Activity of gonadotrophin surge-attenuating factor during the luteal phase in superovulated women

I. E. Messinis¹, A. MacTavish² and A. A. Templeton²

¹Department of Obstetrics and Gynaecology, University of Ioannina, 45332 Ioannina, Greece; and

²Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen AB9 2ZD, UK

Gonadotrophin surge-attenuating factor (GnSAF) is a putative nonsteroidal ovarian factor that attenuates the luteinizing hormone (LH) surge in superovulated women. GnSAF bioactivity was studied during the luteal phase by investigating six normally ovulating women in two cycles – a spontaneous and a follicle-stimulating hormone (FSH)-treated cycle. In both cycles, the pituitary response to an acute intravenous injection (10 µg) of luteinizing-hormone-releasing hormone (LHRH) was investigated in late follicular (follicle size 16 mm), early luteal (day 5 after human chorionic gonadotrophin, hCG), midluteal (day 9 after hCG) and late luteal phase (day 12 or 13 after hCG). FSH was injected daily at the dose of 225 iu on cycle days 2, 3 and 4, and 150 iu thereafter. The increase in LH and FSH (mean $\pm$ SEM) 30 min after LHRH in the spontaneous cycles decreased significantly from early to late luteal phase and remained unchanged in the FSH-treated cycles. Increases in LH and FSH 30 min after LHRH were significantly attenuated in the FSH-treated compared with the spontaneous cycles in late follicular and luteal phases. Serum oestradiol and progesterone concentrations were significantly higher in the FSH than in the spontaneous cycles only in early, but not in mid- and late luteal phase. The pattern of serum oestradiol and progesterone changes during the luteal phase did not correlate with the increases in LH and FSH 30 min after hCG both in the spontaneous and the FSH cycles. These results suggest that GnSAF bioactivity is high during the luteal phase of superovulated cycles.

Introduction

In superovulated women, the endogenous luteinizing hormone (LH) surge is markedly attenuated (Messinis *et al.*, 1985). The attenuation has been related to a putative nonsteroidal ovarian factor called gonadotrophin surge-attenuating factor (GnSAF) (Messinis and Templeton, 1989). The effect of GnSAF is exerted at least at the pituitary level where it reduces luteinizing-hormone-releasing hormone (LHRH)-stimulated gonadotrophin secretion (Messinis and Templeton, 1990a). Activity of GnSAF has been detected during the follicular phase of superovulated cycles (Messinis and Templeton, 1990b). In that respect, the role of GnSAF is not limited to the time of the midcycle LH surge, but is also extended at least to the first half of the cycle when it controls LHRH-induced gonadotrophin release by a negative effect (Messinis and Templeton, 1991a). Several studies using various *in vitro* bioassay systems have shown activity of GnSAF, distinct from inhibin, in steroid-free human follicular fluid from superovulated and spontaneous cycles (Busbridge *et al.*, 1990; Fowler *et al.*, 1990a; Knight *et al.*, 1990).

During the luteal phase of superovulated cycles basal gonadotrophin secretion is markedly reduced (Messinis and Templeton, 1987). In one study, the midluteal pituitary response to a pharmacological dose of LHRH was also decreased (Martikainen *et al.*, 1987). However, it is unclear whether GnSAF is produced

during the luteal phase of superovulated cycles. Previous studies using submaximal doses of LHRH have investigated pituitary sensitivity (first pool) and reserve (second pool) throughout the human menstrual cycle (Wang *et al.*, 1976; Yen and Lein, 1976). In superovulated cycles, the two pools of gonadotrophin secretion are markedly attenuated by GnSAF and this can be used as an *in vivo* bioassay to assess the activity of GnSAF in superovulated women (Messinis and Templeton, 1991b; Messinis *et al.*, 1991).

In the present study a similar approach was used to investigate GnSAF bioactivity during the luteal phase of FSH superovulated cycles.

Materials and Methods

Patients

The study included six normally cycling volunteer women with longstanding unexplained infertility. Approval for the study was obtained from the local Ethical Committee and the patients gave written informed consent. Ovulation was confirmed in all women by serum progesterone measurements and ultrasound scans of the ovaries before admission to the study. The patients were investigated during two cycles – a spontaneous and a follicle-stimulating hormone (FSH)-treated cycle. FSH (75 iu FSH per ampoule; Metrodin; Serono Laboratories, Welwyn Garden City, Herts) was injected intramuscularly once

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a day at the dose of 225 iu on cycle days 2, 3 and 4, and 150 iu thereafter, until the administration of human chorionic gonadotrophin (hCG). The latter was given as a single intramuscular injection of 5000 iu when the largest follicle exceeded 18 mm (19–20 mm) by ultrasound. In each cycle the pituitary response to an acute intravenous injection of 10 µg LHRH was investigated four times – in late follicular phase (largest follicle 16 mm by ultrasound), early luteal phase (day 5 after hCG), midluteal phase (day 9 after hCG) and late luteal phase (days 12 and 13 after hCG, respectively, in the FSH and the spontaneous cycles).

Blood samples in relation to each LHRH injection (time 0) were taken at –15, 0 and 30 min. The point of 30 min after LHRH (pituitary sensitivity) was chosen, as in a recent study (Messinis *et al.*, 1991), because at that time the LH response to a single submaximal dose of LHRH is maximal both during spontaneous and FSH-treated cycles (Wang *et al.*, 1976; Messinis and Templeton, 1991b). LH and FSH were measured in all blood samples taken during the LHRH experiments. Basal concentrations of oestradiol and progesterone (time 0) were also measured in both cycles.

Hormone assays

FSH and LH were measured in blood using immunoradiometric assays incorporating two high-affinity monoclonal antibodies. Kits were purchased from Serono Diagnostics Ltd, Woking (FSH MAIA clone and LH MAIA clone, respectively). The results are expressed as iu l⁻¹ of standards calibrated against the WHO 2nd IRP for human FSH (78/549) and the WHO 1st IRP for human LH (68/40). For oestradiol measurement in serum a solid-phase radioimmunoassay was used. Kits (Coat-A-Count Estradiol) were purchased from DPC (Diagnostics Products Corporation, Los Angeles). Values are expressed as pmol l⁻¹. Progesterone was measured in serum using the Amerlex-M Progesterone RIA kit (Amersham International plc, Amersham) and results are expressed as nmol l⁻¹. The lower limits of detection for FSH, LH, oestradiol and progesterone were 0.5 iu l⁻¹, 0.3 iu l⁻¹, 30 pmol l⁻¹ and 0.25 nmol l⁻¹, respectively, while interassay and intra-assay coefficients of variation were 7.9 and 7.8%, 6.3 and 4.4%, 6.7 and 4.2% and 7.0 and 6.4%, respectively.

Statistical analysis

Statistical analysis of the results was performed using one-way analysis of variance. Variations in gonadotrophin response to LHRH were assessed from the spontaneous to the FSH cycles by calculating the variance ratio (F). Within the same group of cycles, differences in the response to LHRH pulses were assessed using Student's paired *t* test. Although the arithmetic means of the hormone values are presented, in the statistical calculations hormone results were transformed into logarithms.

Results

In each LHRH experiment, basal LH and FSH values were calculated as the mean of the values at –15 and 0 min. The response to LHRH was calculated as the net increase at 30 min in LH

Fig. 1. Serum concentrations of (a) LH and (b) FSH in early, mid- and late luteal phases during (○) spontaneous and (●) FSH superovulated cycles in six normally ovulating women. Values are means ± SEM for six women except for the late luteal phase in the FSH cycles for which *n* = 4. ****P* < 0.05, ***P* < 0.01, **P* < 0.001 compared with corresponding spontaneous cycles.

(Δ LH30) and FSH (Δ FSH30) above the basal value (zero level). Basal LH and FSH values (mean ± SEM) (Fig. 1) in the spontaneous cycles decreased significantly from early (8.2 ± 0.7 and 6.1 ± 0.3 iu l⁻¹, respectively) to midluteal phase (5.0 ± 0.6 and 3.7 ± 0.3 iu l⁻¹, respectively, *P* < 0.01), with no significant change in late luteal phase. In the FSH cycles, basal LH values were significantly lower than in the spontaneous cycles in early (1.0 ± 0.2 iu l⁻¹, *P* < 0.001) and midluteal phase (2.6 ± 0.6 iu l⁻¹, *P* < 0.05), with no significant difference in late luteal phase (2.5 ± 0.4 iu l⁻¹). A significant increase in basal LH values from early to midluteal phase was seen in the FSH cycles (*P* < 0.05). In contrast to LH values, basal FSH values in the FSH cycles decreased significantly from early (2.1 ± 0.4 iu l⁻¹) to midluteal phase (0.8 ± 0.1 iu l⁻¹, *P* < 0.01) with a trend to increase in late luteal phase (1.2 ± 0.2 iu l⁻¹, *P* = 0.06). When, however, only paired samples were compared (*n* = 4), this increase was significant (0.6 ± 0.1 versus 1.2 ± 0.2 iu l⁻¹, *P* < 0.05). FSH values were significantly lower in the FSH than in the spontaneous cycles throughout the luteal phase (Fig. 1).

In the late follicular phase (Fig. 2), basal values of LH (mean ± SEM) in the FSH cycles (2.6 ± 0.5 iu l⁻¹) were significantly lower than in the spontaneous cycles (5.1 ± 0.4 iu l⁻¹,

Fig. 2. (a) Basal LH and FSH concentrations and (b) increases in LH and FSH concentrations (ΔLH and ΔFSH) response (30 min) to an acute intravenous injection (10 μg) of LHRH in late follicular phase of (■) spontaneous and (□) FSH-treated cycles in six normally ovulating women. Values are means $\pm$ SEM and represent net increase above the basal value. *** $P < 0.05$, ** $P < 0.01$, * $P < 0.001$ compared with corresponding spontaneous cycles.

$P < 0.05$), while basal FSH values in the FSH cycles increased significantly owing to the exogenous administration of FSH. ΔLH30 in late follicular phase decreased significantly in the FSH-treated ($5.6 \pm 1.3 \text{ iu l}^{-1}$) compared with the spontaneous cycles ($17.1 \pm 3.6 \text{ iu l}^{-1}$, $P < 0.05$). ΔFSH30 was also significantly reduced in the FSH cycles (Fig. 2); however, this must be interpreted with caution because of the exogenous administration of FSH. The size of the leading follicle (mean $\pm$ SEM) on the day of the LHRH experiment in late follicular phase was similar in the spontaneous ($16.5 \pm 0.2 \text{ mm}$) and FSH cycles ($16.7 \pm 0.3 \text{ mm}$), while serum oestradiol concentrations were significantly higher in the FSH cycles owing to multiple follicular development (1540 ± 353 versus $526 \pm 34 \text{ pmol l}^{-1}$, $P < 0.01$) (mean $\pm$ SEM number of follicles $\geq 12 \text{ mm}$ 3.8 ± 0.6).

ΔLH30 and ΔFSH30 decreased significantly from early (28.4 ± 3.2 and $2.9 \pm 0.4 \text{ iu l}^{-1}$, respectively) to mid- (21.0 ± 2.2 and $2.0 \pm 0.2 \text{ iu l}^{-1}$, respectively, $P < 0.05$) and late luteal phase of the spontaneous cycles ($16.0 \pm 1.5 \text{ iu l}^{-1}$, $P < 0.01$ and $1.4 \pm 0.1 \text{ iu l}^{-1}$, $P < 0.05$, respectively) (Fig. 3). The decrease from mid- to late luteal phase was also significant for both ΔLH30 and ΔFSH30 ($P < 0.05$). In the FSH cycles, ΔLH30 and ΔFSH30 were significantly attenuated compared with the spontaneous cycles in early (6.2 ± 1.1 and $0.5 \pm 0.1 \text{ iu l}^{-1}$, respectively, $P < 0.001$), mid- ($6.3 \pm 1.3 \text{ iu l}^{-1}$, $P < 0.01$ and $0.5 \pm 0.1 \text{ iu l}^{-1}$, $P < 0.001$, respectively) and late luteal ($6.0 \pm 0.7 \text{ iu l}^{-1}$,

Fig. 3. Increases in (a) LH and (b) FSH concentrations (ΔLH and ΔFSH) response (30 min) to an acute intravenous injection (10 μg) luteinizing-hormone-releasing hormone (LHRH) in relation to serum basal (c) oestrogen and (d) progesterone values in early, mid- and late luteal phases during (○) spontaneous and (●) FSH-treated cycles. Values are means $\pm$ SEM for six women except for the late luteal phases in the FSH cycles for which $n = 4$. *** $P < 0.05$, ** $P < 0.01$, * $P < 0.001$ compared with corresponding spontaneous cycles.

$P < 0.05$ and $0.4 \pm 0.1 \text{ iu l}^{-1}$, $P < 0.01$, respectively) phases. ΔLH30 and ΔFSH30 did not change significantly in the FSH cycles from early to late luteal phase. Serum oestradiol and progesterone concentrations in the spontaneous cycles increased significantly from early to midluteal phase ($P < 0.05$) and decreased from mid- to late luteal phase ($P < 0.05$). These two steroids in the FSH cycles decreased significantly from early to mid- ($P < 0.05$ and $P < 0.001$, respectively) and mid- to late luteal phase ($P < 0.01$ and $P < 0.001$, respectively). In early luteal phase, serum oestradiol and progesterone concentrations were significantly higher in the FSH-treated ($1642 \pm 188 \text{ pmol l}^{-1}$ and $80.7 \pm 15.7 \text{ nmol l}^{-1}$, respectively) than in the spontaneous cycles ($444 \pm 55 \text{ pmol l}^{-1}$, $P < 0.001$ and

$23.8 \pm 2.3 \text{ nmol l}^{-1}$, $P < 0.01$, respectively). In mid- and late luteal phases, serum values of these two steroids did not differ significantly between the two cycles.

The duration of the luteal phase (mean $\pm$ SEM) was significantly shorter in the FSH (11.8 ± 0.5 days) than in the spontaneous cycles (13.5 ± 0.2 days, $P < 0.05$). In two women, the late luteal phase LHRH experiments was not performed owing to < 12 day duration of the luteal phase.

Discussion

The present study confirms previous suggestions that the response of the pituitary to LHRH in the late follicular phase of superovulated cycles is markedly attenuated, supporting the contention that GnSAF is produced at this stage of the cycle (Messinis and Templeton, 1989, 1990a, b). This study, however, further demonstrates that pituitary sensitivity to LHRH is also markedly reduced throughout the luteal phase of superovulated cycles. Although oestradiol itself or oestradiol in combination with progesterone can affect pituitary sensitivity and reserve in women (Yen *et al.*, 1974; Lasley *et al.*, 1975; Chang and Jaffe, 1978), in the present study the attenuation of LHRH-induced gonadotrophin release does not seem to be related to such a steroidal effect for the following reasons. First, the higher concentrations of oestradiol and progesterone in the early luteal phase of the FSH-treated compared with the spontaneous cycles could maintain the pituitary in a state of low responsiveness to LHRH (Nippoldt *et al.*, 1989). However, in the present study concentrations of these steroids in mid- and late luteal phase did not differ significantly between the two cycles, although at the same time pituitary sensitivity to LHRH was still markedly reduced. Second, the pattern of pituitary response to LHRH during the luteal phase of the FSH cycles did not correlate with that of steroidal changes, i.e. unchanged response from early to late luteal phase versus gradually decreasing steroidal concentrations. It, therefore, seems unlikely that the decreased pituitary sensitivity to LHRH in the FSH cycles is related to a steroidal effect during the luteal phase and it is probable that the attenuation of LHRH-induced gonadotrophin release represents increased GnSAF bioactivity.

It is difficult to assess whether inhibin produced during the luteal phase contributed to this attenuation. This nonsteroidal substance, which by definition causes the suppression of FSH secretion, under specific conditions also reduces the secretion of LH. However, animal studies *in vitro* and *in vivo* have shown that the secretion of LH, either basal or stimulated by LHRH, is less sensitive to the suppressing effect of inhibin than is the secretion of FSH (Farnworth *et al.*, 1988; Knight *et al.*, 1991). In addition, higher concentrations of inhibin are required for the suppression of LHRH-stimulated than for basal FSH release in rat pituitary cultures (Farnworth *et al.*, 1988). The extent to which these data apply to humans is not known. Nevertheless, in the present study, basal concentrations of FSH in the FSH cycles increased in all women from mid- to late luteal phase (Fig. 1), while at the same time LHRH-induced gonadotrophin release remained markedly attenuated. These findings taken together with the above observations in animals suggest that inhibin is not the principal regulator of this attenuation during the luteal phase. In addition, several studies have shown that

GnSAF contained in steroid-free human follicular fluid is different from inhibin (Busbridge *et al.*, 1990; Fowler *et al.*, 1990a; Knight *et al.*, 1990) and has a molecular mass in the range of 10–30 kDa (Fowler *et al.*, 1992). It is possible, therefore, that the attenuation of LHRH-induced gonadotrophin release during the luteal phase of superovulated cycles is the result of the synergistic effect of GnSAF and inhibin. Furthermore, hCG is probably excluded as a potential inhibitor of gonadotrophin secretion in the present study, since this hormone was administered to all women both in the spontaneous and the FSH-treated cycles.

Different patterns of pituitary response to LHRH and steroidal concentrations were also seen during the luteal phase of the spontaneous cycles. In these cycles, pituitary sensitivity to LHRH decreased significantly from early to late luteal phase and this has previously been reported by others (Wang *et al.*, 1976). Progesterone is known to augment oestradiol-stimulated pituitary sensitivity to LHRH shortly after its administration to women pretreated with oestradiol (Lasley *et al.*, 1975; Chang and Jaffe, 1978), and this can explain the enhanced gonadotrophin secretion in response to LHRH during the midcycle LH surge (Wang *et al.*, 1976). Although the combined effect of oestradiol and progesterone on gonadotrophin secretion becomes suppressive when applied for several days (Soules *et al.*, 1984; Nippoldt *et al.*, 1989), it is difficult to explain how both the increasing concentrations of these steroids during the first half and the decreasing concentrations during the second half of the luteal phase could have similar suppressing effects on the pituitary response to LHRH. It would be expected that pituitary sensitivity would either increase or, as with basal gonadotrophin concentrations, at least remain unchanged towards the end of the cycle when concentrations of these two steroids decline. It is likely, therefore, that apart from steroids, GnSAF is another regulator of LHRH-induced gonadotrophin release during the luteal phase of spontaneous cycles, but this requires further investigation. A previous study has provided preliminary evidence that GnSAF may be produced during the early stages of the human menstrual cycle (Messinis *et al.*, 1991).

The source of GnSAF production during the luteal phase of superovulated cycles is not known. It is possible that, in a similar way to inhibin (McLachlan *et al.*, 1987a, b; Tsonis *et al.*, 1987), GnSAF is produced by the corpus luteum. However, another possibility is that some of the smaller follicles that do not have the capacity to respond to hCG and luteinize continue producing GnSAF during the early luteal phase. A previous study *in vitro* has shown higher activity of GnSAF in the follicular fluid of smaller compared with larger follicles (Fowler *et al.*, 1990b). However, more studies are required to clarify the source of GnSAF production during the luteal phase of the cycle.

Furthermore, the present study confirms previous data that basal LH secretion is suppressed during the luteal phase of FSH superovulated cycles and demonstrates that endogenous FSH is also decreased. However, recovery of basal LH secretion occurred earlier than recovery of FSH secretion (Fig. 1) and this supports a differential control of the two gonadotrophins by the ovaries during the luteal phase. Previous studies have shown that basal LH secretion during the follicular phase is mainly controlled by steroidal factors, while both steroidal and nonsteroidal substances are important for the control of FSH secretion (Messinis and Templeton, 1988, 1989).

In conclusion, the present study demonstrated that LHRH-stimulated gonadotrophin release during the luteal phase of FSH superovulated cycles is markedly reduced. It is suggested that GnSAF is produced both during the follicular and the luteal phase of the human menstrual cycle.

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References

- Busbridge NJ, Chamberlain GVP, Griffiths A and Whitehead SA (1990) Non-steroidal follicular factors attenuate the self-priming action of gonadotrophin-releasing hormone on the pituitary gonadotroph *Neuroendocrinology* **51** 493–499
- Chang RJ and Jaffe RB (1978) Progesterone effects on gonadotropin release in women pretreated with estradiol *Journal of Clinical Endocrinology and Metabolism* **47** 119–125
- Farnworth PG, Robertson DM, de Kretser DM and Burger HG (1988) Effects of 31 kDa bovine inhibin on FSH and LH in rat pituitary cells in vitro: antagonism of gonadotrophin-releasing hormone agonists *Journal of Endocrinology* **119** 233–241
- Fowler PA, Messinis IE and Templeton AA (1990a) Inhibition of LHRH-induced LH and FSH release by gonadotrophin surge-attenuating factor (GnSAF) from human follicular fluid *Journal of Reproduction and Fertility* **90** 587–594
- Fowler PA, Messinis IE and Templeton AA (1990b) Gonadotrophin surge-attenuating factor (GnSAF) is produced preferentially by small follicles in superovulated women *Journal of Reproduction and Fertility Abstract Series* **5** Abstract 6
- Fowler PA, Townsend C, Messinis IE, Cunningham P and Templeton A Gonadotrophin surge attenuating factor attenuates in-vitro LH secretion induced by gonadotrophin-releasing hormone from cultured ovine pituitary cells only during the breeding season *Journal of Endocrinology* **135** 221–227
- Knight PG, Lacey M, Peter JLT and Whitehead SA (1990) Demonstration of a nonsteroidal factor in human follicular fluid that attenuates the self-priming action of gonadotrophin-releasing hormone on pituitary gonadotropes *Biology of Reproduction* **42** 613–618
- Knight PG, Wrathall JHM, Glencross RG and McLeod BJ (1991) Effects of bovine follicular fluid on the secretion of LH and FSH in inhibin-immunized seasonally anoestrous ewes *Journal of Endocrinology* **128** 403–410
- Lasley BL, Wang CF and Yen SSC (1975) The effects of estrogen and progesterone on the functional capacity of the gonadotrophs *Journal of Clinical Endocrinology and Metabolism* **41** 820–826
- McLachlan RI, Robertson DM, Healy DL, Burger HG and de Kretser DM (1987a) Circulating immunoreactive inhibin levels during the normal human menstrual cycle *Journal of Clinical Endocrinology and Metabolism* **65** 954–961
- McLachlan RI, Healy DL, Robertson DM, Burger HG and de Kretser DM (1987b) Circulating immunoreactive inhibin in the luteal phase and early gestation of women undergoing ovulation induction *Fertility and Sterility* **48** 1001–1005
- Martikainen H, Ronnberg L, Ruokonen A and Kauppila A (1987) Anterior pituitary dysfunction during the luteal phase following ovarian hyperstimulation *Fertility and Sterility* **47** 446–449
- Messinis IE and Templeton AA (1987) Disparate effects of endogenous and exogenous oestradiol on luteal phase function in women *Journal of Reproduction and Fertility* **79** 549–554
- Messinis IE and Templeton AA (1988) Blockage of the positive feedback effect of oestradiol during prolonged administration of clomiphene citrate to normal women *Clinical Endocrinology* **29** 509–516
- Messinis IE and Templeton AA (1989) Pituitary response to exogenous LHRH in superovulated women *Journal of Reproduction and Fertility* **87** 633–639
- Messinis IE and Templeton A (1990a) Superovulation induction in women suppresses luteinizing hormone secretion at the pituitary level *Clinical Endocrinology* **32** 107–114
- Messinis IE and Templeton A (1990b) In-vivo bioactivity of gonadotrophin surge attenuating factor (GnSAF) *Clinical Endocrinology* **33** 213–218
- Messinis IE and Templeton AA (1991a) Evidence that gonadotrophin surge-attenuating factor exists in man *Journal of Reproduction and Fertility* **92** 217–223
- Messinis IE and Templeton A (1991b) Attenuation of gonadotrophin release and reserve in superovulated women by gonadotrophin surge attenuating factor (GnSAF) *Clinical Endocrinology* **34** 259–263
- Messinis IE, Templeton A and Baird DT (1985) Endogenous luteinizing hormone surge during superovulation induction with sequential use of clomiphene and pulsatile human menopausal gonadotropin *Journal of Clinical Endocrinology and Metabolism* **61** 1076–1080
- Messinis IE, Hirsch P and Templeton AA (1991) Follicle stimulating hormone stimulates the production of gonadotrophin surge attenuating factor (GnSAF) in vivo *Clinical Endocrinology* **35** 403–407
- Nippoldt TB, Reame NE, Kelch RP and Marshall JC (1989) The roles of estradiol and progesterone in decreasing luteinizing hormone pulse frequency in the luteal phase of the menstrual cycle *Journal of Clinical Endocrinology and Metabolism* **69** 67–76
- Soules MR, Steiner RA, Clifton DK, Cohen NL, Aksel S and Bremner WJ (1984) Progesterone modulation of pulsatile luteinizing hormone secretion in normal women *Journal of Clinical Endocrinology and Metabolism* **58** 378–383
- Tsonis CG, Hillier SG and Baird DT (1987) Production of inhibin bioactivity by human granulosa-lutein cells: stimulation by LH and testosterone in vitro *Journal of Endocrinology* **112** R11–R14
- Wang CF, Lasley BL, Lein A and Yen SSC (1976) The functional changes of the pituitary gonadotrophs during the menstrual cycle *Journal of Clinical Endocrinology and Metabolism* **42** 718–728
- Yen SSC and Lein A (1976) The apparent paradox of the negative and positive feedback control system on gonadotropin secretion *American Journal of Obstetrics and Gynecology* **126** 942–954
- Yen SSC, Vandenberg G and Siler TM (1974) Modulation of pituitary responsiveness to LRF by estrogen *Journal of Clinical Endocrinology and Metabolism* **39** 170–177