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Review

Gonadotropin surge attenuating factor: A physiological regulator

Messinis Ioannis, Messini Christina, Dimitraki Marina, Anifandis George, Dafopoulos Konstantinos

Department of Obstetrics and Gynecology, University of Thessaly, School of Health Sciences, Faculty of Medicine, Larissa, Greece

Correspondence
Messinis Ioannis
Department of Obstetrics and Gynecology, University of Thessaly, School of Health Sciences, Faculty of Medicine, GR - 41110 Larissa, Greece
E - mail: messinis@med.uth.gr

Abstract

Gonadotropin surge attenuating factor (GnSAF) is a non - steroidal ovarian substance, which attenuates the endogenous LH surge in superovulated women. Although several attempts have been made to purify GnSAF from various biological materials, different sequences were found. Only one of them has shown substantial homology of GnSAF to a known portion of the human genome. In particular, GnSAF showed identity to the carboxyl - terminal fragment of human serum albumin (HSA) with a molecular mass of 12.5 kDa. Additional in vitro experiments have confirmed the above finding, since recombinant polypeptides corresponding to the subdomain IIIB of HSA displayed GnSAF bioactivity in an in vitro bioassay system, while the expression of HSA gene was detected in human granulosa cells. Accumulated evidence has indicated that GnSAF may play an important physiological role during the normal menstrual cycle. Thereby, it is assumed, that the bioactivity of GnSAF increases during the intercycle period under the stimulating action of FSH and decreases gradually, thereafter, until the midcycle. This factor seems to be the missing substance in the ovarian feedback system, which maintains the pituitary in a state of low responsiveness to GnRH during the greater part of the follicular phase. The reduced bioactivity of GnSAF in the late follicular phase enhances the sensitizing effect of estradiol on the pituitary response to GnRH, facilitating thus the full expression of the midcycle LH surge.

Key words: LH; GnRH; GnSAF; ovary; pituitary

Gonadotropin surge attenuating factor (GnSAF) is a non - steroidal ovarian substance that attenuates the endogenous LH surge in superovulated women[1]. The first indication for the existence of this factor in women was derived from studies involving ovarian stimulation for in vitro fertilization (IVF). Initially, women superovulated with clomiphene plus either follicle - stimulating hormone (FSH) or human menopausal gonadotropin (HMG) displayed an endogenous luteinizing hormone (LH) surge, which was markedly attenuated both in amplitude and duration[2,3]. Subsequently, it was shown that the endogenous LH surge was also attenuated during superovulation induction with the administration of FSH alone[4]. A few years before, it had been shown that in superovulated monkeys the endoge-

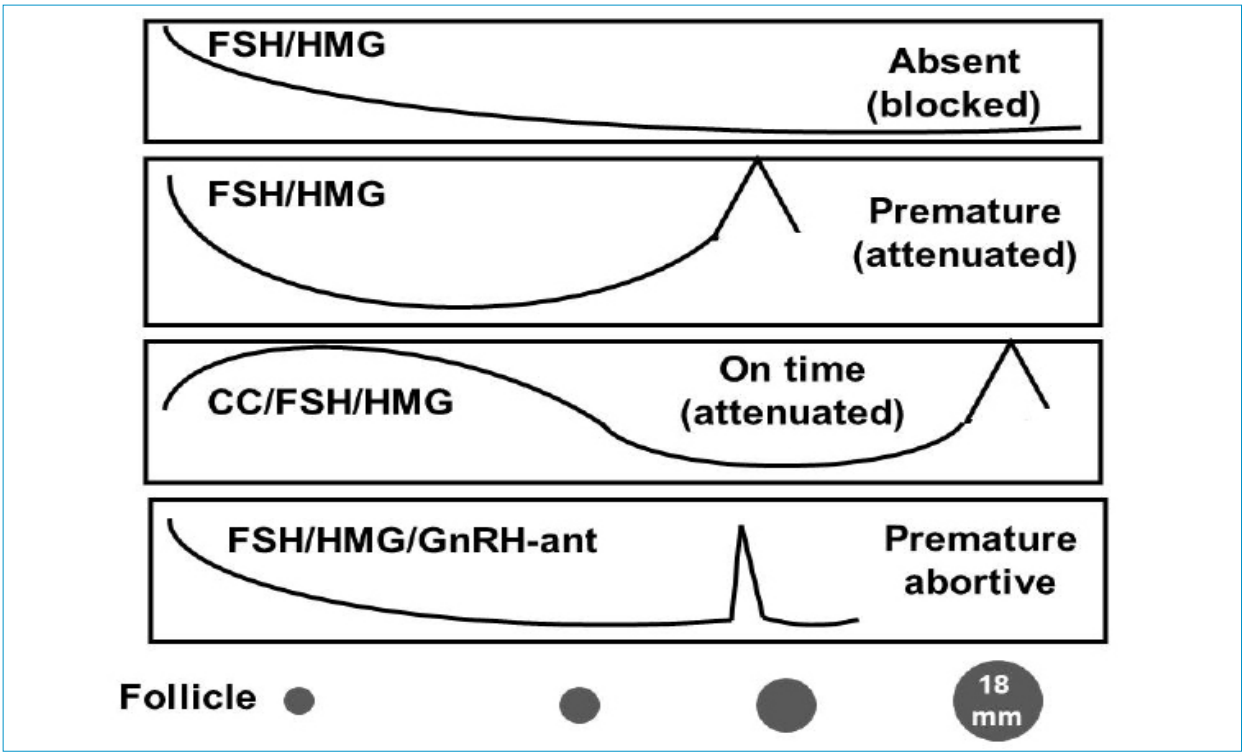

Figure 1. The occurrence of an endogenous LH surge during ovarian stimulation according to the treatment regimen

nous LH surge was inhibited by an ovarian factor that was named gonadotropin surge inhibiting factor (GnSIF)[5]. It is still unclear, if GnSAF and GnSIF are the same or different molecules, although the term GnSAF seems more appropriate in humans. The studies in superovulated women have also shown that the pituitary response to gonadotropin - releasing hormone (GnRH) is markedly reduced as compared to spontaneous cycles and this is probably part of the mechanism via which GnSAF attenuates the endogenous LH surge[1,6].

Clinical research has demonstrated that, in contrast to the normal menstrual cycle, the endogenous LH surge in superovulated women is a variable phenomenon that can be blocked on several occasions. The occurrence of an LH surge in such cycles depends on various parameters including the type of drugs used in the ovarian stimulation regimen, the degree of the ovarian hyperstimulation and the use of GnRH analogues. More specifically, when women are treated with clomiphene citrate alone or in combination with gonadotropins, the endogenous LH surge occurs invariably and on time in relation to the size of the leading follicle (larger than 16 mm in diameter)[2,3,7]. On the other hand, when FSH is used alone, the endogenous LH surge is either blocked (in 50 - 80% of the cycles) or it happens prematurely, i.e. before the leading follicle becomes ovulatory in size[4,8-10]. It is interesting that even with a markedly attenuated LH surge, luteinization of the granulosa cells may happen, while oocytes have been collected following follicle aspiration and conception has taken place after embryo transfer[2]. In clinical terms, organizing the process of egg retrieval taking as a reference point the onset of the endogenous LH surge is rather inconvenient, since follicle aspiration may fall out of working hours.

Although the GnRH agonists invariably prevent the LH surge[10,11], the antagonists are less effective, since several LH peaks can be detected during the period of ovarian stimulation[12-16]. These peaks are usually abortive LH surges, the clinical importance of which is not yet clear, although they can lead to follicle luteinization as confirmed by the elevated

Table 1. Candidate sequences of gonadotropin surge attenuating factor (GnSAF)

Molecular weight (kDa)	Amino acid sequence	Biological material (source)	Species	Reference
37.0	NH2 : SDXXPQL No clear identification	Sertoli cell - conditioned medium	Rat	Tio et al[23]
69.0	NH2 : KPLAE No clear identification	Follicular fluid	Pig	Danforth & Cheng[24]
63.0 59.0	No sequence	Follicular fluid from superovulated ovaries	Human	Mroueh et al[25]
12.5	COOH:ALEVDETYVPK Identification: truncated C - terminus of serum albumin	Follicular fluid from superovulated ovaries	Human	Pappa et al[26]
64.0 64.0	Internal: EPQVYVHAP No clear identification NH2 : XVPQGNAXXN No clear identification	Granulosa - luteal cell - conditioned medium	Human	Fowler et al[30]
64 40	D/NPMYSMITPNILRLES DINGGGATLPQPLYQTSGVLTAFGAP	Follicular fluid	Cows	Hendriks et al[33]

serum progesterone concentrations[12,15,16]. Experiments in women have shown that the occurrence of LH peaks in such cycles is probably due to the inability of the GnRH antagonists to block the positive feedback mechanism[17]. Although LH pulsatility is abolished immediately after the injection of the GnRH antagonist, a normal pattern of pulses is re-established soon after[18]. Therefore, in the context of an IVF program involving daily injections of a GnRH antagonist, there are short intervals during which the pituitary remains unprotected from the stimulating action of estradiol. The fact that only peaks and no actual LH surges occur suggests that during these intervals estradiol only partially activates the positive feedback mechanism. Since at the same time GnSAF is produced in high amounts from the hyperstimulated ovaries, it is probable that the action of the GnRH antagonist, which limits the "window" for estradiol action, combined with the action of GnSAF, which antagonizes the sensitizing effect of estradiol on the pituitary, alleviates the strength of the positive feedback effect which displays an abortive LH surge[17-19] (Figure 1). In spite of the above, previous studies have demonstrated no clear differences in live birth rate, when comparing agonists with antagonists cycles[20].

Characterization of GnSAF

Almost three decades have passed from the first indication of the existence of GnSAF. Nevertheless, its characterization has been only partially achieved and there is no consensus regarding the structure of this molecule[21]. Several attempts have been made to isolate GnSAF from various biological materials obtained from different species, but the results have been inconsistent (Table 1). The first attempt resulted in the detection of GnSAF bioactivity in human follicular fluid obtained from superovulated women. With the use of crude serial ultrafiltration, the bioactivity of GnSAF was detected in the range of 10 - 30 kDa, but the molecule was not purified to homogeneity[22]. In these experiments, an in vitro bioassay system was developed using rat pituitary cells in culture and the GnSAF bioactivity was assessed

by the reduction of the LH secretion in response to GnRH. Following this, Tio et al[23], reported the purification of GnSAF/IF from rat Sertoli cell - conditioned medium as a 37 kDa monomeric protein. Subsequently, purification of GnSAF was reported from porcine follicular fluid and a 69 kDa monomeric protein was identified with no structural similarities with that from the Sertoli cells[24]. These two substances demonstrated different amino (NH2) - terminal sequences. In addition, the GnSAF/IF, which was isolated from Sertoli cell - conditioned medium, showed also potent inhibin activity, since it caused a reduction not only of GnRH - induced LH secretion but also of basal FSH secretion[23]. The latter was not shown in the study in which GnSAF was isolated from porcine follicular fluid[24]. Based on these results, an antibody raised against the 69 kDa porcine protein identified two putative GnSAF/IF-like homologues in human follicular fluid with molecular sizes of 63 and 59 kDa, respectively[25].

Following these attempts for GnSAF purification, a molecule of 12.5 kDa was isolated from human follicular fluid of superovulated women as being identical to the carboxyl (C) terminal fragment of human serum albumin (HSA)[26]. This is the only study that showed substantial homology of the putative GnSAF to a known portion of the human genome. In that study, GnSAF was purified using various biochemical procedures and was identified by amino acid sequence using mass spectrometry. In the in vitro bioassay system, bioactivity of GnSAF in the purified material was demonstrated, while inhibin activity was not detected. Although it sounds interesting that GnSAF is the C - terminal fragment of HSA, this is consistent with previous data showing that several protein fragments exert biological activities, which are different from their intact proteins[27,28]. Given the above information regarding the identity of GnSAF with the C - terminal fragment of HSA, further experiments were performed in which recombinant polypeptides of HSA were produced using the expression - secretion system of pichia pastoris[29]. It was shown then that the subdomain IIIB (residues 490 - 585) of HSA was expressed in secreted forms and supernatants from clones expressing this polypeptide reduced markedly GnRH - induced LH secretion, demonstrating thus GnSAF activity in the in vitro bioassay system. In the same experiments, the remaining subdomains and the full length of HSA were inactive. These results provided further support to the findings of the study of Pappa et al, that GnSAF is the C - terminal fragment of HSA[26]. However, a little later, Fowler et al[30], reported two new candidate GnSAF/IF sequences 60 - 66 kDa, which were present in culture supernatants from human granulosa/luteal cells obtained from superovulated women and from unstimulated cycles.

Although not investigated, it is possible that there may be more than one ovarian protein that expresses activity of GnSAF under in vivo specific conditions. It is worth mentioning, however, that in the majority of the experiments, in which purification of GnSAF was attempted, the purified substance had a molecular mass that was close to or above 60 kDa, i.e. close to the molecular weight of HSA. It is not unlikely, therefore, that in these experiments the purification procedure resulted in the isolation of HSA, which the authors of those studies probably considered the result of contamination. Therefore, a more meticulous observation and analysis of the results might had identified the C - terminal fragment of HSA as the principal GnSAF candidate in agreement with the study of Pappa et al[26].

Following the results of that study[26], a subsequent study revealed the expression of HSA gene in human luteinized granulosa cells obtained from superovulated women undergoing IVF treatment[31]. Using reverse transcription polymerase chain reaction (RT - PCR) analysis of HSA, RNA transcripts and sequencing analysis of cDNA from granulosa cells, only bands for the NH2 - and the C - terminal fragments corresponding to HSA gene were detected in the cytoplasm, while full-length HSA mRNA transcripts were not detectable in granulosa cells. More recently, experimental data in human luteinized granulosa cells after performing in - situ - hybridization and immunocytochemistry showed that, in the presence of FSH, mRNA transcripts corresponding to the C -

terminal fragment of HSA gene were detected in intensity that was comparable to that seen by hepatic HepG2 cells used as a positive control[32].

An attempt to purify and characterize GnSIF/AF from bovine follicular fluid was recently performed by Hendriks et al[33]. Two different bioactive molecules were isolated, one heteromeric and one monomeric of 160 kDA and 40 kDA respectively, which showed activity of GnSAF, i.e inhibition of GnRH - induced LH secretion in an in vitro bioassay system, and attenuated the mitogen - activated protein kinases (MAPK) phosphorylation pathway. These two GnSAF candidates did not show any similarities with the sequence of previous ones. Nevertheless, the NH2 - terminus of the high molecular weight showed high homology with human β-chain of the complement C3, while the low molecular weight with other proteins, members of the DING - family[34]. The authors of that publication, based on the NH2 - terminal sequence were able to produce a synthetic molecule of 26 amino acids, which exerted a significant inhibitory action on GnRH - induced LH secretion in vitro without affecting basal FSH levels[33]. Although the possibility that more than one form of the molecule may exist cannot be excluded, the above findings have contributed further to the existing confusion regarding the nature of GnSAF.

It should be emphasized that all seven putative GnSAF candidates isolated so far are different from each other. Nevertheless, whatever the molecular structure of GnSAF is, it cannot be ignored that the in vivo bioactivity of GnSAF has been demonstrated invariably during ovarian hyperstimulation[35]. By definition, GnSAF attenuates the endogenous LH surge via a significant reduction of the pituitary response to GnRH1. Therefore, any reduction in LH secretion is not due to a direct effect of GnSAF on the tonic secretion but only on the secretion related to the pulsatile action of GnRH.

Physiological role of GnSAF

It has been established that during the normal menstrual cycle, ovarian steroids are the principal mediators of the ovarian effects on the hypothalamic - pituitary system via the feedback mechanisms. Non - steroidal substances, however, produced by the ovaries, such as inhibin, may also participate in these mechanisms.

Negative feedback

The main ovarian steroids mediating the negative feedback effect are estradiol in the follicular phase and progesterone in the luteal phase. The negative feedback mechanism has been studied in various experiments following the administration of exogenous hormones or by manipulating the levels of the endogenous hormones[19,36-39]. Clinical experiments have suggested a differential control of FSH and LH by the ovaries[40]. Although the main steroidal substance produced by the ovaries in the follicular phase of the cycle is estradiol, it has been demonstrated that the ovaries also contribute to the circulating low concentrations of progesterone, which also participates in the negative feedback control especially of LH secretion[38,41].

In the luteal phase, it is the progesterone that controls LH and FSH secretion, however, the contribution of estradiol is also important. In experiments, in which ovariectomy was performed in the midluteal phase of normally cycling women, the postoperative increase in basal FSH and LH levels was prevented by the co - administration of the two steroids[40]. In particular, estradiol alone was able to prevent the increase only for two days, while with the addition of progesterone the increase was prevented for 7 days, which was the total period of observation.

The non - steroidal ovarian substances, inhibin A and inhibin B, seem to be important components of the negative feedback mechanism that controls FSH secretion. The evidence is derived mainly from in vivo experiments following the administration of these substances to animals, while clinical research has proposed that the two inhibins are also important in humans[42-47]. Inhibin B is mainly produced by follicles recruited under the intercycle rise of FSH, while inhibin A by the corpus luteum. Therefore, high levels of inhibin B in serum are found in the first half of the follicular phase, while inhibin A lev-

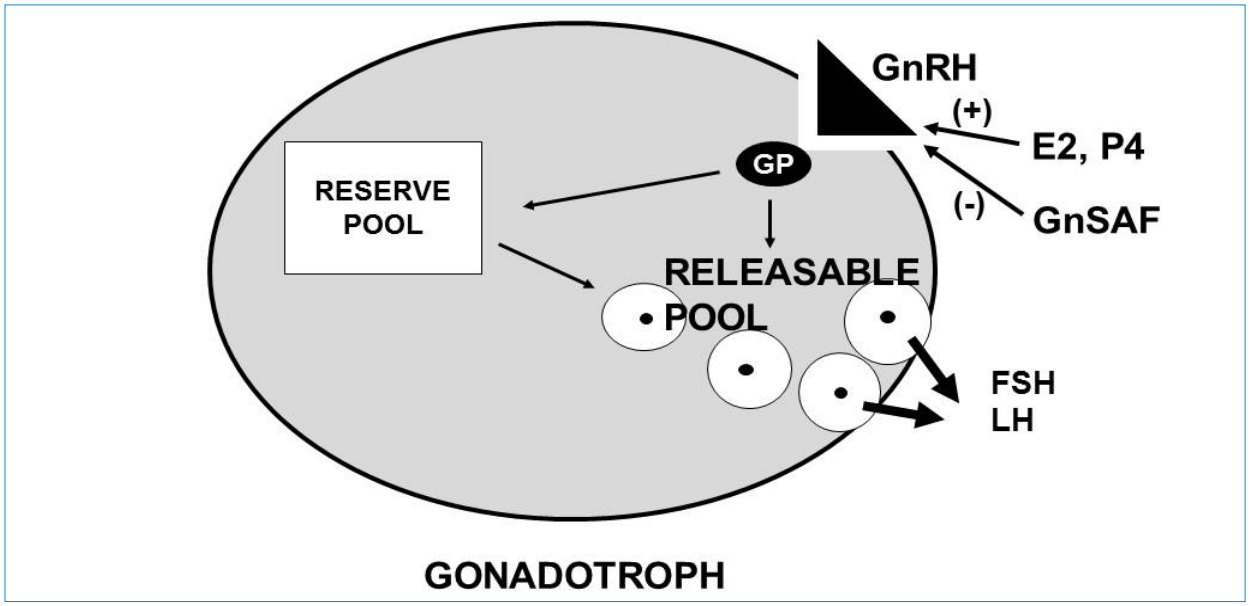

Figure 2. The two gonadotropin pools. GnRH stimulates the secretion of LH and FSH from the pituitary gonadotropic cells. Estradiol (E2) and progesterone (P4) sensitize the cells to GnRH, while GnSAF antagonizes this effect

els are high in the luteal phase[48].

This circulating profile of the two inhibins can explain the intercycle rise of FSH during the luteal - follicular transition. In particular, the decreasing concentrations of inhibin A in parallel with those of estradiol and progesterone from the mid - to the late luteal phase reduces the strength of the negative feedback effect leading to the onset of the FSH rise (FSH window). Thereafter, the increasing FSH levels stimulate follicle recruitment and selection. As a result, serum estradiol and inhibin B concentrations increase, reinstating the strength of the negative feedback effect on FSH secretion and terminating the intercycle rise of this gonadotrophin. It is evident therefore, that inhibin A contributes to the opening and inhibin B to the closure of the FSH window[35].

Other non - steroidal substances produced by the ovaries include activin and follistatin. Although activin is related to inhibin (dimeric product of the 'β' subunit of inhibin), its action is by definition opposite to that of inhibin, i.e. stimulation of FSH secretion at least in animal models[49,50]. Nevertheless, an endocrine role of this substance has not been clarified[51]. On the other hand, follistatin is a carrier protein transferring inhibins and mainly activins to the various tissues for action[52,53]. It is likely that activins mainly play local paracrine roles in different tissues.

Positive feedback

The positive feedback effect of the ovaries on the hypothalamic - pituitary system is mediated by estradiol. During the normal menstrual cycle, estradiol levels increase gradually from the early to the mid - follicular phase and even more rapidly in the late follicular phase leading to the occurrence of the endogenous LH surge. That estradiol is the principal mediator of the positive feedback effect has been shown in several experimental studies following the exogenous administration of this steroid to women either in the follicular phase of the cycle or after menopause[35]. Estradiol sensitizes the pituitary to GnRH and facilitates the "self - priming effect" of GnRH on the anterior pituitary and this is very important for the occurrence of the endogenous LH surge[54,55]. The sensitizing effect of estradiol has been shown in experiments in women with the i.v. administration of two submaximal pulses of GnRH, 10μg each, two hours apart. The response of LH to the second pulse in the late as compared to the early follicu-

lar phase was significantly higher than the response to the first pulse[54]. This was related to the high concentrations of estradiol in the late as compared to the early follicular phase and reflects the increased duration of LH secretory events and consequently the increased LH mass[56]. The sensitizing action of estradiol on the pituitary during the follicular phase of the cycle is building up the pituitary reserves, which from a physiological point of view is required for the occurrence of the endogenous LH surge (Figure 2).

There are several possible mechanisms via which estradiol facilitates the self - priming effect of GnRH on the pituitary. In rats, an estradiol - induced increase in the number of GnRH receptors on the pituitary gonadotropic cells has been shown[57]. Also, estradiol by inhibiting GnRH metabolism may enhance the availability of this peptide on the gonadotropic cells[58]. Additionally, estradiol may decrease the concentration of GnRH that is required for the secretion of LH, while GnRH increases the glycosylation and polypeptide synthesis of LH[59]. It is also possible that an interaction between kisspeptin and GnRH neurons may play a role in the transmission of the action of estradiol, as kisspeptin is known to stimulate LH secretion and the number of LH pulses when injected peripherally in women[60,61].

Apart from estradiol, progesterone is another factor, which sensitizes the pituitary to GnRH during the follicular phase of the cycle. Clinical experiments have provided evidence indicating that the pituitary sensitivity and reserve were reduced in normal women treated from the early to the late follicular phase with the antiprogestagen, mifepristone[62]. It is likely therefore, that progesterone even at low concentrations during the follicular phase enhances the positive action of estradiol and facilitates the expression of the mydcycle LH surge. The action of progesterone may also contribute to the timing of the onset of the LH surge, although data regarding an increase in serum concentrations of this steroid a few hours before the onset of the midcycle LH surge are conflicting[63,64]. Progesterone is also important locally in the hypothalamus where it is produced under the influence of estradiol[65,66]. This neuroprogesterone and the local activation of the progesterone receptors in the hypothalamus are obligatory for the generation of the estrogen - induced LH surge in animals[67,68]. The increasing serum progesterone concentrations following the onset of the endogenous LH surge contribute to the amplification of the LH surge[69]. Nevertheless, the enhancing effect of progesterone on the positive feedback, after the LH peak, is gradually diminishing and the positive effect is converted into a negative action. This results in the decline of LH and FSH levels and the termination of the surge[70]. Therefore, the two principal ovarian steroids, i.e. estradiol and progesterone mediate both the negative and the positive feedback effects of the ovaries on the hypothalamic - pituitary system.

Role of GnSAF

As it was mentioned, the pituitary response to GnRH is markedly reduced in superovulated cycles[1]. In these cases, a dose of 100 μg GnRH was used i.v, which, however, cannot differentiate between the two functionally related pools of the pituitary gonadotropic cells, i.e. the first pool or pituitary sensitivity and the second pool or pituitary reserve. The latter can be achieved with the use of the model of the two submaximal doses of GnRH, 10 μg each, injected two hours apart. In that model, the response at 30 min to the first pulse represents the release of gonadotropins from the first pool (pituitary sensitivity), while the whole area under the curve after the injection of the second pulse represents the second pool or the pituitary reserve[55]. In IVF cycles superovulated with FSH, the response of LH to the two GnRH pulses was markedly suppressed, indicating a reduction of both the pituitary sensitivity and reserve[6]. Based on these findings, the reduction in the 30 - min response of LH to 10 μg GnRH (ΔLH) has been used in several human studies as an in-vivo bioassay for GnSAF35. Changes in the ΔLH response to GnRH are believed to reflect changes in the bioactivity of GnSAF.

By using this method in women under different conditions, interesting information has been obtained regarding the role of GnSAF during the nor-

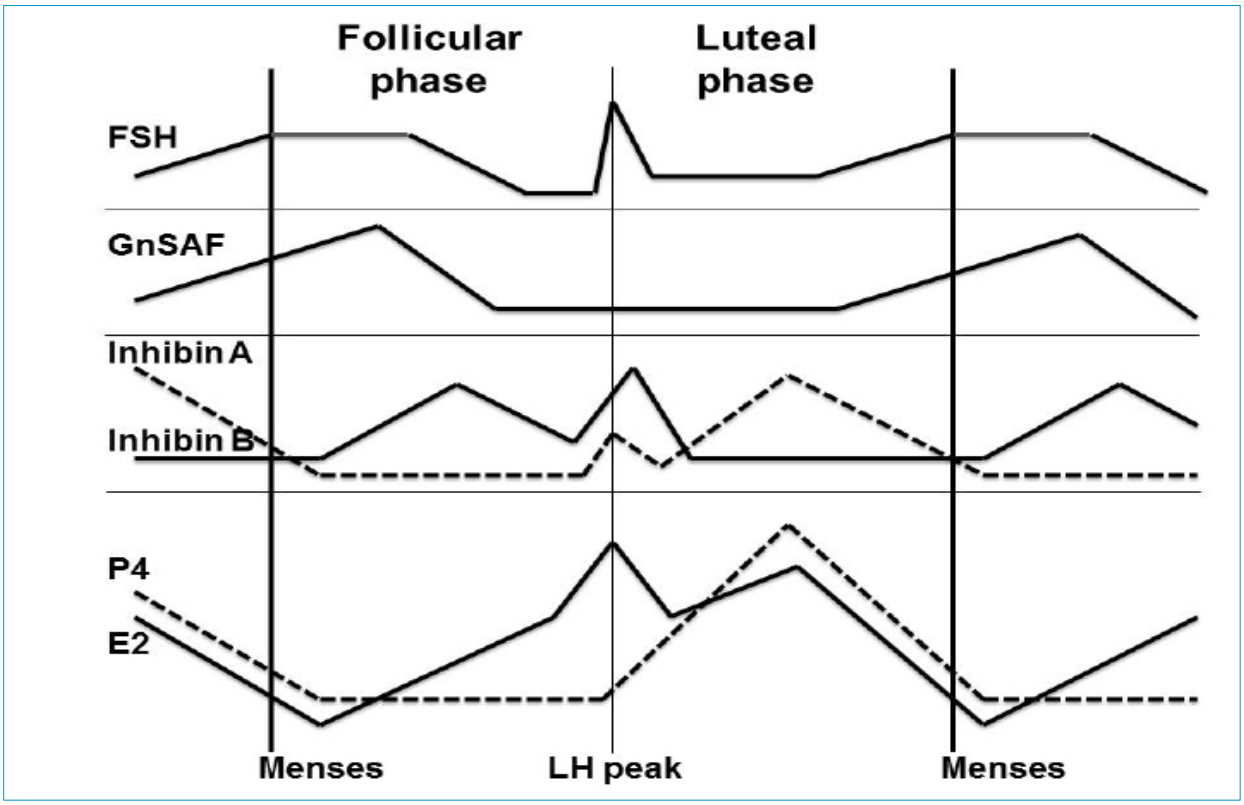

Figure 3. Changes in the profile of FSH and the steroidal and non - steroidal substances during the normal menstrual cycle. GnSAF is produced during the intercycle period under the influence of the intercycle rise of FSH. The activity of GnSAF decreases from the mid - to the late follicular phase, facilitating the positive feedback effect of estradiol. E2 (estradiol), P4 (progesterone)

mal menstrual cycle. Specifically, experiments performed in the early follicular phase of the cycle have demonstrated a gradual decrease in the LH response to GnRH during the first two days at a time when serum estradiol concentrations showed a slight but not significant increase[71]. It was suggested that during that period of time, which is part of the intercycle period, there was a significant increase in GnSAF bioactivity. Since in superovulated cycles the increased activity of GnSAF is detected during the exogenous administration of FSH, it is likely that the intercycle rise of FSH was responsible for the increased production of GnSAF in the early follicular phase. Subsequent experiments in women during the mid - to late follicular phase showed that the pituitary response to GnRH remained unchanged until the preovulatory period, while at the same time serum estradiol concentrations increased steadily[72]. This is compatible with changes in LH pulsatility showing frequent pulses of small amplitude throughout the whole follicular phase, while a marked increase in the amplitude was found at midcycle after the onset of the LH surge[73]. In accordance with above results, it is suggested that the pituitary sensitivity to GnRH was enhanced by estradiol only in the late follicular phase of the cycle.

Nevertheless, estradiol in increasing levels is expected to enhance pituitary sensitivity to GnRH at all times and consequently to do it during the whole follicular phase. The fact that the increase was evident only in the late follicular phase suggests that in the early and midfollicular phase the ovaries produce a substance, which antagonizes the sensitizing action of estradiol on the pituitary response to GnRH. This notion was supported by data in postmenopausal women treated with exogenous estra-

diol and progesterone to simulate the normal menstrual cycle. In these experiments, serum estradiol concentrations increased gradually to reach preovulatory levels within 14 days, simulating thus the normal follicular phase[39]. It was found that under these conditions the pituitary sensitivity to GnRH increased from the beginning to the end of the simulated follicular phase in parallel with the increasing levels of estradiol. These results, in combination with those in the normal menstrual cycle, support the hypothesis of GnSAF, as the ovarian substance, which antagonizes the sensitizing effect of estradiol on the pituitary response to GnRH (Figure 3).

It is interesting that in the normal menstrual cycle, a gradual decrease in the LH response to GnRH has been demonstrated from the early to the late luteal phase[74]. Whether this means that the activity of GnSAF increases during the luteal phase is not yet clear, although high GnSAF bioactivity has been shown during the luteal phase of superovulated cycles[75]. Nevertheless, experiments performed in normal women suggest that an increase in the bioactivity of GnSAF occurs only during the late luteal phase of the normal menstrual cycle[40,74]. The above findings in combination with the results in the early follicular phase, support the notion that increased production of GnSAF takes place during the luteal-follicular transition, i.e. under the influence of the intercycle rise of FSH (Figure 3).

It is evident from the above discussion that GnSAF is possibly part of the mechanism, which controls the secretion of LH during the normal menstrual cycle. The role of GnSAF is restricted to the luteal - follicular transition, while its bioactivity from the mid - to the late follicular phase is gradually diminishing. It sounds logical, therefore, that this factor is produced by the follicles that are recruited under the intercycle rise of FSH, from which the dominant follicle will be selected. Based on the profile of its bioactivity, it is suggested that GnSAF is produced by the small growing follicles rather than by the selected dominant follicle[76,77].

From a physiological point of view, the high bioactivity of GnSAF during the early and midfollicular phase prevents the increase in the response of LH to GnRH by antagonizing the sensitizing action of estradiol on the pituitary gonadotrophs. In this context, GnSAF maintains the pituitary in a state of low responsiveness to GnRH during the greater part of the follicular phase, contributing thus to the preservation of low LH levels at that stage of the cycle. It is only at the end of the follicular phase when high levels of LH in the form of a surge are required for the final oocyte maturation[39]. Although estradiol and progesterone mediate the negative feedback on the tonic secretion of LH in the follicular phase, GnSAF seems to be the missing factor that limits the LH response to GnRH pulses. That LH levels should remain low during the follicular phase of the cycle is in accordance with the "LH ceiling concept", an important issue for the normality of the menstrual cycle. If the ceiling is exceeded, LH will become detrimental to the follicle with suppression of granulosa cells proliferation and initiation of atresia in immature follicles or premature luteinization in preovulatory follicles[78].

It is evident, therefore, that GnSAF is a molecule with a negative action on LH secretion. However, its decreasing bioactivity from the mid- to the late follicular phase seems to facilitate the positive action of estradiol and the full expression of the LH surge[79.] When the bioactivity of GnSAF, instead of decreasing remains high, as during ovarian hyperstimulation with FSH, the positive feedback effect is variably disturbed from slight attenuation to complete inhibition[35]. From a physiological point of view, GnSAF is considered a regulator of the amplitude of the LH surge. This factor may also participate in the timing of the onset of the LH surge. Estradiol is the principal ovarian signal, which activates the positive feedback mechanism and triggers the midcycle LH surge. Progesterone contributes by enhancing the sensitizing action of estradiol on the pituitary response to GnRH. GnSAF may also participate by its reduced production in the late follicular phase, facilitating thus the positive effect of the other two factors. It is evident that the timing of the onset of the midcycle LH surge is driven by a balance between the activity

of estradiol and progesterone on one hand and the activity of GnSAF on the other hand. Nevertheless, the occurrence of a premature LH surge in several superovulated cycles, despite the high bioactivity of GnSAF in the circulation, indicates that this substance is less likely to play a role in timing the onset and more possible to participate in the control of the amplitude of the LH surge.

The importance of GnSAF in the early to midfollicular phase can be also concluded from findings indicating that the estrogen - induced LH surge is significantly reduced both in amplitude and duration as compared to the midcycle LH surge. Under these conditions, the administration of small doses of progesterone amplified the LH surge, which approximated then the dimension of the midcycle surge, although a proper comparison was not performed[80]. Nevertheless, in another study, the LH surge that was induced in the early to midfollicular phase by either estradiol alone or estradiol plus progesterone was significantly smaller than that at midcycle[81]. These data can be explained by the reduced production of GnSAF from the midfollicular phase onwards, although Taylor et al[81], tried to explain the smaller LH surge in the early to midfollicular phase on the basis of missing ovarian factors that are required to amplify the LH surge. As yet, no such factors have been identified in the ovary. Furthermore, experiments performed in postmenopausal women provided similar information with that in the midfollicular phase regarding the dimension of the LH surge[80].

The mechanism of action of GnSAF at the hypothalamic - pituitary level is not known. A reduced response of LH to GnRH has been shown in several studies[35]. As it was mentioned, the progesterone receptors in the hypothalamus play an important role in the expression of the positive feedback mechanism[67]. Several studies have attempted to investigate the mechanism of action of GnSAF at the central nervous system. Overall, it has been shown that a reduced phosphorylation/activation of progesterone receptors in the pituitary gonadotropic cells may be involved in the decreased synthesis and release of LH that is induced by GnSAF in animals[82-84].

Conclusions

Accumulated evidence has demonstrated that GnSAF is the missing ovarian factor in the normal menstrual cycle that is responsible for the unchanged sensitivity of the pituitary to GnRH during the early and midfollicular phase of the cycle, so that the release of LH is limited to amounts creating a certain low level of this hormone, which by any means is adequate for normal folliculogenesis. It is evident that GnSAF is not part of the classical negative feedback mechanism, which is responsible for the reduced tonic secretion of gonadotropins, a process that is controlled by the ovarian steroids. This factor seems to affect the pulsatile process of LH release that is controlled by GnRH. Therefore, GnSAF is a factor the action of which affects the dynamic aspects of LH secretion, being a negative influence in the context of the positive feedback mechanism.

Conflict of interest

All authors declare no conflict of interest.

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