

# RESPONSIVENESS OF RAT PITUITARY GLAND IN VITRO TO PULSATILE LUTEINISING HORMONE-RELEASING HORMONE(LHRH) DURING THE OESTROUS CYCLE

Pages with reference to book, From 237 To 241

M. Aslam, M.A. Hameed ( Department of Physiology, Army Medical College, Rawalpindi. )  
S. Nicholson, M.T. Jones ( Upnited Medical Schools of Guy's and St. Thomas's Hospital, London (UK), )

## Abstract

Anterior pituitary gland fragments removed from female rats at various days of the oestrous cycle were perfused into small Biogel columns. After 2 hours stabilisation, the tissues were exposed at the beginning of each of five hours of perfusion to a volley of six 1-min pulses of LHRH (10M1 8, given 4 min.apart. Pituitary glands removed during oestrus or met-oestrus showed little change in the sensitivity to pulses of LHRH, LH release being similar in response to all volleys. Pituitaries removed during di-oestrus showed a priming response to the second volley of LHRH but this was followed by desensitisation thereafter. In contrast, tissues removed at various times during pro-oestrus and exposed to LHRH pulses of different frequencies exhibited only progressive sensitisation. In conclusion, sensitivity of pituitary gland to LHRH varies throughout the oestrous cycle which plays an important role in the control of episodic secretion of LH (JPMA 38: 237, 1988).

## INTRODUCTION

Since the introduction of neuro-hormonal theory<sup>1</sup>, it is being increasingly evident that LHRH is secreted episodically in the hypothalamo-hypophyseal portal system for delivery to the anterior pituitary gland<sup>2</sup>. Furthermore, it is now established that rhythmicity of menstrual or oestrous cycle in mammals is the consequence of a reciprocal inter-relationship between hypothalamus, pituitary and gonads.<sup>3-4</sup> Various workers have characterised the secretory pattern of LH in vivo throughout the oestrous cycle in the female rat. We have investigated the effects of giving volleys of pulses of LHRH in vitro to the perfused pituitaries removed from female rats on specific days of the cycle.

## MATERIALS AND METHODS

### Experimental Animals

All animals used were 150-250 gram female Wistar-derived rats and were housed on 14h light: 10h dark schedule (lights on 0700-2 100h). They had access to food and water ad libitum. Only animals which had exhibited two consecutive 4-day oestrous cycle as shown by the daily vaginal smearing were selected for these experiments.

### Perfusion of Pituitary Tissue

The method for perfusion of anterior pituitary gland fragments (1mm<sup>3</sup>) in a small Biogel column was as described previously.<sup>5</sup> Pituitaries were removed from rats during oestrus, metoestrus, di-oestrus and pro-oestrus. The tissues were packed into the columns and perfused at a rate of 0.5ml/min. With Kreb's bicarbonate medium at 37C° containing 11mM glucose and gassed with 95% O<sub>2</sub>: 5% CO<sub>2</sub>. After 2 hours equilibrium period, the tissues were exposed at the beginning of each of 5 hours of perfusion to a volley of six 1min. pulses of IOM-8 synthetic LHRH (Hoechst, Hounslow, Middx, UK), pulses being 4 min. apart. Pituitary perfusates were collected in 2 min, (1ml) fractions and analysed for

rat LH (rLH) by radioimmunoassay (RIA)

### Radioimmunoassay of Rat LH

The rLH from pituitary perfusate was measured<sup>6</sup> by a double antibody RIA kit supplied by the National Hormone and Pituitary Programme (University of Maryland School of Medicine, USA) NIADDK. The concentration of rLH was expressed in sensitivity terms of NIADDK rLH-RP-2 as the reference preparation. The sensitivity of this assay was 0.39mg/ml. The intra-assay variation was found to be 6.6% at a level of 0.78ng/ml and 8.7% at a level of 50 ng/ml, and the inter-assay variation at same levels was found to be 8.0% and 123% respectively, where % variation is sem/mean for 5 estimations.

## RESULTS

### Pattern of tissues removed during Oestrus and Met-Oestrus

Pituitaries removed during oestrus or met-oestrus showed no specific pattern of LH release in response to the repeated volleys of LHRH. Each volley caused a similar fold increase in LH secretion (4.5 fold increase for oestrous tissue, 5 fold increase for met oestrus) above the corresponding basals (Figure 1 a & b).

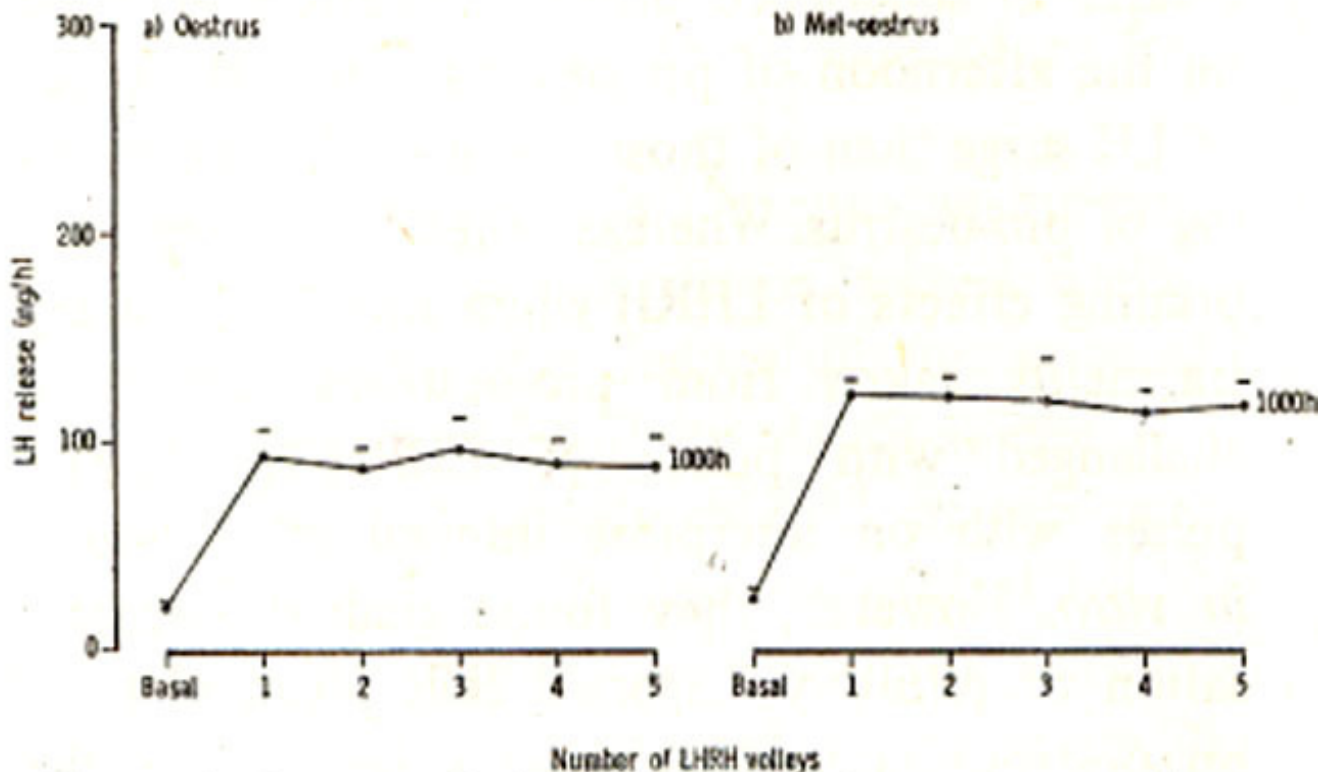


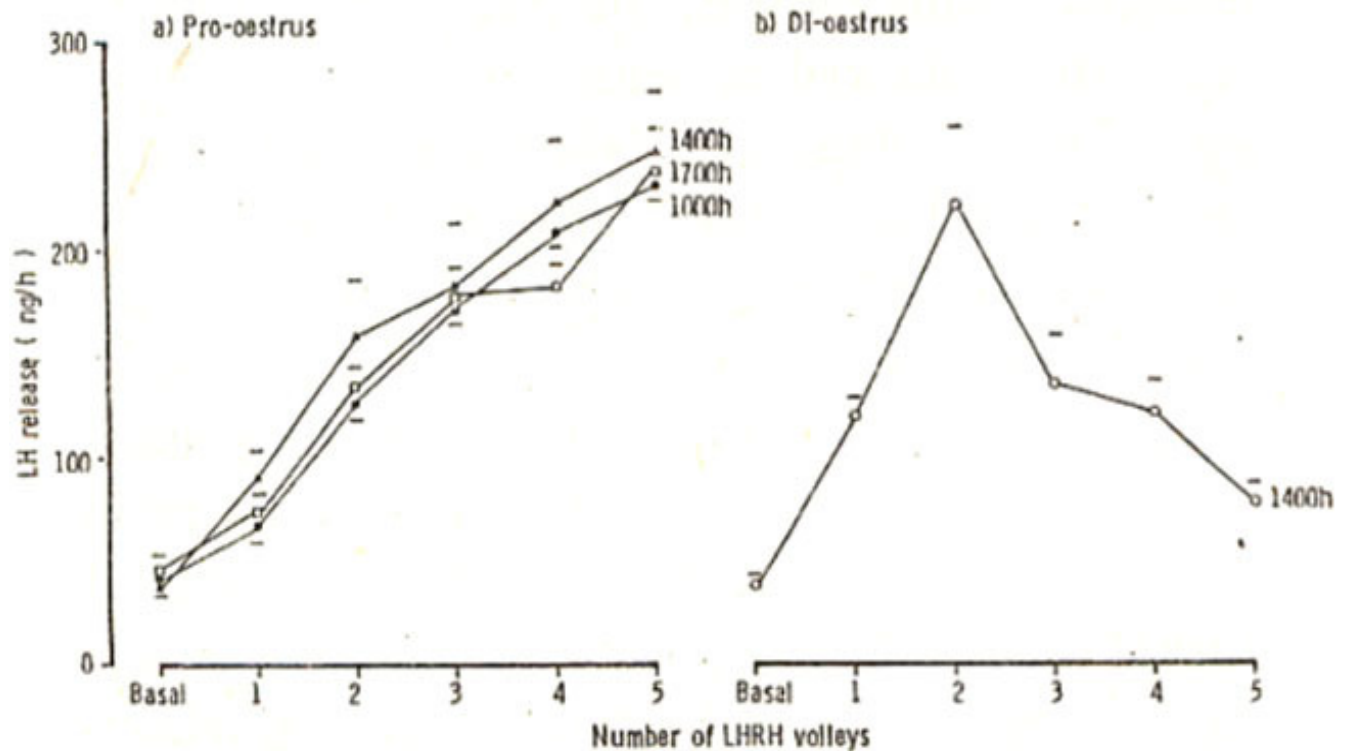
Figure 1. Pattern of pituitary responsiveness removed on oestrus or met-oestrus.

Pituitaries were removed at 1000h on (a) Oestrus (b) met-oestrus and exposed to hourly volleys of LHRH.

Di-Oestrus and Pro-Oestrus

For pituitaries removed during di-oestrus, release of LH in response to the second volley of LHRH was significantly greater ( $P < 0.005$  one way ANOVA) than to the first, that is, sensitisation occurred. Thereafter, the responses gradually declined (difference between responses to second and third volleys,  $P < 0.01$ ), such that less LH was released in response to the fifth volley than to the first (desensitisation occurred).

In contrast, the pattern of response of tissues removed either in the beginning (1000h), middle (1400h) or end (1700h) of the pro-oestrus (Figure 2 a)



**Figure 2. Pattern of pituitary responsiveness removed on pro-oestrus or di-oestrus. Pituitaries were removed at (a) different times on pro-oestrus or (b) 1400h on di-oestrus and exposed to repeated volleys of LHRH.**

**n = 3-5 experiments**

**mean  $\pm$  sem**

showed no evidence of desensitisation, amount of LH secretion increased in response to successive volleys to LHRH (sustained priming effect of the releasing hormone). This change in LH release with time in each case was significant ( $P < 0.01$ , one way ANOVA). This was a specific response to LHRH as hourly stimulations of pro-oestrous pituitaries with volleys of 48mM Potassium ( $K^+$ ) ions according to exactly the same protocol produced no evidence of changing responsiveness (Table).

**TABLE. Release of LH from Tissues removed from pro-oestrous Rats in response to hourly volleys of pulses of 48mMK<sup>+</sup> ions.**

Number of K <sup>+</sup> volley	LH release (ng/h)
Basal	58.8 ± 3.0
1	265.0 ± 10.6 *
2	259.4 ± 12.5 *
3	267.5 ± 13.5 *
4	263.3 ± 13.4 *
5	265.3 ± 9.3 *

\*P < 0.001 vs basal values.

**Effect of different modes of LHRH Pulses on ProOestrus**

LHRH pulses were given according to three different protocols to tissues removed during prooestrus. Firstly, the protocol used in the above experiments was that of volleys of six 1min. pulses of LHRH, given 4min. apart of the beginning of each hour of perfusion. The.. response to this regimen of LHRH pulses has already been shown in Secondly, to ascertain whether the tissue became refractory. to LHRH, short pulses (1min) were given at different (20min) intervals over a long time-course, and thirdly, much longer (5min) pulses with longer rest periods in between (35min) were given. LH release in response to these single LHRH pulses (as opposed to volleys) is shown in Figure 3.

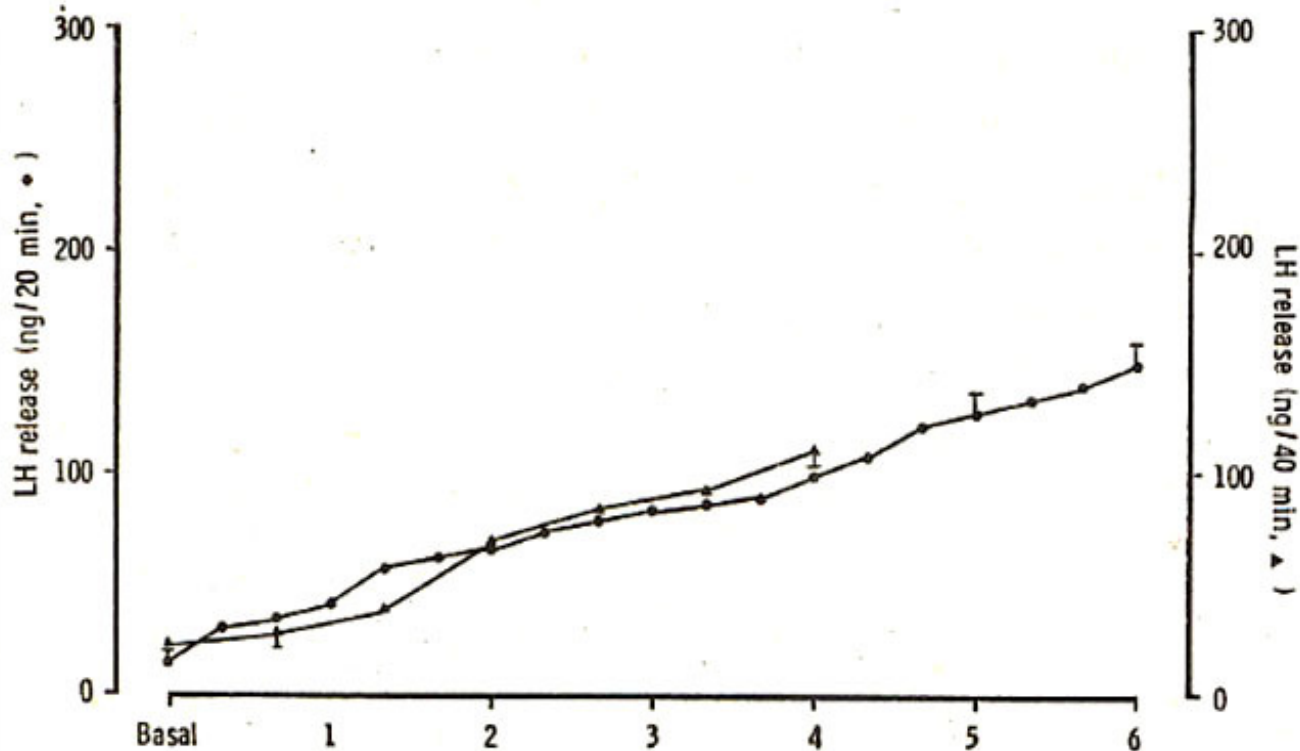


Figure 3. Effect of different frequencies of LHRH pulses on pro-oestrus.

Pituitaries were removed at 1000h on pro-oestrus and exposed to single pulses of LHRH given for 1min. every 20 min (●) or for 5 min. every 40 min (▲). For clarity, sems are included only where they exceed 10% of the mean. Note that release is expressed for entire period following each pulse.

n = 3-4 experiments

mean  $\pm$  sem

There was no evidence of desensitisation to 1min. pulses with 20 min. interval in between, given over 6 hours (18 pulses), LH release in response to each pulse being slightly greater than that to the preceding one. The response to 5min. pulses of LHRH separated by 35min. rest periods was also similar showing a gradual rise in LH release.

#### Expression of data and statistical analysis

As each column contained tissues from 6 different animals and the values obtained from experiment to experiment were consistent, observations for a given set of conditions were repeated 3-5 times (n=5), equivalent to the response of tissues obtained from 18-30 animals. Values and bars represent mean  $\pm$  sem but in some figures, sems less than 10% of the mean have been omitted for clarity.

The data from these experiments were expressed in terms of total LH released during each hour after the onset of a volley of LHRH pulses (that is, release per 30 fractions). Temporal differences across

several groups were analysed by one-way analysis of variance (ANOVA). Differences were considered significant if P values from the test were less than 5%.

## DISCUSSION

This pituitary characteristics of changing LHRH responsiveness and LHRH self-priming are components of a system which produces the cyclicity of LH release during the oestrous cycle and the pro-oestrous LH surge. In these experiments, pituitaries removed in the beginning, middle or at the end of pro-oestrous phase showed a sustained priming effect to the volleys of LHRH 300 over 5 hours of perfusion.

These results are in contrast with those of Waring and Turgeon<sup>7</sup> who showed that the 200priming effect of LHRH (2x10min. pulses with a 2 hour interval in between) in vitro was significantly greater in superfused pituitary quarters removed on the afternoon of pro-oestrus before the onset of LH surge than of those taken out in the morning of pro-oestrus. Whereas others<sup>8</sup> have reported priming effects of LHRH when anterior pituitary fragments taken from pro-oestrous rats were challenged with pulses of LHRH (2x30min. pulses with an interpulse interval of 2 hours) in vitro. However, they found gradual desensitisation of pituitary dispersed cells prepared from pro-oestrous rats on continuous exposure to the releasing hormone for 5 hours.

To ascertain whether the tissues became refractory, LHRH pulses were given to pituitary fragments removed from pro-oestrous rats according to the protocol described by various workers<sup>9-10</sup> over a long time-course. There was no evidence of desensitisation with both the frequencies of LHRH in vitro on pro-oestrus. The fact that very similar amounts of LH were recorded over the 40 min. following a 5min. pulse as during the 20min. after a 1min. pulse suggests that the tissue became refractory to the effect of this concentration of LHRH within 5min.

Since pro-oestrus in rat is not a long phase<sup>11</sup> (12h), it was thought that LHRH pulses given particularly with long intervals may not truly reflect the pituitary responsiveness peculiar of this phase of the cycle. However, pituitaries removed during oestrus, the phase which follows pro-oestrus, showed no sensitisation to the releasing hormone. Pituitaries taken even during metoestrus exhibited little change in the responsiveness, not even priming, to repeated LHRH stimulations in vitro. In another study,<sup>12</sup> a lesser concentration of LHRH-receptors in the pituitary gland on oestrus than that on pro-oestrus was found in the rat. In the present study, the inability of LHRH to prime the pituitaries in vitro on the day of oestrus and met-oestrus may be related to the prior LH release during pro-oestrous with partial depletion of glandular content or to the long interval since the occurrence of high oestrogen titres.<sup>13</sup> Prolonged sensitisation was a specific response to LHRH as hourly stimulation of prooestrous tissue with volleys of pulses of 48mMK<sup>+</sup> ions to exactly the same protocol produced no evidence of changing sensitivity, the magnitude of LH release being similar to all volleys of K<sup>+</sup> ions. The LH release induced by the raised C ions in the perfusion medium is presumably the result of depolarisation of the plasma membrane of the gonadotrophs which causes an elevation of cytosolic calcium leading to the release of readily releasable pool of the hormone.<sup>14</sup>

The exact mechanism of sustained priming to LHRH is unknown. However, several studies suggest that LHRH may induce the formation of additional specific high affinity plasma membrane receptors on the gonadotrophs<sup>14</sup> or this may increase the synthesis of releasable pool of LH.<sup>15</sup> Recently, protein kinase C & phosphatidylinositol have reported to be involved in the priming effects of LHRH.<sup>16</sup>

In this study, tissue removed on di-oestrous showed the well-known self-priming effect in response to the second volley of LHRH followed by subsequent desensitisation. A similar study<sup>17</sup> carried out using static incubation of hemipituitaries from di-oestrous rats showed a rise in LH release in response

to the second hour of exposure to U-IRH in vitro, but thereafter the LH release became smaller. It has been suggested that down-regulation by LHRH of the number of its receptors or receptor-occupancy are the basis of this phenomenon of desensitisation.<sup>18</sup>

The data discussed here, show that sensitisation followed by subsequent desensitisation of anterior pituitary tissue in response to repeated pulses of LHRH in vitro is a characteristic of dioestrous.

However, on pro-oestrous, when, the pre-ovulatory LH surge takes place, there occurs only progressive sensitisation, specific to LHRH. This suggests that there is a basic difference in the response of anterior pituitary gland to LHRH on various days of the oestrous cycle and this presumably plays a role in maintaining the LU surge.

## REFERENCES

1. Harris, G.W. The induction of avulation in the rabbit by electrical stimulation of the hypothalamo-hypophysial mechanism. *Proc. Roy. Soc.*, 1973; 122: 374.
2. Sarkar, D.K. and Fink, G. Luteinising hormone releasing factor in pituitary stalk plasma from long-term ovariectomised rats; effects of steroids. *J.Endocrinol.*, 1980;86 :511.
3. Butcher, R.L., Collins, W.E. and Fugo, N.W. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17 throughout the 4-days estrous cycle of the rat., *Endocrinology*, 1974;94: 1704.
4. Gallo, R.V. Pulsatile LH release during periods of low level LH secretion in the rat estrous cycle. *Biol. Reprod.*, 1981;24: 771.
5. Aslam, M., Hameed, M.A., Nicholson, S.A. and Jones, M.T. Perfusion of pituitary gland a methodologic analysis, (Submitted for publication).
6. Monroe, S.E., Parlow, A.F. and Midgley, A.R. Radioimmunoassay for rat luteinizing hormone. *Endocrinology*, 1968;83: 1004.
7. Warling, D.W. and Turgeon, J. Luteinising hormone releasing hormone induced luteinising hormone secretion in vitro; cycle changes in responsiveness and self-priming.. *Endocrinology*, 1980; 106: 1403.
8. Evans, W.S., Uskaviteh, D.R., Kaiser, D.L., Hellmann, P., Borges, J.L.C. and Thorner, M.O. The self-priming effect of gonadotrophin releasing hormone on luteinising hormone release; observations using rat anterior pituitary fragments and dispersed cells continuously perfused in parallel. *Endocrinology*, 1984; 114 : 861.
9. Buckingham, J.C. and Cover, P.O. Changes in the responsiveness of perfused rat adenohypophysial cells to repeated stimulation with luteinizing hormone-releasing hormone. *Acta Endocrinol.*, 1986; 113: 479.
10. Edwardson, J.A. and Gilbert, D. Application of an in-vitro perfusion technique to studies of luteinizing hormone release by rat anterior hemipituitaries; self-potential by luteinizing hormone releasing hormone. *J. Endocrinol.*, 1976; 68:197.
11. Fox, R.R. and Laird, C.W. Sexual cycles, in reproduction and breeding techniques for laboratory animals. Edited by Hasez. Philadelphia, Lea & Febiger, 1970, p. 107.
12. Clayton, R.N. and Catt, K.J. Gonadotrophin-releasing hormone receptors; characterization, physiological regulation, and relationship to reproductive function. *Endocr. Rev.*, 1981; 2 : 186.
13. Schwartz, N.B. A model for the regulation of ovulation in the rat. *Recent Progr. Horm Res* 1969; 25:1.
14. Mitchell, R.H., Jaffeji, S.S. and Jones, M.L. The possible involvement of phosphatidylinositol breakdown in the mechanism of stimulus response coupling at receptors which controls cell-surface calcium gates, in functions and biosynthesis of lipids. Edited by Bazan et al. New York., Plenum Press, 1977,p.447.
15. Smith, P.S. Release of luteinizing hormone and follicle stimulating hormone and high potassium

concentration. *Endocrinology*, 1985; 116: 1826.

16. Noor, Z. Integration of phosphoinositide turnover, calcium mobilisation and protein kinase C activation in GnRH action. *J. Endocrinol.*, 1986; III (Suppl) Abs.4.

17. Conn, P.M., Hsueh, A.J. and Crowley, W.F. Jr. Gonadotrophin-releasing hormone; molecular and cell biology, physiology, and clinical applications. *Fed. Proc.*, 1984;43 :2351.

18. Witcher, J.A., Nearhoof, K.F. and Freeman, M.E. Secretion of luteinizing hormone (LH) and pituitary receptors for LH - releasing hormone as modified by the proestrous surge of progesterone. *Endocrinology*, 1984; 115: 2189.