td>
<td>Tran et al. (2013)</td>
</tr>
<tr>
<td>Smad4/Foxl2 double knockout</td>
<td>Phenocopy of Fshb-null mice</td>
<td>Fortin et al. (2014)</td>
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they have been reported (Huhtaniemi & Themmen 2005, Narayan et al. 2018). While mouse models lacking Fshb are somewhat discordant, those lacking Fshr mostly phenocopy human mutations in the corresponding gene (Kumar 2016).

**Extragonadal actions of FSH**

The central dogma of classical FSH actions in gonads has been challenged in the past decade by studies that demonstrated a direct involvement of FSH in bone physiology (Sun et al. 2006). FSH levels increase sharply in contrast to declining estrogen levels as observed in post-menopausal women with osteoporosis. A direct effect of FSH on the skeletal system was discovered using Fshb- and Fshr-null mice, which have increased bone density (Sun et al. 2006). GÎ2α-coupled FSH receptors were identified in osteoclasts and their precursors. An increase in osteoclast formation and function was demonstrated by an FSH-FSHR-dependent activation of MEK/Erk, Akt and NF-KB pathways, suggesting a positive correlation of circulating FSH with hypogonadal bone loss (Sun et al. 2006). FSH was also shown to regulate FSHR-induced alveolar bone loss in rats by an estrogen-independent process (Liu et al. 2010). The mechanism of FSH-induced increase alveolar bone loss was addressed in a recent study that showed that the FSH effect was mediated by upregulation of the cyclooxygenase-2 (COX-2) protein and the process involves Akt, Erk and p38 signaling pathways (Zhu et al. 2016a). A new concept advocated that a reciprocal cross signaling operates between FSH signaling by FSH and bone morphogenetic protein-9 (BMP-9)-induced activation of BMP/Smad signaling in mouse embryonic fibroblasts. This study identified the cross signaling between FSH and BMP-9 promoted osteogenic differentiation (Su et al. 2017). Controversy exists with regard to FSH actions on bone (Kumar 2018) as data obtained using both mice and humans, contradict the original observations made by Sun et al. (2006).

In addition to its previously discussed functions in skeletal system, FSH was reported to act on the endothelial cells of human umbilical cord and monocytes (Robinson et al. 2010, Cannon et al. 2011, Stilley et al. 2014b). FSH receptors have been identified in female reproductive tract and the developing placenta, and the functional relevance on this finding was confirmed by the observation that Fshr-knockout mice exhibit feto-placental defects (Stilley et al. 2014a). Low levels of Fshb expression was also detected in various non-ovarian tissues, maternal uterine decidua, placenta and uterine myometrium in pregnant women. Tumor blood vessels were also shown to express FSHRs (Radu et al. 2010) and FSH action has been implicated in endometriosis (Ponikwicka-Tyszko et al. 2016). Data are also available on FSH action on prostate tumors (Ide et al. 2013, Siraj et al. 2013, Zhu et al. 2016b). Finally, a blocking antibody against FSH shown to be efficacious in reducing white fat accumulation and conversion to brown fat accompanied by mitochondrial biogenesis in ovariectomized mice (Liu et al. 2017). The biochemical identity and the key downstream components of extragonadal FSH receptors are not clear. At least in mouse bone osteoclasts and adipocytes, FSH signaling pathways appear to be different and involve non-cAMP-mediated effects (Sun et al. 2006, Liu et al. 2017). The physiological relevance of FSH actions in at least some extragonadal tissues was challenged recently (Stelmaszewska et al. 2016), yet to be rigorously tested and may require development of novel genetic models (Kumar 2018).

**Conclusion**

Over the past several decades, studies on FSH have identified the molecular mechanisms for its overall regulation. Starting from signals mediating the initial synthesis to those needed for exerting successful biological actions, every step of FSH regulation is under tight control. Thus, the regulation FSH is highly complex and multilayered. Crosstalk between various signaling pathways modulate specific steps and further adds up to the complexity. As discussed in this review, novel findings related to the transcriptional, translational, post-translational and functional regulatory mechanisms validate that FSH plays a more comprehensive role in controlling various physiological functions than just being classified as a reproductive effector.

Appreciation of diverse functions of FSH and their regulation would be instrumental in developing effective therapies related to both reproductive disorders and other pathological conditions known to be directly or indirectly affected by FSH. For example, with the new understanding that FSH may be a key player in age-related bone loss, effective estrogen and related therapies for bone loss treatment could be implemented. One concept is to develop novel antagonists for both FSH and its receptor. Our knowledge of novel FSH functions in extragonadal is currently limited. More comprehensive investigations are needed to decipher the underlying molecular mechanisms that interweave FSH regulation across different physiological systems. A deeper understanding of such mechanisms of the overall FSH regulation will

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facilitate the development of novel hormonal therapies in the coming decade.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as hindering the impartiality of this review.

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