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Review

Neuroendocrine, autocrine, and paracrine control of follicle-stimulating hormone secretion

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A B S T R A C T

Follicle-stimulating hormone (FSH) is a glycoprotein hormone produced by gonadotropes in the anterior pituitary that plays a central role in controlling ovarian folliculogenesis and steroidogenesis in females. Moreover, recent studies strongly suggest that FSH exerts extragonadal actions, particularly regulating bone mass and adiposity. Despite its crucial role, the mechanisms regulating FSH secretion are not completely understood. It is evident that hypothalamic, ovarian, and pituitary factors are involved in the neuroendocrine, paracrine, and autocrine regulation of FSH production. Large animal models, such as the female sheep, represent valuable research models to investigate specific aspects of FSH secretory processes. This review: (i) summarizes the role of FSH controlling reproduction and other biological processes; (ii) discusses the hypothalamic, gonadal, and pituitary regulation of FSH secretion; (iii) considers the biological relevance of the different FSH isoforms; and (iv) summarizes the distinct patterns of FSH secretion under different physiological conditions.

1. Introduction

Follicle-stimulating hormone (FSH) is a heterodimeric glycoprotein hormone secreted by gonadotropes in the anterior pituitary that plays a central role in reproduction. In female mammals, FSH stimulates antrum formation in secondary ovarian follicles, growth and maturation of antral follicles, and proliferation of granulosa cells and estradiol production (Hunzicker-Dunn and Maizels, 2006; Richards, 1994). The requirement for FSH in female reproduction is evidenced by clinical and animal studies. Women with loss-of-function mutations in the genes encoding the FSH beta subunit (*FSHB*) or the FSH receptor (*FSHR*) manifest arrest in follicle development at the preantral stage and associated amenorrhea (Huhtaniemi and Themmen, 2005). Transgenic mouse models with deficiency (knockout) in those genes exhibit similar ovarian perturbations (Danilovich et al., 2000; Kumar et al., 1997).

In males, FSH mediates induction of aromatase and acts upon Sertoli cells to support spermatogenesis (Pomerantz, 1979; Ramaswamy and Weinbauer, 2014). Despite these important roles, the absolute requirement for FSH on male reproduction has been a topic of debate. Men with loss-of-function mutation in the *FSHR* gene present clinically with different levels of oligozoospermia and may manifest normal fertility, subfertility, or complete infertility (Tapanainen et al., 1997). In male mice, deficiency in the *FSHβ* gene does not impair fertility despite reduced testes size and sperm counts (Kumar et al., 1997). Therefore, these observations suggest that FSH contributes to spermatogenesis, however, it may not be critically required for fertility in males.

In addition to the classical regulation of reproductive organs, recent evidence indicates that FSH also exerts important extragonadal effects (Sun et al., 2006; Kumar 2017). The FSHR is expressed on different extragonadal tissues, including bone (Sun et al., 2006) and adipose tissue (Liu, et al. 2017). Epidemiological and clinical observations suggest that FSH directly regulates bone mass. During perimenopausal transition, women experience a drastic increase in bone turnover, which is highly correlated to elevated circulating concentrations of FSH independent of estrogen levels (Sowers et al., 2003).Similarly, there is a strong correlation between elevated concentrations of FSH and low bone mass in women with amenorrhea (Devleta et al., 2004). Moreover, women with polymorphisms in *FSHR* that result in constitutively active FSHR exhibit rapid reduction in bone density and higher prevalence of osteoporosis (Rendina et al., 2010). In female mice, deletion of the FSHR prevents the negative effects of ovariectomy on bone density (Sun et al., 2006), further indicating that the rise in FSH levels after ovariectomy (or menopause in women) drives bone loss. This premise was corroborated recently by observations that immunoneutralization of FSH prevents the ovariectomy-induced bone loss in mice (Zhu et al., 2012). Interestingly, FSH immunoneutralization also results in a reduction in total, visceral, and subcutaneous fat volume in wild-type mice (Liu et al. 2017). Administration of the FSH antibody also prevents the increase in adiposity seen after ovariectomy in female mice (Liu et al. 2017). Therefore, FSH immunoneutralization could act as a dual-

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purpose intervention with promising future clinical applications for treating both obesity and osteoporosis in women during the perimenopausal transition (Liu et al. 2017).

While the studies mentioned above provide evidence that FSH may contribute directly to the regulation of bone resorption and, thereby, bone mass, it is important to note that several studies present contradicting results. More recently, Allan et al. (2010) reported no detectable *FSHR* mRNA in mouse bone or cultured osteoblasts or osteoclasts, suggesting that FSH regulates bone mass indirectly, likely via ovary-dependent mechanisms. Moreover, Danilovich et al. (2000) reported that *FSHR* knockout mice have elevated androgen levels, raising the possibility that changes in bone mass reported by Sun et al. (2006) could result from local aromatization of these androgens. Therefore, future studies are required to confirm the putative direct effect of FSH on bone mass and other extragonadal targets. For additional information regarding the extragonadal actions of FSH, readers are referred to Kumar (2017) and Zhu et al. (2018).

2. FSH structure, isoforms, and biological activity

FSH is a heterodimeric glycoprotein comprised of a common α subunit noncovalently linked with a hormone-specific β subunit (FSH-β) (Baenziger and Green, 1988; Ryan et al., 1988). The α subunit (chorionic gonadotropin alpha [CGA]) is common to 3 pituitary glycoprotein hormones, FSH, luteinizing hormone (LH) and thyroid stimulating hormone (TSH), whereas the β chain is unique to each hormone and confers specific biological function (Baenziger and Green1988). Separately, the two FSH chains are not able to bind and activate receptors and need to be associated in a dimeric structure to exhibit biological activity (Andersen, 2002). After the dimeric structure is formed and before release into the circulation, oligosaccharide structures are added to two *N*-linked glycosylation sites present on each subunit resulting in the glycosylation of FSH (Andersen, 2002). The structure of oligosaccharides attached to the FSH peptide backbone is highly variable resulting in a variety of different hormone isoforms (Creus et al., 2001). Additionally, each carbohydrate branch may or may not terminate in a negatively charged sialic acid residue, which results in a wide array of isoforms with different isoelectric points (Andersen, 2002). Consequently, more acidic isoforms have higher numbers of sialic acid residues, reflecting a more complex branching pattern, whereas less acidic isoforms have fewer sialic acid residues (Ulloa-Aguirre and Timossi, 2000). As discussed below in the section "4. *Pattern of FSH secretion during different physiological states*", different regulators and physiological states control the pattern of FSH glycosylation, thus modulating the availability of the different FSH isoforms.

The metabolism and biological activity of different FSH isoforms are influenced by the glycosylation pattern and content of sialic acid residues. FSH molecules with higher number of sialic acid residues (acidic isoforms) have lower metabolic clearance rates compared to isoforms with lower sialic acid content (less acidic isoforms) (Blum and Gupta, 1985; Padmanabhan et al., 1999; Ulloa-Aguirre and Timossi, 2000). Consequently, the plasma half-life of less acidic isoforms is markedly shorter than that of more acidic isoforms (Blum and Gupta, 1985; Andersen, 2002). However, despite the shorter half-life, the less acidic isoforms of FSH have been shown to have higher binding affinity for the FSHR and greater efficiency to stimulate proliferation of granulosa cells and rapid preantral follicular growth (Barrios-De-Tomasi et al., 2002). Additionally, less acidic FSH isoforms are significantly more effective than more acidic isoforms in stimulating ovarian synthesis and secretion of estrogens *in vivo* and *in vitro* (Barrios-De-Tomasi et al., 2002). In contrast, more acidic FSH isoforms induce higher ovarian synthesis of inhibin-A when compared to less acidic isoforms (Ulloa-Aguirre et al., 2003). Collectively, most *in vitro* an *in vivo* studies indicate that less acidic FSH isoforms exhibit higher potency compared to more acidic isoforms, although there are a few exceptions in which the reverse occurs. This may occur because in addition to sialic acid composition, the complexity of oligosaccharide branching also plays a role in determining the biological activity of the different FSH isoforms (Creus et al., 2001). For additional information regarding FSH structure, glycobiology, and the biological activity of the different FSH isoforms, readers are referred to previously published reviews (Bousfield and Harvey, 2019; Padmanabhan et al., 1999; Smitz et al., 2016).

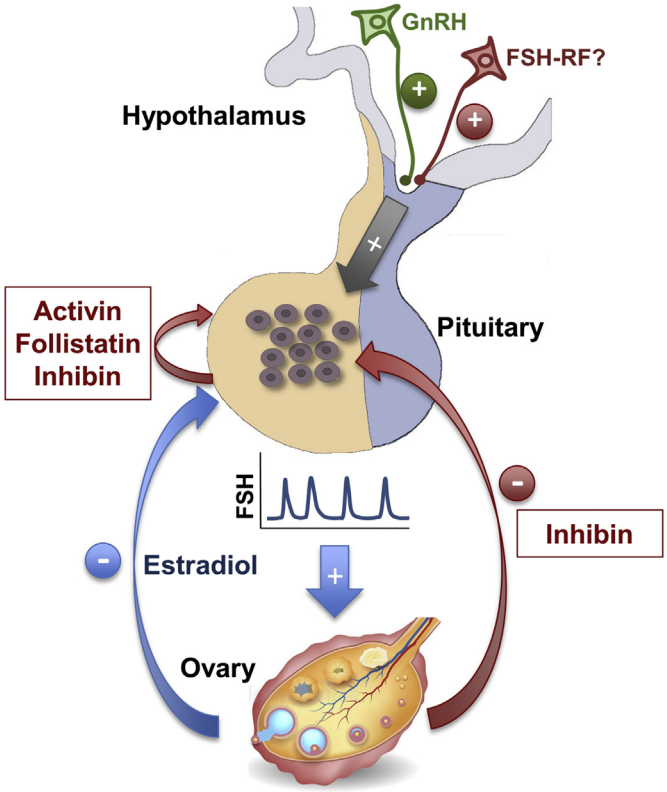

Fig. 1. Schematic diagram of the hypothalamic, pituitary (local), and ovarian regulation of FSH secretion in female mammals. At the hypothalamic level, GnRH pulses are transported to the anterior pituitary by the hypothalamic-hypophyseal portal vasculature to stimulate FSH synthesis and secretion by gonadotrope cells. There is also evidence suggesting the existence of a hypothalamic FSH-releasing factor (FSH-RF). At the anterior pituitary level, a local loop involving activin, inhibin, and follistatin regulates FSH secretion in an autocrine/paracrine fashion. At the ovarian level, estradiol and inhibin are two key negative feedback regulators of FSH secretion. While the ovary also produces activin and follistatin, these hormones are not believed to play an endocrine role in controlling FSH secretion.

3. Regulation of FSH secretion

Regulation of FSH production and release is an intricate process that involves hypothalamic (neuroendocrine), gonadal (endocrine), and pituitary (autocrine and paracrine) factors (Fig. 1). Importantly, the different levels of FSH regulation not only control synthesis and secretion, but also posttranslational modifications of FSH, such and glycosylation and a sialylation (Sairam and Bhargavi, 1985; Ulloa-Aguirre et al., 1995). Several factors pose challenges for investigators to better understand the mechanisms underlying the regulation of FSH secretion. First, as discussed previously, FSH is secreted as a mixture of numerous isoforms with different half-life (Padmanabhan et al., 1999; Padmanabhan and Sharma, 2001). Second, while different stimulus promotes FSH secretion, most FSH present in the peripheral circulation is secreted soon after synthesis in a constitutive manner (Padmanabhan et al., 1997; McNeilly et al., 2003; Nicol et al., 2004; Wang et al., 2014). This differential secretion of gonadotropins is possible, at least in part, because FSH and LH are stored into different secretory granules within

gonadotropes (Nicol et al., 2004; McNeilly et al., 2003). The relatively long half-life coupled with the constitutive mode of FSH secretion result in sustained levels of FSH in the peripheral circulation that hinder the detection of FSH secretory pulses, which are predominantly comprised of short-lived less acidic FSH isoforms (Padmanabhan et al. 2002). Third, existing assays to measure FSH are unable to discriminate between the different FSH isoforms (Padmanabhan et al. 2002; Stanton et al., 1996). Therefore, unlike LH, pulsatile patterns of FSH secretion from the anterior pituitary cannot be characterized effectively based on peripheral FSH measurements. In this regard, large animal models, such as the female sheep, represent valuable biological models to investigate the neuroendocrine control of FSH secretion. This is primarily because these animals are suitable for surgical procedures that allow parallel monitoring of hypothalamic and pituitary hormone levels near the site of their release (*e.g.*, portal vasculature cannulation) and allow collection of repetitive blood samples due to their large blood volume (Padmanabhan et al. 2002).

The following sections will discuss: (i) the hypothalamic regulation of FSH; (ii) the ovarian factors controlling FSH; (iii) the autocrine and paracrine regulation of FSH at the pituitary level; and (iv) the differential regulation of LH and FSH, the two gonadotropins co-synthesized within the gonadotropes, by these hypothalamic-pituitary-gonadal factors. While relevant findings from different species will be presented, these sections will highlight important contributions that the sheep model has made to advance our understanding of the regulation of FSH secretion and the potential benefits that the use of large animal models can provide to this research field. Additionally, this review will focus primarily on FSH secretory aspects; detailed information regarding FSH synthesis can be found elsewhere (Bernard et al., 2010; Bousfield and Dias, 2011; Das and Kumar 2018; Thompson and Kaiser, 2014).

3.1. Hypothalamic regulation of FSH secretion

Gonadotropin-releasing hormone (GnRH). Since its discovery, GnRH, a hypothalamic decapeptide secreted into the pituitary portal vasculature, is arguably the most important regulator of FSH secretion (Schally et al., 1971). With the exception of the preovulatory GnRH/gonadotropin surge, GnRH is secreted in a pulsatile fashion to stimulate LH and FSH secretion by gonadotrope cells. The pattern of GnRH pulsatile secretion, including pulse frequency and amplitude, changes throughout the reproductive cycle and is largely modulated by ovarian steroids (Karsch et al., 1987). Perifusion studies have clearly shown that administration of GnRH pulses induces the pulsatile secretion of both LH and FSH from cultured pituitary cells from rodents (Ishizaka et al., 1992; Weiss et al., 1990) and sheep (Padmanabhan et al. 2002). However, a clear association between GnRH and FSH pulsatile secretion was not evident in whole-animal studies in which blood samples were collected from the peripheral circulation. The primary reasons for this lack of clear association are discussed above and include the relative long half-life of FSH and the predominantly constitutive pattern of FSH release.

The development of surgical procedures to collect blood samples directly from the hypothalamic-hypophyseal portal system of conscious and physiologically uncompromised sheep (Caraty et al., 1982; Clarke et al., 1983) has proved invaluable in elucidating the associations between GnRH and FSH secretion. With this approach, portal blood vessels in the anterior limit of the pituitary gland are cut and blood containing undiluted, freshly secreted hypothalamic and pituitary hormones can be sampled. Using this surgical approach, studies in in the female sheep have shown that FSH is indeed secreted in a pulsatile manner and that a clear temporal association exists between GnRH and FSH pulses (Padmanabhan et al., 1997). While studies monitoring peripheral concentrations of gonadotropins in GnRH-immunoneutralized rats had shown similar findings (Culler and Negro-Vilar, 1987), characterization of GnRH and FSH secretory patterns in the sheep pituitary portal vasculature confirmed the interrelationship between GnRH and FSH pulses.

FSH-releasing factor. Despite the well-characterized association between GnRH and FSH pulsatile secretion, anatomical, physiological, and biochemical data suggest that a second hypothalamic factor may regulate the secretion of FSH. Evidence for the existence of an FSH-releasing factor (FSH-RF) is briefly discussed below. For detailed information on this topic, readers are referred to earlier reviews (McCann et al., 2001; Padmanabhan and McNeilly, 2001).

Evidence supporting the existence of FSH-RF derives from neuroanatomical studies that demonstrated that hypothalamic regions that do not contain GnRH neurons are involved in the control of FSH secretion. Ablation (Lumpkin and McCann, 1984) or de-afferentation (Lamperti and Hill, 1987) of the dorsal anterior hypothalamic area (DAHA) selectively suppressed FSH secretion. A subsequent study confirmed that radiofrequency lesions of the DAHA suppressed FSH secretion in female rats (Lumpkin et al., 1989). Collectively, these data suggest that separate mechanisms regulate LH and FSH secretion and that the DAHA is an important brain region controlling the latter.

Physiological evidence that supports the existence of FSH-RF derives from animal studies that report the secretion of FSH pulses that are not associated with GnRH pulses. Studies demonstrating episodic pattern of FSH in the peripheral circulation after blockage of GnRH action with GnRH antagonists in rats or immunization against GnRH in ovariectomized rabbits provide strong evidence for the existence of GnRH-independent FSH pulses (Culler and Negro-Vilar, 1987; Pau et al., 1991). While the persistence of FSH pulses in peripheral blood might result from different clearance rates of the various FSH isoforms, our studies in the female sheep demonstrate the existence of GnRH-independent FSH pulses also in the pituitary portal blood, providing evidence to the contrary (Padmanabhan et al., 1997). These studies in portal-vasculature cannulated sheep demonstrate: 1) a clear one-to-one relationship between GnRH and LH pulses; 2) that all GnRH pulses are associated with FSH pulses; and 3) the existence of additional FSH pulses that are not associated with GnRH pulses (GnRH-independent pulses of FSH). The existence of a GnRH-independent component of episodic FSH secretion was further confirmed by the observation that administration of Nal-Glu, a GnRH antagonist, eliminated LH but not FSH pulsatility in the sheep pituitary portal vasculature (Padmanabhan et al., 2003).

Finally, biochemical evidence also supports the notion that a hypothalamic factor other than GnRH controls FSH secretion by the anterior pituitary. Using pig and sheep hypothalamic tissues, McCann et al. (1983) were able to partially separate different hypothalamic extracts that had more FSH-releasing activity than could be accounted for by the content of GnRH. A preparation with strong FSH-releasing biological activity free of GnRH was obtained subsequently after ion exchange chromatography of ovine hypothalamic extracts (Lumpkin et al., 1987). Identification of different GnRH isoforms in lower vertebrates (Lin et al., 1998) raised the possibility that one of the GnRH variants could be the long sought after FSH-RF. This possibility was supported by the findings that receptors for a second isoform of GnRH (GnRH-II) were present in gonadotropes and that GnRH-II does not potently stimulate LH release (Millar et al., 2001; Padmanabhan et al., 2003). In sheep, GnRH-II administration produced a higher ratio of FSH to LH secretion than that achieved with GnRH treatment, although GnRH was markedly more effective than GnRH-II in stimulating secretion of either of the gonadotropins (Millar et al., 2001). However, more recent studies contradict these earlier findings. Studies in sheep and rhesus monkeys reported no selective FSH-releasing activity for GnRH-II (Densmore and Urbanski, 2003; Gault et al., 2003). Similar observations were reported *in vitro* using cultured primate pituitaries (Okada et al., 2003). Collectively, these observations suggest that GnRH-II has only weak selective actions inducing FSH secretion and, unlike GnRH, the primary role of GnRH-II in the brain is not to stimulate gonadotropin secretion (Kauffman and Rissman, 2006). While other studies implicated a third GnRH isoform (lamprey GnRH-III) as a

FSH-RF candidate (Wen et al., 1997; Yu et al., 2002), several studies have reported no selective FSH-releasing action of lamprey GnRH-III (Amstalden et al., 2004; Kovacs et al., 2002), thus leaving this issue currently unresolved.

3.2. Ovarian regulation of FSH secretion

Gonadal Steroids. Gonadotropins stimulate sex steroid production by the gonads, which in turn feedback to the hypothalamus and pituitary to regulate gonadotropin secretion. In general, androgens, progestogens, and estrogens have the potential to exert negative feedback effects and suppress FSH release, either via direct effects in the pituitary (estrogens) or indirectly by suppressing GnRH secretion by the hypothalamus (Gharib et al., 1990; Nett et al., 2002). Estrogens also have positive feedback actions on GnRH secretion prior to ovulation. In the female sheep, a large body of work demonstrated that sex steroids exert negative feedback effects on the GnRH neurosecretory system resulting in diminished GnRH release and subsequent reduced gonadotropin secretion (Clarke et al., 1989; Karsch et al., 1997). In addition to hypothalamic actions, gonadal steroids act directly at the anterior pituitary level in both males and females (Gharib et al., 1990; Martin et al., 1988). The importance of direct steroid actions in the pituitary are clearly demonstrated by the infertile phenotype observed in female mice lacking the estrogen receptor α specifically in gonadotropes (Gieske et al., 2007). The effects of gonadal steroids on the pituitary are mediated via several processes, including modifications in the expression of the GnRH receptor (Gregg and Nett, 1989), transcription of *FSHβ* mRNA and FSH secretion (Bernard et al., 2010), and FSH post-translational modifications (Bousfield and Harvey, 2019; Ulloa-Aguirre et al., 1992). For detailed information regarding the steroid regulation of FSH biosynthesis, readers are referred to Gharib et al. (1990) and Bernard et al. (2010).

Activins, Inhibins, and Follistatins. In addition to gonadal steroids, regulatory proteins secreted by the gonads, specifically activin, inhibin, and follistatin are also important contributors to the amount of FSH secreted (Carroll et al., 1989; Ling et al., 1986; Ueno et al., 1987; Vale et al., 1986; Vale et al.,). Activin and inhibin are members of the transforming growth factor β superfamily and are structurally related (Ling et al., 1986; Ying, 1988). Inhibin α, β_A, and β_B subunits are encoded by different genes and these subunits dimerize to form inhibin A (α-β_A), inhibin B (α-β_B), activin A (β_A-β_A), activin B (β_B- β_B), and activin AB (β_A-β_B) (Ying, 1988). Follistatin is a monomeric protein that binds to the β subunit of both activin and inhibin (Phillips and de Kretser, 1998). Activin stimulates intracellular signaling in gonadotropes that results in enhanced expression of *FSHB* mRNA and subsequent FSH release primarily due to its constitutive pattern of secretion (Ling et al., 1986; Pangas and Woodruff, 2000). Inhibin and follistatin appear to regulate FSH secretion primarily by antagonizing activin's action rather than by directly initiating signaling events (DePaolo et al., 1991; Padmanabhan and West, 2001). Inhibins, which are secreted by granulosa and luteal cells in the ovary, act in an endocrine manner to suppress synthesis and consequently the amount of FSH secreted (Padmanabhan and West, 2001; Woodruff et al., 1996). Inhibins bind to activin receptors on gonadotropes and, via competitive antagonism, prevent activins from triggering intracellular signaling pathways (Cook et al., 2004). Follistatins are structurally different from activins and inhibins, but bind to activins with high affinity preventing their receptor binding (Thompson et al., 2005). Follistatins also inhibit FSH secretion by promoting internalization and degradation of activins, thus reducing their bioavailability (Cash et al., 2009).

The endocrine role of these regulatory proteins has been demonstrated in different studies. A negative relationship between inhibin and FSH concentrations in the peripheral circulation supports this premise (Padmanabhan and West, 2001). Moreover, at the time of menopause, a decrease in inhibin B is associated with the hallmark increase in circulating concentrations of FSH, consistent with a negative feedback relationship between inhibin and FSH (Klein and Soules, 1998). In support for an endocrine role for follistatin are the observations that administration of recombinant human follistatin to sheep result in a marked suppression in FSH but not in LH concentrations (Padmanabhan and Sharma, 2001). Additionally, these studies in the female sheep reported that after administration of follistatin the clearance of total follistatin was slower than that of free follistatin, providing evidence for activin-bound follistatin and thus indirect evidence for the presence of free activin in the circulation (Padmanabhan and Sharma, 2001). Additional information on the ovarian regulatory proteins that control FSH synthesis can be found elsewhere (Bernard et al., 2010; Bilezikjian et al., 2006; Das and Kumar 2018; DePaolo et al., 1991).

3.3. Pituitary regulation of FSH secretion

Studies in the last two decades have confirmed that inhibin, activin, and follistatin are produced in many tissues including the anterior pituitary gland (Bilezikjian et al., 2004; Padmanabhan et al. 2002). Therefore, these proteins have been postulated to act in an autocrine and paracrine fashion to locally control FSH production and secretion (Besecke et al., 1996; DePaolo et al., 1991; Padmanabhan and West, 2001). Moreover, changes in the gonadal steroid profile have been shown to result in modifications in the expression patterns of activin, inhibin, and follistatin in the anterior pituitary (Bilezikjian et al., 2001).

One of the first evidences supporting local pituitary modulation of FSH secretion came from studies that showed that exposure of cultured pituitary cells from rats to an antiserum that neutralized the effects of activin B resulted in marked suppression of FSH secretion (Corrigan et al., 1991). Additional support for a paracrine control of FSH came from studies that showed a negative relationship between the expression pattern of follistatin and *FSHβ* mRNA expression, and a positive relationship between activin and *FSHβ* expression (Besecke et al., 1996; Dalkin et al., 1999). Using follistatin to neutralize locally produced activin, our studies in perifused ovine pituitary cells provide direct evidence that FSH secretion can be considerably modulated by changes in activin tone in the pituitary (Padmanabhan et al. 2002). In agreement, other studies have found that activin stimulates *FSHβ* mRNA expression and FSH secretion in fetal human pituitary cultures as well as in some pituitary adenomas (Blumenfeld and Ritter, 2001; Takano et al., 1992).

3.4. Differential control of LH and FSH

The numerous endocrine and local regulators of both constitutive and pulsatile secretion of FSH act in concert to mediate selective release of FSH. Different endocrine and molecular mechanisms have been proposed to explain the differential regulation of FSH and LH in different physiological conditions. As mentioned previously, GnRH is secreted in pulses and the nature of these pulses (both frequency and amplitude) impacts the relative synthesis and secretion of both gonadotropins (Burger et al., 2004). Rapid GnRH pulses (every 30–60 min) tend to favor LH release, whereas slower GnRH pulses (every 2–4 h) preferentially stimulate synthesis and secretion of FSH (Kaiser et al., 1997). Therefore, physiological changes in GnRH pulse frequency may explain situations in which FSH and LH are differently regulated. Notably, FSH does not appear to depend on GnRH pulsatile stimulation to the extent required for LH secretion, since daily injections of GnRH are sufficient to stimulate marked increases in FSH content in the pituitary and plasma concentrations, but not LH in GnRH-deficient mice (Charlton et al., 1983). Studies in the rhesus monkey also indicate that higher frequency of GnRH pulses favor LH secretion, while lower frequency favors FSH secretion (Wildt et al., 1981). While changes in GnRH pulse characteristics may explain changes in the overall amount of FSH released, they do not explain the presence of FSH pulses that are not associated with GnRH, particularly after pharmacological blockage

of GnRH actions. There are two plausible explanations for the persistent episodic pattern of FSH secretion in the absence of GnRH pulses. First, as discussed previously, the existence of other hypothalamic factors that selectively regulate FSH secretion (*e.g.*, FSH-RF) could explain the presence of FSH pulses in the absence of GnRH and LH pulses (Padmanabhan and McNeilly, 2001). Second, a time lag in the response of activin, inhibin, and follistatin to GnRH input could result in increased or decreased FSH secretion in the absence of changes in LH secretion that would culminate in what appears to be GnRH-independent pulses of FSH (Padmanabhan et al. 2002).

Regarding the molecular mechanism that likely contribute to the differential regulation of LH and FSH, studies have shown that the GnRH receptor has several regulatory elements and binding of GnRH to its receptor can activate multiple intracellular signaling pathways that could result in differential transcription of *FSHβ* and *LHβ* (Bernard et al., 2010). Additionally, while intracellular calcium influx is well known to regulate LH release by gonadotropes, it does not appear to be involved in FSH secretion (Kile and Nett, 1994). While the exact mechanisms involved remain unknown, it is possible that FSH-bound sialic acid residues may target translocation of FSH to different secretory granules than those containing LH, thereby providing a regulatory mechanisms for the differential secretion of both gonadotropins (Baenziger and Green 1988). For additional information regarding the molecular regulation of FSH synthesis and secretion, readers are referred to Das and Kumar (2018).

4. Pattern of FSH secretion during different physiological states

4.1. Pubertal Development

The beginning of puberty is associated with increased GnRH activity and subsequent gonadotropin secretion in girls (Sizonenko et al., 1970). The pattern of gonadotropin response to exogenous administration of GnRH also changes during pubertal development. Initially, the FSH response is relatively greater, but as puberty advances, the LH response increases and the relative FSH decreases, leading up to the adult pattern (Cumming, 1990). This change in pattern of gonadotropin response to GnRH likely reflects the increased ovarian feedback at the pituitary level by sex steroids and protein regulators of FSH. In support of this, are the observations that the circulating concentrations of inhibin A and total follistatin, two negative regulators of FSH secretion, change in opposite directions during pubertal maturation in girls (Foster et al., 2000). Concentrations of inhibin increase while follistatin levels decrease during pubertal development (Foster et al., 2000). Total concentrations of activin A, a positive regulator of FSH, remain unchanged during pubertal progression in girls. Therefore, the reduction in follistatin, a binding neutralizer of activin, in the face of relatively constant concentrations of total activin A suggests that the bioavailability of activin (free activin A) increases with pubertal maturation, such that activin could override the inhibin increase and contribute to the rise in FSH secretion that occurs during puberty (Foster et al., 2000).

In addition to changes in FSH secretory pattern, the proportion of the different FSH isoforms also changes during pubertal progression. While no changes are observed for LH isoforms, FSH composition shifts to more acidic isoforms during pubertal progression in girls (Phillips et al., 1997). Although the underlying mechanisms regulating the changes in FSH isoforms during puberty remain unclear, changes in GnRH secretory pattern, circulating concentrations of gonadal steroids, and endocrine or paracrine effects of protein regulators are all suspects (Ulloa-Aguirre et al., 1995).

4.2. Menstrual cycle

The reduction in circulating levels of estradiol, progesteroneand inhibin A in the beginning of the menstrual cycle due to the demise of the corpus luteum reduces the inhibitory effects on the pituitary and allows FSH to rise in the early follicular phase and stimulate follicular growth and selection (Lee et al., 1988; Mishell et al., 1971; Yding Andersen, 2017). At approximately Day 7 of the menstrual cycle, when selection of the dominant follicle occurs, FSH concentrations have already peaked and started to decline, with a continued slow decline until ovulation occurs (Lee et al., 1988; Yding Andersen, 2017). The general view is that this down regulation of FSH in the follicular phase of the menstrual cycle occurs due to increased estradiol production by the selected follicle, which in turn exerts a negative feedback at the anterior pituitary level. However, there appears to be a spatial-temporal issue with this premise since levels of FSH begin to decline several days prior to the rise in circulating concentrations of estradiol, suggesting that other ovarian factors, such as inhibin, activin, and follistatin may also play a role (Schneyer et al., 2000; Yding Andersen, 2017). The observations that inhibin B concentrations increase gradually during the follicular phase to reach a mid-cycle peak coincident with the preovulatory gonadotrophin surge are supportive of this premise (Muttukrishna et al., 1994).

During the midcycle, FSH and LH concentrations rise rapidly in response to the increased estradiol production by the preovulatory follicle (Reed and Carr, 2015; Yding Andersen, 2017). While a GnRH surge is critical to drive the preovulatory gonadotropin surge in rodents (Sarkar et al., 1976) and sheep (Moenter et al., 1991),the gonadotropin surge in women appears to unfold in the absence of a midcycle GnRH discharge being generated instead by the interaction between a pulsatile GnRH input to the pituitary and an action of estradiol (Martin et al., 1998; Plant TM, 2012). After falling immediately after the pre-ovulatory gonadotropin surge, inhibin A increases in parallel with serum progesterone to reach a peak during the mid-luteal phase (Muttukrishna et al., 1994). While concentrations of inhibin B rise immediately after the gonadotropin surge, they rapidly decrease during the luteal phase (Groome et al., 1996; Welt, 2004). Concentrations of FSH remain relatively low through most of the luteal phase primarily due to the inhibitory effects of inhibin A and the suppressive effects of progesterone on GnRH secretion (Lee et al., 1988; Reed and Carr, 2015). Activin A concentrations vary in a biphasic manner during the menstrual cycle, with highest levels occurring around the midcycle and the late luteal phase (Muttukrishna et al., 1996).While it is possible that activin A plays an endocrine role during the menstrual cycle, findings that virtually all detectable activin A in the peripheral circulation is associated with binding proteins raise questions about its relative bioavailability for acting at the pituitary level (Muttukrishna et al., 1996).

During the luteal phase, progesterone also plays a role modulating FSH heterogeneity. In the presence of high progesterone levels, estradiol fails to increase the presence of less acidic isoforms of FSH in the circulation (Wide et al., 1996). Moreover, during the luteal phase of the menstrual cycle, the predominant circulating isoform of FSH is acidic (Padmanabhan et al., 1988). Contrarily, during the follicular phase of the menstrual cycle, when estradiol levels are relatively high, the less acidic FSH isoforms predominate (Padmanabhan et al., 1988). Findings that estradiol decreases the expression of pituitary α 2,3-sialyltransferase, which incorporates sialic acid residues into the FSH molecule, provide a potential mechanism by which estradiol can stimulate increased production and secretion of less acidic isoforms of FSH (Damian-Matsumura et al., 1999). As discussed in detail in other review articles (Bousfield and Harvey, 2019; Padmanabhan et al., 1988; Ulloa-Aguirre et al., 2003), these changes in FSH heterogeneity are likely to play important biological roles in controlling ovarian processes during the menstrual cycle.

4.3. Perimenopause transition

A hallmark alteration observed during the perimenopause period is a continuous rise in FSH levels in the face of normal basal concentrations of LH, presumably due to declining estrogenic effects in the neuroendocrine system (Bäckström et al., 1982; Reame et al., 1996). It

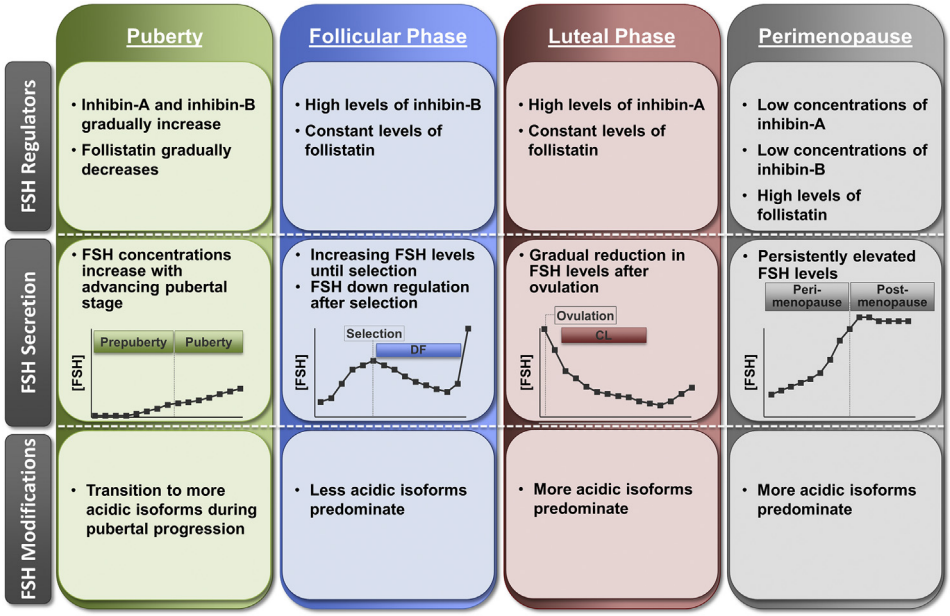

Fig. 2. Schematic summarizing the general changes in circulating levels of FSH regulators, FSH secretory patterns, and posttranslational modifications of FSH during pubertal progression, menstrual cycle, and perimenopause transition in women (reviewed in Cumming, 1990; Padmanabhan et al. 2002; Ulloa-Aguirre et al., 2003; and Yding Andersen, 2017). CL, corpus luteum; DF, dominant follicle.

is believed that FSH secretion, due to its elevated sensitivity to the inhibitory effects of estradiol and inhibin, increases first in response to the decline in negative feedback from the aging ovary as the total number of responsive follicles markedly reduces during the perimenopause transition (Ferin et al., 1993). While a reduced ovarian steroidogenic capacity could be involved in this FSH rise, enhanced FSH concentrations are observed in perimenopausal women with normal levels of ovarian steroids (Reame et al., 1996), suggesting that other ovarian regulators could be involved. In agreement, a substantial decrease in circulating levels of inhibin-B with no significant changes in estradiol or inhibin-A were observed in women during the early perimenopausal phase (Burger et al., 1998). Later during the perimenopausal period, inhibin-A and estradiol also fall markedly, further contributing to the FSH rise (Burger et al., 1998). An age-dependent reduction in follistatin was also reported in women during the perimenopause transition (Reame et al., 2007). Collectively, these findings suggest that changes in the secretory pattern of these regulatory proteins, which occur before significant changes in circulating levels of estradiol, are consistent with enhanced activin bioavailability and may contribute to the perimenopausal rise in concentrations of FSH (Burger et al., 1998; Reame et al., 2007).

In addition to a rise in FSH concentrations, the perimenopausal transition is also characterized by changes in the FSH heterogeneity. The predominant FSH glycoforms during the perimenopausal transition are the least complex and more simple, yet very acidic isoforms (Anobile et al., 1998). Because the perimenopausal phase is characterized by decreased circulating levels of estradiol, these findings further support the notion that estradiol increases the presence of less acidic FSH isoforms in the circulation (Wide et al., 1996). In conjunction with the observations during the normal menstrual cycle, these findings in perimenopausal women suggest that changes in the steroidal *milieu* and ovarian production of protein regulators of FSH (*e.g.,* inhibin and activin) modulate the carbohydrate complexity and charge of FSH (Fig. 2). Therefore, this FSH heterogeneity may provide a secondary level of control by which FSH regulates ovarian function (Padmanabhan and Sharma, 2001).

5. Conclusions

This review provides an overview of the neuroendocrine, endocrine, paracrine, and autocrine regulation of FSH secretion and heterogeneity during different physiological states. At the hypothalamic level (neuroendocrine), GnRH pulses clearly stimulate FSH pulsatile secretion, particularly detectable when FSH is monitored close to the site of secretion (*e.g.,* portal vasculature in sheep). Yet, GnRH-independent pulses of FSH suggest that other neuroendocrine factors, such as a putative FSH-RF, may also regulate the episodic release of FSH. At the ovarian level, sex steroids and inhibin act in an endocrine fashion to provide the primary negative feedback control of FSH biosynthesis and consequently to the constitutive release of FSH. It remains uncertain whether activin and follistatin produced by the ovary play an endocrine role in controlling FSH secretion. At the pituitary level, activin, inhibin (Bilezikjian et al., 1996; Peeters et al., 1997; Roberts et al., 1989), and follistatin produced locally act to form a paracrine/autocrine loop that contributes to the amount of FSH released. Hypothalamic, ovarian, and pituitary factors act in concert to control not only secretion of FSH but also heterogeneity, and changes in these regulators during different physiological states can ultimately modulate FSH biological activity and its effects in the gonads and extragonadal targets.

While it is well established that FSH has great therapeutic potential and it represents an indispensable part of fertility treatment in women, there are numerous FSH preparations commercially available or in development that have some differences related to the glycosylation patterns and biological activity (Smitz et al., 2016). Both urinary-derived products and FSH produced through recombinant techniques are currently available. Future studies are needed to provide additional information regarding their source, purity, potency, and biological activity and to guide clinicians to choose which preparation or combination of preparations will be administered to women undergoing assisted reproductive technologies and/or fertility treatment. Additionally, a better understanding of the extragonadal actions of FSH is warranted, particularly in light of the recent findings that suggest a potential causal link between FSH hypersecretion, bone loss, and increased adiposity in perimenopausal women (Kumar 2017; Liu et al. 2017; Sun et al., 2006).

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