

## SERUM INHIBIN LEVELS DURING THE PERIOVULATORY INTERVAL IN NORMAL WOMEN: RELATIONSHIPS WITH SEX STEROID AND GONADOTROPHIN LEVELS

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*(Received 20 March 1989; returned for revision 12 May 1989; finally revised 19 June 1989;  
accepted 4 July 1989)*

### SUMMARY

Inhibin is a gonadal glycoprotein believed to be important in the regulation of pituitary FSH secretion and/or to function as a paracrine factor within the ovary and testis. We studied serum levels of inhibin, oestradiol ( $E_2$ ), progesterone (P), FSH and LH during the periovulatory interval in order to determine whether there is differential control of sex steroid and inhibin secretion by the mature follicle and the emerging corpus luteum. Seven normal cyclic women were admitted 3–4 days prior to midcycle and blood samples drawn every 3 h for 5–7 days. Serum  $E_2$ , P, FSH, LH and inhibin were measured by radioimmunoassay. Data were normalized around the peak LH value (0 h). Serum  $E_2$  and inhibin rose in parallel ( $r=0.92$ ,  $P<0.001$ ) between –69 and –18 h,  $E_2$  reached a peak of  $1296 \pm 154$  (mean  $\pm$  SEM) pmol/l at –18 h, then fell to  $1050 \pm 139$  pmol/l at 0 h. Serum inhibin, on the other hand, continued to rise to a peak of  $837 \pm 95$  U/l at –6 h, fell to  $455 \pm 48$  U/l at +45 h, then rose again. On average, the peak inhibin level occurred  $10.4 \pm 5.1$  h after the peak  $E_2$  ( $P<0.05$ ). Inhibin levels were positively correlated with both serum LH and FSH between –24 and +24 h ( $P<0.01$ ). Serum  $E_2$  was negatively correlated with LH, FSH and inhibin between –24 and 0 h ( $P<0.01$ ). Serum P levels increased from  $1.8 \pm 0.3$  nmol/l at –24 h to  $14.3 \pm 1.0$  nmol/l at +60 h. Serum inhibin was positively correlated with serum P from –24 to 0 h ( $P<0.01$ ) and +45 to +60 h ( $P<0.01$ ), but was inversely correlated from 0 to +45 h ( $P<0.01$ ). We conclude that the maturing follicle secretes both  $E_2$  and inhibin in parallel until –18 h, at which time the process of luteinization is initiated by the onset of the midcycle LH surge, as evidenced by the rise in P.  $E_2$  secretion then falls while inhibin secretion rises, indicating different regulation of secretion of these two hormones by the maturing follicle. Furthermore, the close positive correlation

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between inhibin and gonadotrophin levels around midcycle suggests that FSH and/or LH stimulate inhibin secretion and that the presumed negative feedback effect of inhibin on FSH secretion is overcome at this time. After midcycle, inhibin secretion initially falls, then rises, while P rises progressively. This transient divergence of P and inhibin secretion may occur during the transformation of the preovulatory follicle into the corpus luteum.

The glycoprotein hormone, inhibin, is a disulphide-linked heterodimer, consisting of two subunits termed  $\alpha$  and  $\beta$ , which is believed to have an important role in the physiological regulation of pituitary FSH secretion (Franchimont *et al.*, 1979; McLachlan *et al.*, 1988; Ying, 1988) and/or to function as a paracrine modulator of ovarian function (Ying *et al.*, 1986; Hutchinson *et al.*, 1987). Serum inhibin levels during the normal human menstrual cycle show a late follicular phase rise, in parallel with oestradiol ( $E_2$ ) (McLachlan *et al.*, 1987a). This is consistent with in-vitro studies showing that the granulosa cell secretes inhibin under the influence of FSH (Channing *et al.*, 1984; Bicsak *et al.*, 1986; Zhiwen *et al.*, 1987) via a cAMP-dependent mechanism (Bicsak *et al.*, 1986). In women, inhibin levels rise markedly when FSH is administered in the form of human menopausal gonadotrophin (McLachlan *et al.*, 1986; Tsonis *et al.*, 1988) and correlate with the induced increases in serum  $E_2$  levels and follicular number (McLachlan *et al.*, 1986).

During the human luteal phase, serum inhibin levels undergo a marked rise in parallel with progesterone (McLachlan *et al.*, 1987a,b) which suggests the corpus luteum secretes inhibin. Human corpus luteal tissue also contains inhibin subunit mRNA and inhibin immuno and bio-activity (Davis *et al.*, 1987). Luteinizing hormone appears to regulate luteal inhibin secretion based on both in-vitro studies of cultured human granulosa-lutein cells (Tsonis *et al.*, 1987) and in-vivo studies utilizing GnRH antagonist treatment combined with gonadotrophin replacement (McLachlan *et al.*, 1989).

The only previous report of inhibin levels during the normal human menstrual cycle was based on daily blood sampling in six women (McLachlan *et al.*, 1987a). This report suggested there was a complex relationship between inhibin, sex steroids and gonadotrophin levels around midcycle (during the transition from the FSH-dependent follicle to the LH-dependent corpus luteum). For example, serum  $E_2$  appeared to peak a day prior to inhibin which itself rose to a peak coincident with the midcycle gonadotrophin surge. Immediately after midcycle, inhibin levels fell transiently prior to a marked rise along with progesterone. In this report we expand our previous data regarding daily inhibin and other reproductive hormone levels in a large number of normal menstrual cycles; and then particularly describe the relationships between inhibin,  $E_2$ , P, 17-OH-progesterone (17-OH-P), FSH and LH during the periovulatory interval. Specifically, we sought to examine (1) the potential gonadotrophic control of inhibin secretion by the follicle and corpus luteum; (2) the proposed feedback role of inhibin on FSH before, during and after the midcycle gonadotrophin surge; and (3) whether there is differential control of sex steroid and inhibin secretion by the mature follicle and the emerging corpus luteum.

## MATERIALS AND METHODS

### *Subjects*

Forty normal young women, aged 21 to 35 years volunteered to participate in the study.

Their normality was established according to the following criteria: (1) normal body mass index; (2) history of regular menstrual periods (their cycles were 25–32 days in duration); (3) a normal ovulatory basal body temperature chart and luteal phase progesterone greater than 25 nmol/l in a screening cycle; (4) no past history of significant illnesses and no regular medications; (5) normal blood chemistry and haematological parameters. Informed consent was obtained and the study was approved by the University of Washington Human Subjects Committee.

Seven of the subjects were admitted to the Clinical Research Center 3–4 days prior to their anticipated midcycle gonadotrophin surge. Daily serum  $E_2$  levels and ovarian follicular diameter by ultrasound were used to predict the probable day of the LH surge (either an  $E_2$  level  $\geq 550$  pmol/l or a follicle  $\geq 15$  mm in mean diameter were considered sufficient to initiate the study). After admission, an intravenous catheter was inserted into a forearm vein and blood samples (10 ml) were obtained every 3 h until 48 h after the disappearance of the dominant follicle as detected on twice-daily ovarian ultrasounds. Ovulation was presumed to have occurred when two of the following three sonographic criteria were observed: (1) an abrupt decrease in size or disappearance of a preovulatory sized follicle, (2) a hyperechoic border or increased echoes in a follicle-like structure, and/or (3) an acute increase in free intraperitoneal fluid. An Acuson 128 (Mountain View, CA) with a 5-MHz phased-array endovaginal transducer was used to perform the ultrasound scans.

Daily blood samples were obtained between 0800 and 1000 h from the remaining 33 normal women during one complete menstrual cycle. Serum samples from each subject were stored at  $-20^\circ\text{C}$  and were then analysed in the same assay.

### Hormone analysis

Serum FSH and LH were measured by double antibody RIA using reagents supplied by the NIH with LER-907 as the reference preparation (Midgley, 1966; Bremner *et al.*, 1981). The assays were performed at 200  $\mu\text{l}$  in duplicate. The sensitivity of the LH assay was 3.2  $\mu\text{g/l}$  with intra and inter-assay coefficients of variation (CV) of 5.5 and 8.4%, respectively. The sensitivity of the FSH assay was 25  $\mu\text{g/l}$  with intra and inter-assay CVs of 7.3 and 9.7%, respectively.

Serum  $E_2$  was measured at 50  $\mu\text{l}$  in duplicate using reagents obtained from ICN Biomedicals, Inc., RSL Division, Carson, CA. This assay had cross-reactivities reported by the manufacturer of 20% with oestrone and 1.5% with oestriol. The sensitivity of the assay was 37 pmol/l and the intra and inter-assay CVs were 6.9 and 14.2%, respectively.

Serum P was measured at 100  $\mu\text{l}$  in duplicate using reagents supplied by Diagnostic Products Corporation, Los Angeles, CA. This assay had cross-reactivities reported by the manufacturer of 0.3% with 17-OH-P and no detectable cross-reactivity with testosterone,  $E_2$ , pregnenolone and cortisol. The sensitivity of the assay was 0.01 nmol/l and the intra and inter-assay CVs were 9.5 and 13.2%, respectively.

Serum 17-OH-P was measured by a solid-phase, coated-tube immunoassay (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA). The sensitivity of the assay was 0.03 nmol/l, with a cross-reactivity reported by the manufacturer of less than 0.07% with P and 0.002% with  $E_2$ , respectively. The intra and inter-assay CVs were 4.6 and 5.1%, respectively.

Serum inhibin was measured in a heterologous double antibody RIA based on purified 31kDa bovine follicular fluid inhibin as previously described (Robertson *et al.*, 1988). The rabbit antiserum (As 1989) was raised to crude, and then increasingly pure, preparations of bovine inhibin and  $^{125}\text{I}$ -31kDa bovine inhibin was used as tracer. Transforming growth factor beta, bovine activin A, bovine Mullerian inhibitory substance and free inhibin subunits obtained following reduction and alkylation of 31kDa bovine inhibin showed less than 1% cross-reactivity in the assay. A serum pool obtained from women undergoing ovulation induction was used as the standard and was calibrated in the RIA against a partially purified human follicular fluid preparation described previously (McLachlan *et al.*, 1987a), which itself was calibrated in terms of its bioactivity in a rat *in vitro* bioassay against an ovine testicular lymph preparation of defined unitage 1 unit per mg (Eddie *et al.*, 1979). Serum samples were assayed at 200 and 100  $\mu\text{l}$  (luteal samples only) in duplicate. The sensitivity of the assay was 100 U/l. The intra-assay CVs in the upper ( $\text{ED}_{12}$ ), mid ( $\text{ED}_{50}$ ) and lower ( $\text{ED}_{88}$ ) regions of the standard curve were 10.7, 2.8 and 6.0%, respectively from ten assays. The interassay CV was derived from the repeated measurement of multiple dilutions of a quality control serum (obtained from normal women during the midluteal phase) covering the range 90–20%  $B/B_0$  and was 10.9% from ten assays.

### Statistics

The daily values from each patient were normalized around the day of the peak LH value (day 0). For the periovulatory frequent samples the data were normalized around the peak LH value and are expressed as mean  $\pm$  SEM. Linear regression analysis was performed using the mean data for the group and significance was regarded to exist with  $P < 0.05$ . For the purpose of data analysis, follicular rupture was presumed to have occurred at the time of the scan showing ovulation.

## RESULTS

### Daily sampling

Mean ( $\pm$  SEM) daily serum inhibin,  $\text{E}_2$ , P, LH and FSH levels from normal menstrual cycles in 33 women are shown in Fig. 1. Inhibin levels were relatively constant during the early and midfollicular phases until a rise occurred after day - 5. Inhibin levels then rose towards a midcycle peak ( $751 \pm 33$  U/l), then fell significantly ( $P < 0.01$ ) to a nadir on day + 2 ( $587 \pm 30$  U/l), then rose further to a midluteal peak on day + 7 ( $1631 \pm 87$  U/l). Serum FSH levels fell by a mean 71% between days - 10 and - 2, then rose on day - 1, along with LH, towards the midcycle peak. Serum  $\text{E}_2$  rose gradually from day - 10 toward a mean peak level of  $1090 \pm 66$  pmol/l on day - 1. Relative to the inhibin peak,  $\text{E}_2$  consistently peaked 1–2 days prior to inhibin in each individual. Serum  $\text{E}_2$  and P rose during the luteal phase in parallel with inhibin (Fig. 1). All three gonadal hormones exhibited reciprocal relationships with both FSH and LH during the luteal phase (days + 2 to + 12).

### Periovulatory frequent sampling

Serum inhibin, LH, FSH,  $\text{E}_2$  and P levels between - 69 and + 60 h relative to the peak LH value are shown in Fig. 2 and represent data (mean  $\pm$  SEM) from six or seven subjects at

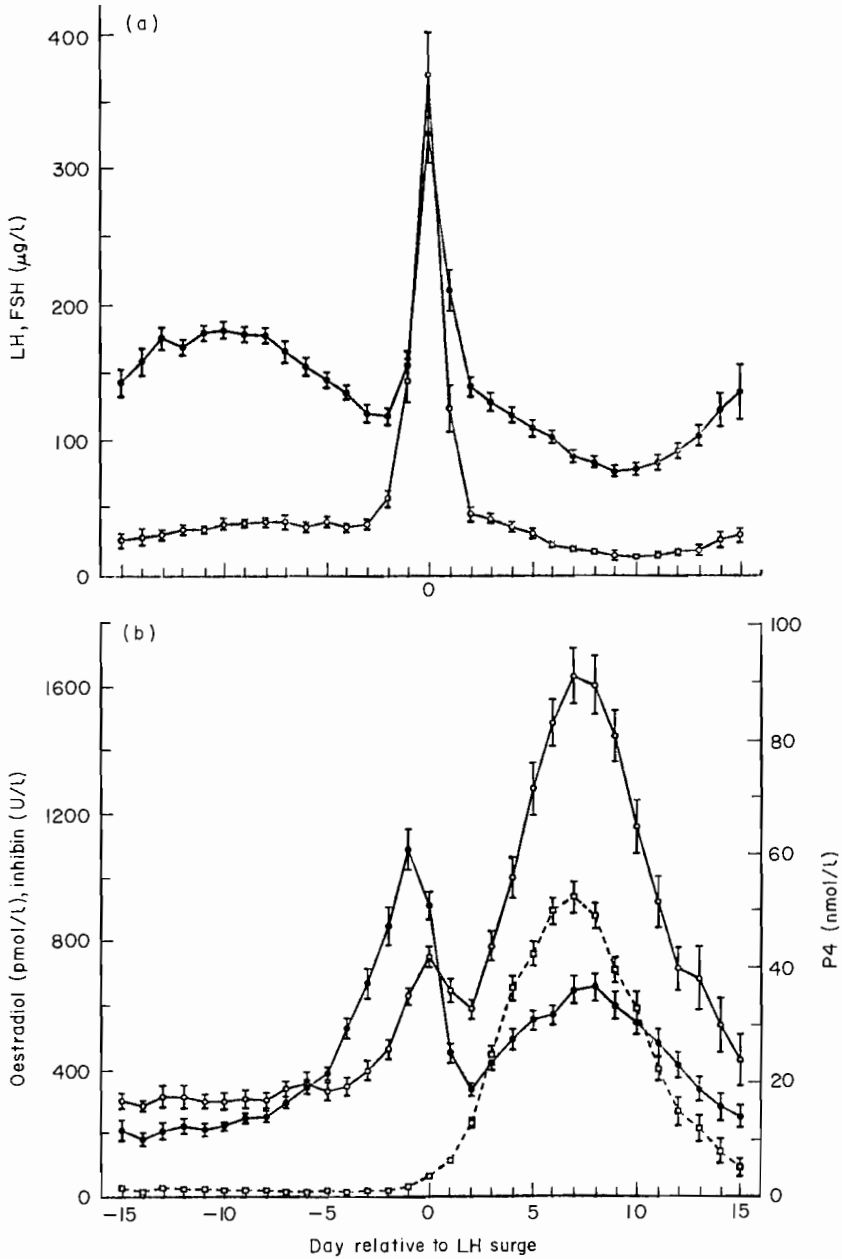


Fig. 1. The daily mean ( $\pm$ SEM) levels of serum a,  $\circ$ , LH,  $\bullet$ , FSH; b,  $\circ$ , inhibin,  $\bullet$ ,  $E_2$  and  $\square$ , P during the menstrual cycle are shown for 33 normal women. Data have been normalized around the day of the LH surge (day 0).

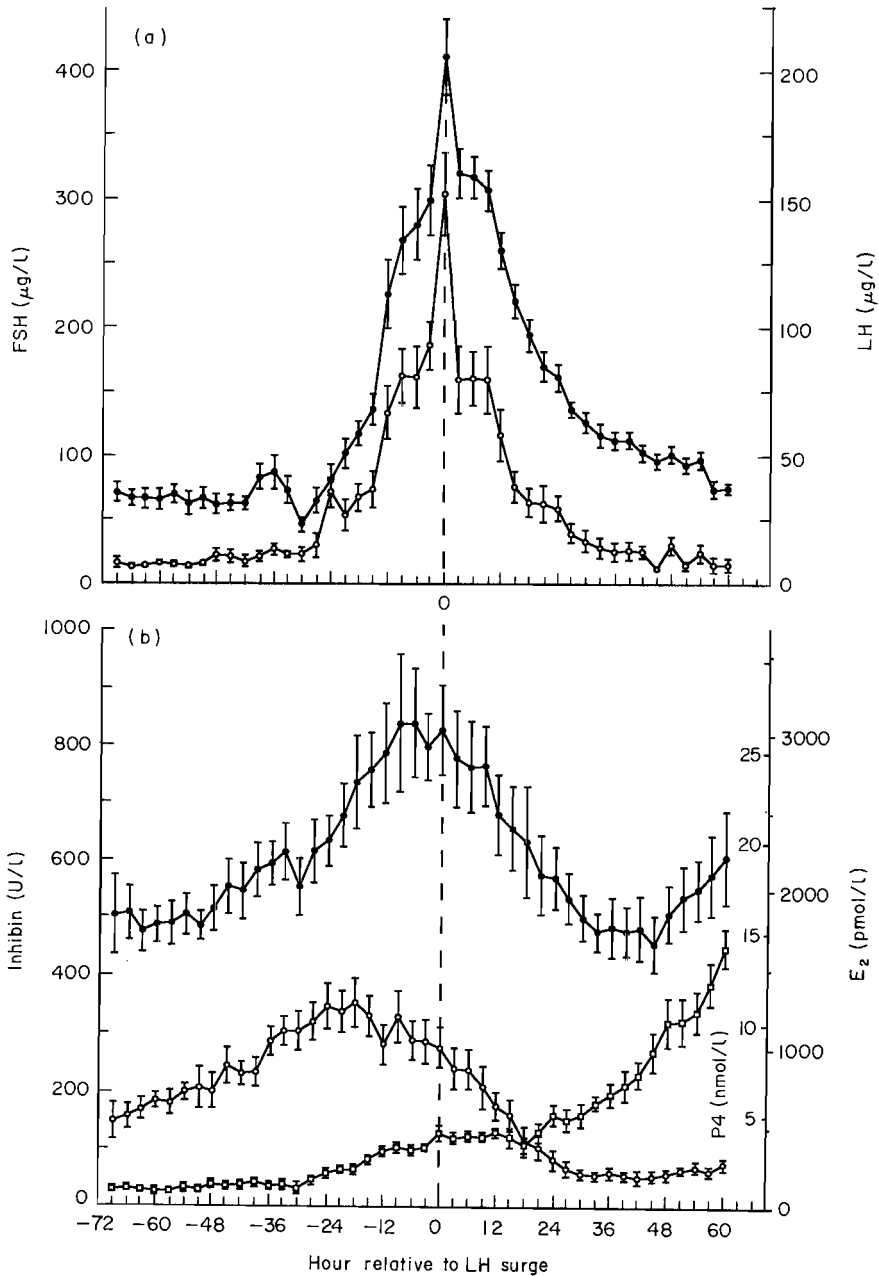


Fig. 2. Mean ( $\pm$ SEM) serum, a, O, LH, ●, FSH; b, ●, inhibin, O,  $\text{E}_2$  and  $\square$ , P levels between  $-69$  h and  $+60$  h relative to the peak LH value are shown for the seven normal women who had blood samples drawn every 3 h around midcycle (each point consists of data from at least six women).

each point. Mean serum  $E_2$  increased progressively from  $543 \pm 114$  pmol/l at  $-69$  h to a peak of  $1296 \pm 154$  pmol/l at  $-18$  h, then fell progressively to  $1050 \pm 139$  pmol/l at 0 h and  $301 \pm 59$  pmol/l at  $+24$  h. Serum inhibin levels rose from  $504 \pm 68$  U/l at  $-69$  h to a peak of  $837 \pm 95$  U/l at  $-6$  h, fell to  $455 \pm 48$  U/l at  $+45$  h, then rose sharply. Serum  $E_2$  and inhibin levels were closely correlated ( $r=0.92$ ,  $P<0.001$ ) between  $-69$  and  $-18$  h. Subsequently, inhibin levels rose to a peak on average  $10.4 \pm 5.1$  h ( $P<0.05$ ) after the  $E_2$  peak.

In the 24 h prior to midcycle, inhibin levels were positively correlated with both serum LH and FSH ( $P<0.01$ ), while serum  $E_2$  was negatively correlated with LH, FSH and inhibin ( $P<0.01$ ). Serum inhibin levels continued to be positively correlated with both serum LH and FSH between 0 and  $+24$  h ( $P<0.01$ ).

Serum P levels were unchanged between  $-69$  and  $-27$  h with a mean of  $1.08 \pm 0.03$  nmol/l, then increased to  $1.84 \pm 0.25$  nmol/l at  $-24$  h,  $4.04 \pm 0.38$  nmol/l at 0 h, and  $14.25 \pm 1.02$  nmol/l at  $+60$  h. The rise in serum P at  $-24$  h was simultaneous with the onset of the LH surge. Serum inhibin levels were positively correlated with serum P levels from  $-24$  to 0 h ( $P<0.01$ ) and from  $+45$  to  $+60$  h ( $P<0.01$ ), but were inversely correlated from 0 to  $+45$  h ( $P<0.01$ ). Serum 17-OH-P levels also rose from a mean of  $85.6 \pm 3.0$  nmol/l between  $-69$  and  $-27$  h to  $127.7 \pm 12.7$  nmol/l at  $-24$  h and  $255.1 \pm 28.4$  nmol/l at 0 h, but did not rise further subsequently (data not shown).

The dominant follicle reached a mean maximum diameter of  $22.8 \pm 0.8$  mm and had disappeared, on average,  $19.0 \pm 2.8$  h after the peak serum LH value.

## DISCUSSION

In this study, we describe the relationships between serum inhibin levels and those of the serum gonadotrophins and sex steroids during the normal menstrual cycle, particularly around midcycle. We have extended our previous description based on daily blood samples and examined the hypothesis that the secretion of inhibin and sex steroids by the preovulatory follicle and emerging corpus luteum is discordant. We have confirmed that inhibin secretion is not closely coupled to either  $E_2$  or P at midcycle, suggesting that the secretion of this glycoprotein hormone is under different control from that of the steroids.

In confirmation of our earlier study (McLachlan *et al.*, 1987b), inhibin levels do not rise during the early to mid-follicular phase. The substantial fall in serum FSH during this period would therefore seem not to be attributable simply to rising inhibin levels. Serum  $E_2$ , on the other hand, rose progressively after day  $-10$  and seems a better candidate for the inhibition of FSH during this period. This is not to say, however, that inhibin has no role in FSH regulation. There is substantial evidence from animal studies for the proposed endocrine role of inhibin in FSH regulation (see reviews: McLachlan *et al.*, 1988; Ying, 1988). It remains possible, therefore, that a stable serum inhibin level and a rising  $E_2$  act synergistically to reduce FSH over this time interval in the menstrual cycle. Inhibin levels begin to rise after day  $-4$  toward an initial peak at midcycle, then rise further to a marked midluteal peak. Throughout the luteal phase, serum inhibin demonstrated a strong correlation with both  $E_2$  and P, in support of the concept that all are products of the corpus luteum.

We then examined the perioovulatory period more closely by performing frequent (3-hourly) blood sampling. During the late follicular phase, serum inhibin and  $E_2$  levels

increased in parallel from -69 to -18 h. At the end of this interval, the process of luteinization was being initiated, as evidenced by the rise in serum 17-OH-P and P. Oestradiol levels then fell, while inhibin secretion rose further to a peak just prior to the gonadotrophin surge. These data suggest that the luteinizing granulosa cells maintain their ability to secrete inhibin in response to FSH and/or LH while  $E_2$  secretion is falling and P secretion is rising. The relative contributions of FSH and LH to the stimulation of inhibin secretion around midcycle are unclear. It is well established that FSH increases inhibin secretion during the mid to late follicular phase, while LH is its primary secretagogue in the luteal phase (McLachlan *et al.*, 1989). Therefore, there is a transition from FSH to LH control of inhibin secretion, but the exact timing of this transition remains unclear. We speculate this transition commences along with the onset of luteinization.

After midcycle, inhibin secretion fell to a nadir at +45 h while P secretion increased progressively from the day prior to midcycle. It thus appears that inhibin secretion falls around the time of ovulation while P secretion (an index of luteal cell function) is rising throughout. This transient fall in inhibin secretion suggests a loss of the granulosa cell component of its production. It has been shown that androgens stimulate inhibin secretion by granulosa cells *in vitro* (Henderson & Franchimont, 1981; Tsonis *et al.*, 1987) and also that androgen levels decline in human follicular fluid in the days leading up to ovulation (McNatty *et al.*, 1976). A decline in androgen stimulation of granulosa cell inhibin secretion may also be a component in the transient decline of inhibin levels immediately after the LH surge. In summary, serum inhibin levels at this time may represent the sum of inhibin secretion from two sources, i.e. a declining granulosa cell and an increasing luteal cell component.

The relationships between serum LH, FSH,  $E_2$  and P at midcycle were similar to those described by Hoff *et al.*, (1983) based on 2-hourly sampling. The similarities were that  $E_2$  reached a peak around the time of the onset of the LH surge, then fell, and the duration of the LH surge was approximately 48 h. We found serum P levels began to increase around the time of the onset of the LH surge, continued to rise until 9 h prior to the LH peak when they plateaued for 24 h, and then increased steeply. In contrast, Hoff *et al.* (1983) reported the rise in P preceded the onset of the LH surge by 12 h (a triggering role for P in the onset of the LH surge was speculated), although the other changes we noted were similar to their findings. Finally, follicular rupture occurred approximately 44 and 19 h after the onset of the LH surge and the peak LH value, respectively.

It is evident from the coincident rises of inhibin and gonadotrophins at midcycle that the proposed suppressive action of inhibin on FSH or LH is overcome at midcycle. It has recently been shown that purified inhibin reduces the number of binding sites for GnRH on the gonadotrope (Wang *et al.*, 1988). It is possible that rapid increases in GnRH pulse frequency and amplitude at midcycle may overcome the inhibitory effect of increasing inhibin concentrations.

In this report we have shown evidence that inhibin secretion during the periovulatory interval is not coupled closely to that of either  $E_2$  or P. Inhibin levels in serum are, however, positively correlated with those of both gonadotrophins. Finally, the absolute level of inhibin in serum may represent the sum of its secretion by the preovulatory follicle and the emerging corpus luteum. The possible endocrine and/or paracrine role(s) for inhibin during this time interval requires further study.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the laboratory assistance of Florida Flor, Dorothy McGuinness, Arlen Sarkissians and Dianne Schoenberg, and the secretarial assistance of Cindy Schuler. Drs D. M. de Kretser, D. M. Robertson and H. G. Burger are thanked for their provision of reagents used in the inhibin radioimmunoassay.

This research was supported by CONRAD grant 88-032, National Institutes of Health grants RO1-HD-18967 (MRS), P50-HD-12629 (WJB), Clinical Research Center grant RR-37 and the Veterans Administration. Robert McLachlan is a Neil Hamilton Fairley Fellow from the Australian National Health and Medical Research Council.

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