

Evidence for Decreased Luteinizing Hormone-Releasing Hormone Pulse Frequency in Men with Selective Elevations of Follicle-Stimulating Hormone*

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ABSTRACT. To examine the hypothesis that the frequency of endogenous pulsatile LHRH stimulation controls the relative secretion of FSH and LH from the pituitary, we studied men with elevated FSH levels and normal LH levels to determine whether they have an altered frequency of pulsatile LHRH secretion compared to normal men. Because peripheral blood measurements of LHRH do not reflect the pulsatile characteristics of hypothalamic LHRH secretion, and it is generally accepted that the pulse frequency of LH secretion is an index of the frequency of endogenous LHRH pulsation, we used LH pulse frequency as the indicator of LHRH pulse frequency. Frequent blood sampling was performed to characterize LH pulse patterns in five men with selective elevations of FSH and seven age-matched normal men. Beginning at 0800–0930 h, blood samples were obtained every 10 min for 24 h through an indwelling iv catheter. Serum LH and FSH levels were measured by RIA in each sample, and the pattern of LH secretion was determined.

Testosterone (T), estradiol, sex hormone-binding globulin, and free T were measured in a pooled serum sample from each man. Men with selective elevations of FSH had fewer LH pulses per 24 h (mean \pm SEM, 10.6 ± 0.5) than the control group (12.9 ± 0.6 ; $P < 0.01$). There was no statistically significant difference in LH pulse amplitude (23 ± 4 vs. 17 ± 3 ng/ml). There were no statistically significant differences in T (4.9 ± 0.5 vs. 6.1 ± 0.5 ng/ml), estradiol (23 ± 7 vs. 31 ± 5 pg/ml), sex hormone-binding globulin (7.7 ± 1.4 vs. 7.7 ± 1.2 ng bound dihydrotestosterone/ml), or free T (0.16 ± 0.02 vs. 0.23 ± 0.04 ng/ml) in these men vs. normal subjects. We conclude that 1) compared to normal men, men with selectively elevated FSH levels have decreased LH pulse frequency, which suggests decreased LHRH pulse frequency; and 2) the relative secretion rates of LH and FSH by the pituitary may be regulated by the frequency of pulsatile LHRH secretion from the hypothalamus. (*J Clin Endocrinol Metab* 60: 197, 1985)

SEVERAL clinical and experimental states are characterized by a selective increase in FSH levels with normal or near-normal LH levels (1). This elevation of FSH occurs despite the fact that the only known gonadotropin-releasing hormone, LHRH, when administered as a single dose, stimulates LH secretion to a greater extent than FSH. This apparent paradox has led to the proposal of several mechanisms to explain selective FSH increases, such as increased pituitary stimulation by a

separate FSH-releasing factor (2), decreased gonadal production of inhibin (3), and decreased gonadal production of sex steroids (4).

An alternative mechanism for the selective control of FSH secretion was suggested by the studies of Wildt *et al.* (5). Using ovariectomized, hypothalamus-lesioned monkeys, these investigators demonstrated that the relative blood levels of LH and FSH could be controlled separately by the frequency of pulsatile exogenous LHRH administration. Slower pulse frequencies favored FSH secretion, while more rapid pulses favored LH secretion.

Based on these observations, we hypothesized that men with selective elevations of FSH have a slower than normal pulse frequency of LHRH, which might result in their high FSH levels. To test this hypothesis, we determined the LH pulse patterns in men with elevated FSH levels and normal LH levels compared to those in normal men. We assumed that endogenous LHRH pulses are reflected in LH pulses that are measurable in peripheral blood (6, 7). If men with selectively increased FSH levels

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had decreased LH pulse frequency compared to normal men, this would be evidence supporting the role of endogenous LHRH pulse frequency in the control of the relative secretion of LH and FSH by the pituitary gland.

Materials and Methods

Subjects

Men with selective FSH elevations. Five men, aged 32–44 yr, with selective elevations of serum FSH levels were recruited and volunteered to participate in this study. Three of these men were first seen during evaluation for infertility; two had azoospermia, and one had a mean sperm count of 5.5 million/ml (six measurements). The other two men were discovered while undergoing screening laboratory testing before enrollment as normal volunteers in a separate study. On six measurements, their mean sperm counts were 20.8 and 52 million/ml (8). All five men had single random serum FSH levels above 230 ng/ml (normal range in adult men, 30–230 ng/ml) and normal serum LH (11–80 ng/ml) and testosterone (T; 2.7–10 ng/ml) levels (Fig. 1). Twenty-four-hour mean FSH levels during the sampling studies described below ranged from 200–400 ng/ml, and LH levels were less than 60 ng/ml in these men. They were all otherwise healthy and were not receiving any medications. None of the men had received prior treatment for infertility.

Normal men. Seven normal men, aged 29–44 yr, were recruited by newspaper advertisement and volunteered to participate in this study. These men had normal single random serum FSH, LH, and T levels (Fig. 1). All had sperm counts above 20 million/ml (six measurements). Their mean 24-h FSH and LH levels (in sampling studies described below) were within the normal adult male range. These men also were healthy and were receiving no medications.

Protocol

Subjects were admitted to the Special Studies Unit of the Seattle V.A. Medical Center or to the Clinical Research Center at the University of Washington Hospital. Beginning at 0800–0930 h, blood samples were obtained every 10 min for 24 h through an indwelling catheter placed in an arm vein. The iv line was kept open with a continuous infusion of heparinized normal saline. Before taking each 3-ml blood sample, the iv line was cleared by removing the first 6 ml of blood via a three-way stopcock. After obtaining each blood sample, the 6 ml of blood originally removed were reinfused, and the line was flushed with heparinized saline. Samples were allowed to clot at room temperature, and the blood was centrifuged. The serum was separated, frozen, and stored at -20°C until assayed.

Serum LH and FSH levels were measured in duplicate by RIA in all samples; all samples from an individual man were analyzed at the same time in each assay. Serum T, estradiol (E_2), sex hormone-binding globulin (SHBG), and free T were determined in a pool composed of equal volumes of serum taken from the first five samples from each subject.

The study protocol was reviewed and approved by the Human Subjects Review Committee of the University of Washington. Informed consent was obtained from each subject before their participation in the study.

Hormone assays

LH and FSH RIA. The RIA for LH (9, 10) used a reference standard (LER 907) and first antibody (antihuman LH batch 2) supplied by the National Pituitary Agency. The tracer was purified hCG (supplied courtesy of Dr. C. Alvin Paulsen) radioiodinated with ^{125}I using chloramine-T (11). The limit of detectability of this assay was 6 ng/ml, and the intra- and interassay coefficients of variation were 5.5% and 8.4%, respectively.

The RIA for serum FSH (9, 10) used reagents distributed by the National Pituitary Agency. The reference standard was LER 907; the first antibody was antihuman FSH (batch 5), and tracer was HS-1, radioiodinated with ^{125}I using chloramine-T (11). The limit of detectability of FSH in this assay was 25 ng/ml. The intraassay variability was 7.3%, and the interassay variability was 9.7%. Assay results for both LH and FSH RIAs were calculated using the computer program of Burger *et al.* (12).

T and E_2 RIA. The RIA for serum T and E_2 used reagents supplied by the WHO Matched Reagent Programme (13). The antisera were raised in rabbits against BSA conjugates of T and E_2 . Anti-T antiserum exhibited cross-reactivity of 14% with 5α -dihydrotestosterone (DHT), 6% with 5α -androstane-diol, and less than 2% with the other steroids tested. Anti- E_2 antiserum exhibited 17% cross-reactivity with estrone. The T assay was preceded by ether extraction, and the E_2 assay was preceded by ether extraction and Celite chromatography using 40% ethyl acetate eluant. In both assays, separation of bound from free hormone was accomplished using dextran-coated charcoal. The assay sensitivity was 0.1 ng/ml for T and 12 pg/ml for E_2 . The intra- and interassay variabilities were 5.1% and 9.8%, respectively, for T and 8.2% and 8.8%, respec-

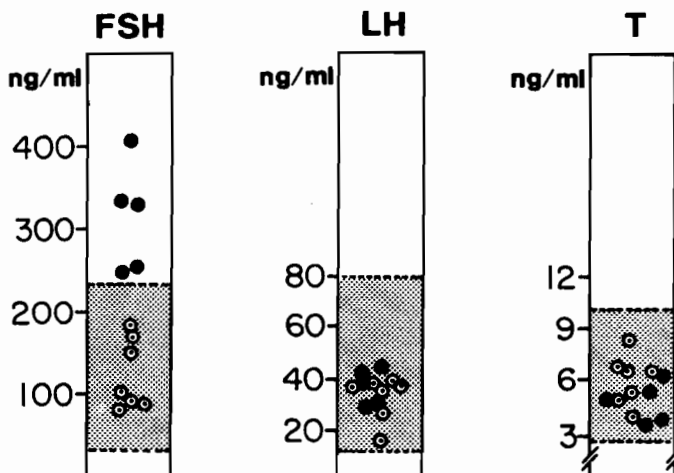


FIG. 1. FSH, LH, and T levels in normal men (○) and men with selective elevations of FSH (●) at entry to the study. Values were obtained from single random blood samples. The normal adult male range for each hormone is within the shaded areas. Note the similarity of LH and T levels and the distinct difference in FSH levels.

tively, for E_2 .

SHBG and free T determination. SHBG determinations were performed by Dr. Stephen R. Plymate, Madigan Army Medical Center (Tacoma, WA), using a modification (14) of procedures used to measure androgen-binding protein described by Musto and Bardin (15). Serum samples were incubated with charcoal for 16 h at 4 C before DHT saturation analysis so as to remove circulating steroids. Free T levels were calculated from the formula of Pearlman (16) using the equilibrium association constant of T for SHBG reported by Moll *et al.* (17).

LH pulse analyses. LH pulses were analyzed using a computerized method in which duplicate determinations of LH on sequential blood samples collected from each subject were first assessed by analysis of variance. This procedure was used to determine the relative contribution of measurement variability (as indicated by the coefficient of variation of assay duplicates) to the overall pattern of hormone fluctuations in the experimental data. Before pulse analysis, a moving average of each two successive data points was performed to smooth the baseline. A pulse was defined as an increment in hormone level from nadir to peak greater than 4 times the coefficient of variation of assay duplicates. The number of LH pulses per 24 h and pulse amplitudes (increment in hormone level from nadir to peak per pulse) then were determined by the computer.

Statistical analysis. Mean gonadotropin levels and mean LH pulse amplitude were determined for the 24 h of sampling for each subject. Student's unpaired *t* test was used to compare the mean hormone levels and LH pulse parameters of the group of men with selective elevation in FSH with those of the normal men.

Results

LH and FSH levels

Mean gonadotropin values in the 24-h sampling studies are shown in Table 1. In men with selective elevations of FSH, the mean 24-h LH level was 40 ± 4 (\pm SEM) ng/ml compared to 31 ± 2 ng/ml in the normal men ($P = \text{NS}$). The 24-h mean FSH level in the group with selective elevations of FSH was 289 ± 35 ng/ml compared to 112 ± 15 ng/ml in the normal group ($P < 0.001$). The ratio of FSH to LH levels was significantly increased in

TABLE 1. Serum FSH and LH levels and LH pulse parameters

	FSH (ng/ml)	LH (ng/ml)	LH pulse frequency (no./24 h)	LH pulse amplitude (ng/ml)
Elevated FSH (n = 5)	289 ± 35^a	40 ± 4	10.6 ± 0.5^b	23 ± 4
Normal men (n = 7)	112 ± 15	31 ± 2	12.9 ± 0.6	17 ± 3

All values are the mean \pm SEM. Values were obtained from sampling performed every 10 min for 24 h.

^a $P < 0.001$ compared to normal men.

^b $P < 0.01$ compared to normal men.

men with selective FSH elevations (7.4 ± 0.9) compared to that in the normal men (3.9 ± 0.7 ; $P < 0.01$).

LH pulse patterns

The 24-h LH secretory pattern with designation of the pulses found by our method is shown for each of the subjects with elevated FSH in Fig. 2 and for each of the normal men in Fig. 3. As can be seen, the men with selective elevations of FSH as a group had fewer LH pulses than the normal men.

LH pulse parameters for the groups are shown in Table 1. In men with selective elevations of FSH, the mean LH pulse frequency was 10.6 ± 0.5 pulses/24 h compared to 12.9 ± 0.6 pulses/24 h in the normal men ($P < 0.01$). The mean LH pulse amplitude of men with selective elevations of FSH was 23 ± 4 ng/ml compared to 17 ± 3 ng/ml in normal men ($P = \text{NS}$).

T and E_2 values

Mean T and E_2 values are shown in Table 2. In the group with selective elevations of FSH, serum T levels were 4.9 ± 0.5 ng/ml compared to 6.1 ± 0.5 ng/ml ($P =$

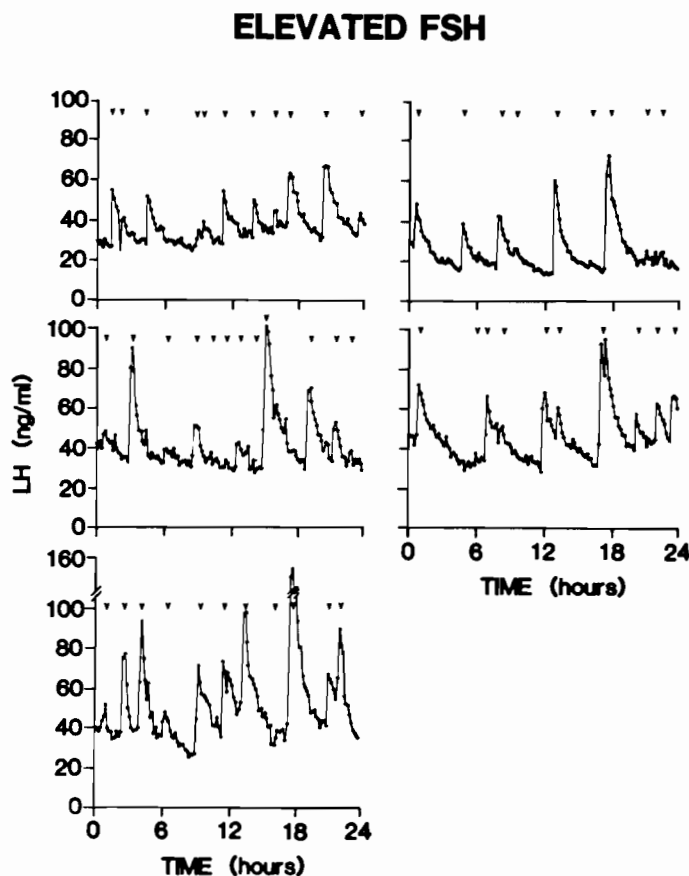


FIG. 2. Twenty-four-hour LH pulse patterns of men studied with selectively increased FSH levels. Arrows denote pulses, as detected by the computer program (see *Materials and Methods*).

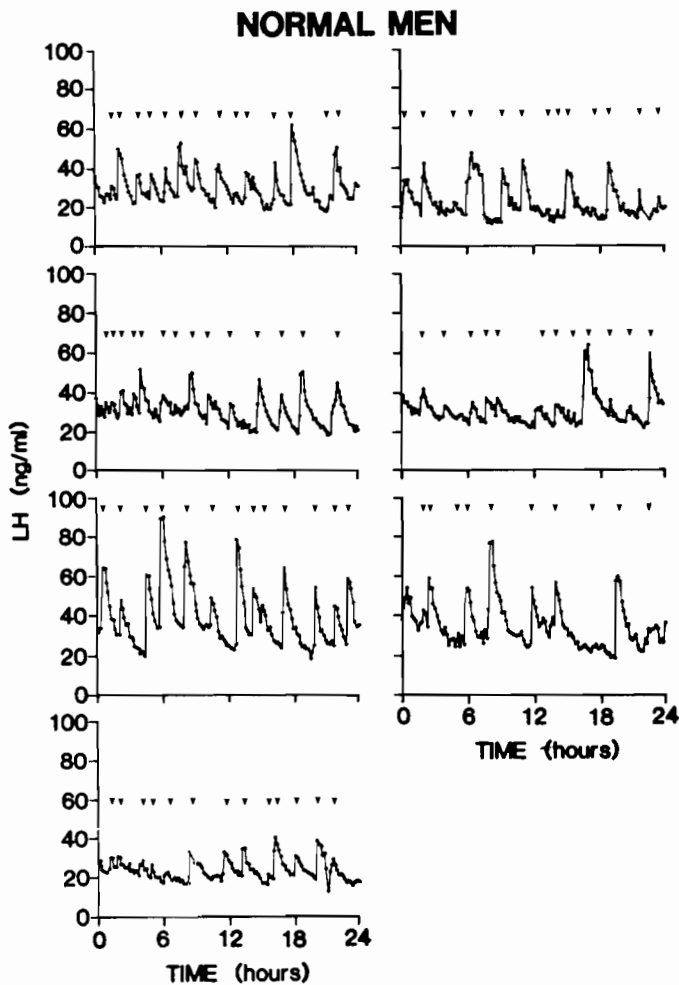


FIG. 3. Twenty-four-hour LH pulse patterns of normal men studied. Arrows denote pulses, as detected by the computer program (see Materials and Methods).

TABLE 2. Serum T, E₂, SHBG, and free T levels

	T (ng/ml)	E ₂ (pg/ml)	SHBG (ng bound DHT/ml)	Free T (ng/ml)
Elevated FSH (n = 5)	4.9 ± 0.5	23 ± 7	7.7 ± 1.4	0.16 ± 0.02
Normal men (n = 7)	6.1 ± 0.5	31 ± 5	7.7 ± 1.2	0.23 ± 0.04

All values are the mean ± SEM. Values were obtained from pooled samples at the beginning of frequent sampling.

NS) in the normal group. E₂ values were 23 ± 7 pg/ml in the men with selective elevations of FSH compared to 31 ± 5 ng/ml in the normal group (P = NS).

SHBG and free T

Mean SHBG and free T values are shown in Table 2. SHBG levels were 7.7 ± 1.4 ng bound DHT/ml in the group with selective elevations of FSH compared to 7.7

± 1.2 ng bound DHT/ml (P = NS) in the normal group. The free T level was 0.16 ± 0.02 ng/ml in the men with selective FSH elevations compared to 0.23 ± 0.04 ng/ml in the normal group (P = NS).

Discussion

These findings demonstrate that men with selective elevations of FSH have a decreased LH pulse frequency compared to normal men. Based on previous observations that LH pulsatility is a reflection of LHRH secretory activity (6, 7), our results imply that men with selective elevations of FSH have decreased hypothalamic LHRH pulse frequency. These results give support to the concept of endogenous LHRH pulse frequency having a role in modulating the relative rates of LH and FSH secretion from the anterior pituitary gland.

That the frequency of pulsatile LHRH stimulation can selectively regulate LH and FSH secretion was first demonstrated by Wildt *et al.* (5). Using ovariectomized rhesus monkeys bearing radiofrequency-induced lesions of the LHRH-producing arcuate nucleus, they found that by varying the frequency of pulsatile LHRH administration, they could affect the relative amounts of FSH and LH produced by the pituitary gland, with slower frequencies favoring FSH secretion. We recently extended this finding to men by varying the frequency of pulsatile LHRH administration to men with endogenous LHRH deficiency and demonstrating that mean FSH levels rise but mean LH levels remain constant as the frequency of LHRH administration decreases (18).

How alterations in the frequency of LHRH selectively modulate FSH and LH secretion is a question of considerable interest. Although no cellular mechanism has been established to account for this process, the longer half-time of disappearance of FSH from blood (4–6 h) compared to that of LH (30–60 min) (19, 20) as well as differences between FSH and LH in their responsiveness to LHRH stimulation probably are involved. Lincoln (21) demonstrated in sexually quiescent rams receiving LHRH that FSH levels remained elevated for at least 24 h after stopping LHRH treatment, whereas LH levels declined to low baseline values within 4 h. The persistent elevation of FSH could not be accounted for by half-life considerations alone, and the conclusion of the study was that once stimulated by LHRH, FSH synthesis and release proceed for a longer time than do LH synthesis and release. Wildt *et al.* (5) found that with a decrease in LHRH pulse frequency from once per hour to once per 3 h, the amount of FSH released from the pituitary gland with each LHRH stimulus increased 4-fold, while LH release increased only 2-fold per pulse. This finding is likely to be accounted for by relatively more FSH synthesis during the interstimulus period, resulting in an

increased readily releasable pool available for secretion when the next LHRH stimulus occurred. Thus, it seems that FSH and LH not only have different half-lives, but also that the cellular mechanisms involved in their synthesis and release have different response patterns to LHRH stimulation. The differences probably contribute to the ability of a decreased LHRH pulse frequency to generate a selective elevation of FSH.

The presumably decreased LHRH pulse frequency in our subjects may be either a primary or a secondary phenomenon. Primary disorders of hypothalamic LHRH pulse generation have been described. Patients with endogenous LHRH deficiency (*e.g.* Kallmann's syndrome) have absent or minimal LH pulsatility (22). Adolescents with the polycystic ovary syndrome have a primary abnormality in the circadian rhythm of LH pulsation (23). It is possible that our subjects have a primary abnormality in their LHRH pulse generation processes and are part of a spectrum of such disorders. If so, the spermatogenic defect in these men could be due to the alteration in LHRH production, mediated by an imbalance in circulating LH and FSH levels.

Alternatively, the decreased LHRH pulse frequency could be secondary to altered feedback mechanisms. Men with oligo- or azoospermia and selective elevations of FSH have either normal T levels (3, 24–26) or decreased T levels (27, 28), whereas E_2 levels are either normal (24, 25, 27) or elevated (29). Booth *et al.* (30) reported that men with selective elevations of FSH have a decreased production rate of T despite normal T levels. In the present study we found normal levels of T, free T, and E_2 , although there was a tendency for all to be reduced in the men with high FSH levels. If our men do have decreased Leydig cell function, altered feedback mechanisms could be acting on the hypothalamus or pituitary. The available evidence suggests, however, that loss of T feedback results in an increased LH pulse frequency (31), making it unlikely that T deficiency accounts for the decreased LHRH pulse frequency of our subjects.

Another gonadal product, inhibin, needs to be considered as an explanation for the slowed LHRH pulse frequency. However, LH pulse frequency has been found to be unaffected by peripheral infusions of inhibin-containing material, which caused selective decreases in FSH in monkeys (32) and rats (33). Such evidence suggests that inhibin has no effect on LH pulse frequency and that inhibin deficiency cannot explain the decreased LHRH pulse frequency of our subjects.

Although this study demonstrates an association between decreased LHRH pulse frequency and selectively elevated FSH levels, it was not designed to establish a cause and effect relationship between these two parameters. There is evidence that T, E_2 , and inhibin all can affect the pituitary gland to selectively inhibit FSH se-

cretion (34–38). Because we did find a trend toward lower steroid levels and we did not measure inhibin levels, we cannot rule out the possibility that deficiencies of these substances contributed to the selective elevations of FSH seen in our subjects.

In summary, we demonstrated that men with selective elevations of serum FSH levels have a decreased frequency of LH pulsation, which strongly suggests decreased LHRH pulse frequency. This finding is consistent with the hypothesis that the frequency of hypothalamic LHRH pulsation differentially modulates pituitary LH and FSH secretion. It will be possible to test this hypothesis by administering LHRH in a pulsatile fashion and determining gonadotropin levels at increased LHRH pulse frequencies. It is possible that the slowed LHRH pulse frequency of these subjects is a primary hypothalamic disorder and may cause their spermatogenic defect.

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References

1. Franchimont P, Bourguignon JP, Demoulin A, Turbridge M 1978 Mechanisms which control the specific secretion of FSH and LH. In: Fabbrini A, Steinberger E (eds) Recent Progress in Andrology. Academic Press, New York, p 21
2. Bowers CY, Currie BL, Johansson KNG, Folkers K 1973 Biologic evidence that separate hypothalamic hormones release the follicle stimulating and luteinizing hormones. *Biochem Biophys Res Comm* 50:20
3. Rosen SW, Weintraub BD 1971 Monotropic increase of serum FSH correlated with low sperm count in young men with idiopathic oligospermia and aspermia. *J Clin Endocrinol Metab* 32:410
4. Kulin HE, Reiter EO 1972 Gonadotropin suppression by low dose estrogen in men: evidence for differential effects upon FSH and LH. *J Clin Endocrinol Metab* 35:836
5. Wildt L, Häusler A, Marshall G, Hutchinson JA, Plant TM, Belchetz PE, Knobil E 1981 Frequency and amplitude of gonadotropin releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. *Endocrinology* 109:376
6. Clarke IJ, Cummins JT 1982 The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology* 111:1737
7. Levine JE, Pau K-YF, Ramirez VO, Jackson GL 1982 Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology* 111:1449
8. Karpas AE, Matsumoto AM, Paulsen CA, Bremner WJ 1983 Elevated serum follicle stimulating hormone levels in men with normal seminal fluid analyses. *Fertil Steril* 39:333
9. Bremner WJ, Matsumoto AM, Sussman A, Paulsen CA 1981

- Follicle stimulating hormone and human spermatogenesis. *J Clin Invest* 68:1044
10. Midgley AR 1967 Radioimmunoassay for human follicle-stimulating hormone. *J Clin Endocrinol Metab* 27:295
 11. Greenwood FC, Hunter WM, Glover JSC 1963 The preparation of ¹³¹I-labelled human GH of high specific radioactivity. *Biochem J* 89:114
 12. Burger HG, Lee VWK, Rennie GC 1972 A generalized computer program for the treatment of data from competitive protein-binding assays including radioimmunoassays. *J Lab Clin Med* 80:302
 13. Matsumoto AM, Paulsen CA, Hopper BR, Rebar RW, Bremner WJ 1983 Human chorionic gonadotropin and testicular function: stimulation of testosterone, testosterone precursors and sperm production despite high estradiol levels. *J Clin Endocrinol Metab* 56:720
 14. Plymate SR, Fariss BL, Bassett ML, Matej L 1981 Obesity and its role in polycystic ovary syndrome. *J Clin Endocrinol Metab* 52:1246
 15. Musto N, Bardin CW 1976 Decreased levels of androgen binding protein in the reproductive tract of the restricted (H^m) rat. *Steroids* 28:1
 16. Pearlman WH 1970 Measurement of testosterone binding sites. In: Diczfaulusy E (ed) *Karolinska Symposium on Research Methods in Reproductive Endocrinology, 2nd Symposium: Steroid Assay by Protein Binding*. Karolinska Institute, Stockholm, p 225
 17. Moll GW, Rosenfeld RC, Helke VH 1981 Estradiol-testosterone binding interactions and free plasma estradiol under physiological conditions. *J Clin Endocrinol Metab* 52:868
 18. Gross KM, Matsumoto AM, Southworth MB, Bremner WJ 1984 The pattern of luteinizing hormone releasing hormone (LHRH) administration controls the relative secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in man. *Clin Res* 32:74A (Abstract)
 19. Yen SCC, Llerena LA, Pearson OH, Littell AS 1970 Disappearance rates of endogenous follicle-stimulating hormone in serum following surgical hypophysectomy in man. *J Clin Endocrinol Metab* 30:325
 20. Pepperell R, deKretser DM, Burger HG 1975 Studies on the metabolic clearance rate and production rate of human luteinizing hormone and on the initial half time of its subunits in man. *J Clin Invest* 56:118
 21. Lincoln GA 1979 Differential control of luteinizing hormone and follicle stimulating hormone by luteinizing hormone releasing hormone in the ram. *J Endocrinol* 80:133
 22. Santen RJ, Bardin CW 1973 Episodic luteinizing hormone secretion in man. Pulse analysis, clinical interpretation, physiological mechanisms. *J Clin Invest* 52:2617
 23. Zumoff B, Freeman R, Coupey S, Saenger P, Markowitz M, Kream J 1983 A chronobiologic abnormality in luteinizing hormone secretion in teenage girls with the polycystic-ovary syndrome. *N Engl J Med* 309:1206
 24. Ruder HJ, Loriaux DL, Sherins RJ, Lipsett MB 1974 Leydig cell function in men with disorders of spermatogenesis. *J Clin Endocrinol Metab* 38:244
 25. Nankin HR, Castaneda E, Troen P 1977 Endocrine profiles in oligospermic men. In: Troen P, Nanken HR (eds) *The Testis in Normal and Infertile Men*. Raven Press, New York, p 529
 26. Wu FCW, Edmond P, Raab G, Hunter WM 1981 Endocrine assessment of the subfertile male. *Clin Endocrinol (Oxf)* 14:493
 27. Purvis K, Brenner PF, Landgren BM, Cekan Z, Diczfaulusy E 1975 Indices of gonadal function in the human male. I. Plasma levels of unconjugated steroids and gonadotrophins under normal and pathological conditions. *Clin Endocrinol (Oxf)* 4:237
 28. Foulter R, Mattei A, Duivier J, Franchimont P 1978 Measurement of gonadotrophins, testosterone, $\Delta 4$ androstenedione and dihydrotestosterone in idiopathic oligospermia. *Clin Endocrinol (Oxf)* 9:303
 29. Wu FCW, Swanston IA, Baird DT 1982 Raised plasma oestrogens in infertile men with elevated levels of FSH. *Clin Endocrinol (Oxf)* 16:39
 30. Booth JD, Sherins RJ, Loriaux DL, Low testosterone production in men with a selective increase in plasma FSH: an alternative to the inhibin hypothesis. 64th Annual Meeting of The Endocrine Society, San Francisco CA, 1982, p 949 (Abstract)
 31. Matsumoto AM, Bremner WJ 1984 Modulation of pulsatile gonadotropin secretion in man by testosterone. *J Clin Endocrinol Metab* 58:609
 32. Stillman RJ, Williams RF, Lynch A, Hodgen GD 1983 Selective inhibition of follicle-stimulating hormone by porcine follicular fluid extracts in the monkey: effects on midcycle surges and pulsatile secretion. *Fertil Steril* 40:823
 33. Lumpkin MD, DePaolo LV, Negro-Vilar A 1984 Pulsatile release of follicle-stimulating hormone in ovariectomized rats is inhibited by porcine follicular fluid (inhibin). *Endocrinology* 114:201
 34. Valk TW, Corley KP, Kelch RP, Marshall JC 1981 Pulsatile gonadotropin releasing hormone in gonadotropin deficient and normal men: suppression of follicle stimulating hormone responses by testosterone. *J Clin Endocrinol Metab* 53:184
 35. Marshall JC, Case GD, Valk TW, Corley KP, Sauder SE, Kelch RP 1983 Selective inhibition of follicle stimulating hormone secretion by estradiol. *J Clin Invest* 71:248
 36. Baker HWG, Bremner WJ, Burger HG, deKretser DM, Dulmanis A, Eddie LW, Hudson B, Keogh EJ, Lee VWK, Rennie GC 1976 Testicular control of follicle stimulating hormone secretion. *Recent Prog Horm Res* 32:429
 37. Steinberger A, Steinberger E 1976 Secretion of an FSH-inhibiting factor by cultured Sertoli cells. *Endocrinology* 99:918
 38. deJong FM, Sharpe RM 1976 Evidence for inhibin like activity in bovine follicular fluid. *Nature* 263:71