30

THE CONTROL OF PROLACTIN SECRETION AND THE ROLE OF GONADOTROPHIN RELEASING HORMONE IN THE PRODUCTION OF CONCORDANT SECRETORY SPIKES OF LUTEINIZING HORMONE AND PROLACTIN IN THE LUTEAL PHASE OF THE MENSTRUAL CYCLE

Hilton Kaplan

MB.ChB. (RAND) F.C.P (SA)

A thesis submitted in partial fulfilment

of the requirements for

the degree of Master of Medicine (Internal Medicine)

University of Cape Town

1988

ACKNOWLEDGEMENTS

I would like to thank Professor Bob Millar who provided me with the concept for this work, for his assistance with the design of the protocol and for supplying the materials which made the study possible. Furthermore, I wish to acknowledge the assistance of my supervisor, Professor F. Bonnici and Sister Francis Kovecs (nee Hardy) for supervising the taking of the blood samples during the test period and for helping with literature searches and with the collection of data and plotting the graphs.

I would also like to thank Mr. K Samsodien for helping to collect blood samples, Mr. Sidique Isaacs for helping with the statistics, Mr. Salie and Mrs. M. Pane for the assays. Susan Abrahamson did the art work and Charlene Thomson provided invaluable secretarial assistance in typing the manuscript and offering advice on layout.

Glossary of Abbreviations

DA	Dopamine
FSH	Follicle Stimulating Hormone
GABA	Gamma Amino Butyric Acid
GnRH	Gonadotrophin Releasing Hormone
LH	Luteinizing Hormone
MCP	Metoclopramide
PIF	Prolactin Inhibiting Factor
PRL	Prolactin
PRF	Prolactin Releasing Factor
PROG	Progesterone
RIA	Radioimmuno assay
SEM	Standard Error of the Means
TIDA	Tuberoinfundibular dopamine

ABSTRACT

The control of prolactin secretion is a complex interaction of peptides and neurotransmitters acting either in an inhibitory or stimulating way to effect final secretion of this hormone from the lactotrope cell in the anterior hypothalamus. These factors may act either directly on the lactotrope cell or indirectly by changing either dopamine restraint of prolactin secretion or by modulating peptide substances or neurotransmitters higher up in the hypothalamus. Gonadal steriods may also modulate the effect of peptides or dopamine at the level of the lactotrope.

Prolactin's major role in the female rat is one of milk production post-partum, nurturing the young. It probably also has other physiological functions and may play a part in the menstrual cycle although this is controversial.[1] Certainly pulsatile secretion of prolactin during the menstrual cycle is well established[2,3] and in the luteal phase this is concommitant with the secretion of luteinizing hormone.

Theories explaining the synchronous surges seen during this phase of the menstrual cycle have been proposed and GnRH has been implicated in the genesis of the concordance of these secretory spikes.[4]

Using a potent GnRH antagonist an experiment was undertaken to establish the role of GnRH by blocking this hypothalamic peptide and observing the effect that this had on luteinizing hormone, prolactin and follicle stimulating hormone. In the first part of the thesis

the control of prolactin secretion is reviewed. In the following section, an experiment was performed using a potent GnRH antagonist. A dose response curve was established for the antagonist action on LH. Then a twice maximum dose of this peptide was administered to three subjects in the midluteal phase of the menstrual cycle and the response of LH, prolactin and FSH was measured. The results indicate that although the GnRH antagonist significantly blocked LH secretory peaks, this action was not observed for either prolactin or FSH. This result is perhaps at variance with previous data which suggested that GnRH was responsible for concordant secretory spikes of LH and prolactin in the midluteal phase of the menstrual cycle.

CONTENTS

REVIEW OF PROLACTIN SECRETION

Introduction:

Prolactins, somatotrophins and placental lactogens form a group of protein hormones characterised by a high degree of homology in their Amino Acid sequence. There is evidence that this group of biologically related proteins has arisen from a shorter primordial peptide by intragenic duplication preceding extragenic duplication. Prolactin is less related to growth hormone than to placental lactogen. This evolution of the two separate hormones produced by extragenic duplication probably occurred early on before the evolution of lactation as the pituitaries of birds, reptiles and amphibians seem to contain separate prolactin-like and somatotrophin-like hormones. However, prolactin could conceivably have been a growth promoting substance and may have promoted growth in lower vertebrates including some teleost fishes and reptiles.[5]

The early anterior pituitary hormones with the exception of prolactin were committed early on in vertebrae phylogeny to the control of a single or, at most, a few physiological processes. Prolactin differed from these other hormones in controlling a wide variety of different physiological mechanisms which varied according to the species in which the action of this versatile hormone was manifest[6, 7]

Thus, in fish prolactin is concerned with the regulation of water and electrolyte metabolism[5, 6 & 7]

In amphibians, for example, urodeles (particularly when they are aquatic), prolactin may have osmotic regulating and growth promoting functions. It has even been suggested that these effects may be important ontogenetically in mammals i.e. in foetuses and neonates.[8]

In some eutheria, for example the rat, prolactin maintains the corpus luteum, while in other species evidence is scant but would suggest that a similar situation may apply, although only minimal levels of prolactin are necessary.[1]

Other actions of prolactin include effects on parental behaviour, various metabolic effects in birds, amphibians and mammals, effects on the rat testis, and female amphibian reproductive tract, and an antigonadotrophic effect in nonmammalian species; in birds the formation of crop "milk", release of "milk" production and the brood patch; and probably its most important function, that of nurturing the young and the production, in mammals, of milk.[8] What has brought about the change of function of prolactin through evolution in these different species is perhaps uncertain but it seems likely to have been a change in the target cells.[5]

Four different molecular variants of prolactin are found on gel electrophoresis and these have been named according to their sedimentation rates.[9] A specific variant may be secreted under certain situations and may give rise to a specific physiological

action.[10 & 11]

In pathological states particular forms of prolactin are secreted[12, 13 & 11] and this preferential release of one or other form(s) is found in situations contrasted by high or low secretory rates,[11 & 14] and after stimulation with Thyrotrophic Hormone.[11 & 14]

Not only may prolactin exist in different forms, but lactotroph cells in the pituitary gland are heterogenous in morphology and function[10] and immunocytochemistry has distinguished four different types of cells distinguished by the contour of the cell and the size and shape of the secretary granules in rats.[15]

At present the possible relationship between the molecular diversity of prolactin and the cellular diversity of the lactotrophs is speculative.[10]

It is possible that most prolactin in the resting state in normal people circulates as "Big-Big" prolactin which is biologically less active than e.g. "small" prolactin.[11]

A circadian rhythm exists both in the male rat and in the human which is abolished by drugs which block 5-HT.[16] The basal secretion of the gonadotrophins and prolactin is pulsatile and in the former this is thought to reflect the importance of frequency modulation of

gonadal function by the brain-pituitary system,[20] seen most convincingly in seasonal breeders.[12]

The pulsatile pattern of tonic prolactin release has been recognized for some time.[17, 3 & 18] The Nycthemeral maximum occurs in late sleep under normal conditions of health and this pattern is absent in the last trimester of pregnancy and in some pathalogical states and may be related to either pulsatile GnRH secretion or to another mechanism which originates within the pituitary.[10 & 19]

To support the view that GnRH is responsible for prolactin pulsatility is the observation that synchrony exists between prolactin and LH in the luteal phase of the menstrual cycle[8] and in sleep associated peaks of LH during puberty.[20]

Although the lactotroph and gonadotroph cells may communicate information via intercellular gap junctions or in a paracrine fashion,[10] and although a common neuronal pathway may account for GnRH and prolactin pulsatility, the role of GnRH directly influencing tuberoinfundibular dopamine (TIDA) neurons was thought to be one of the more attractive theories to explain these observations (of LH and prolactin synchrony).[4]

Prolactin inhibiting factors:

Everett[21] in 1954, first reported that prolactin secretion was stimulated by the transplantation of the pituitary gland beneath the kidney capsule, and seven years after, in the early 1960's, it

became apparent that the hypothalamus in some way, inhibited prolactin secretion in vitro.[22] More work and cogent observation over the next ten years consolidated this finding[22] but the factors responsible for inhibition of prolactin by or from the hypothalamus remained obscure.[22 & 23]

It became apparent that monoamines had an important role in some or other way in this control although the mechanism appeared obscure.[24] Anatomical data had shown that tuberoinfundibular dopaminergic nuerons terminated in the median eminence, adjacent to portal blood vessels leading to the anterior pituitary and that other dopaminergic neurons originating in the arcuate nucleus terminated in the neurointermediate lobe of the pituitary gland.[25]

It was also known that catecholamines were present in the hypothalamus in relatively high concentrations, but the significance of this was uncertain.[24] However, Bridge et al in 1970 showed that catecholamines inhibited prolactin secretion in isolated rat pituitary glands. This was also shown by other workers.[24] Dopamine was thought of as the main PIF but the site of action was controversial.[22 & 23] In vivo studies[26] showed that dopamine inhibited prolactin secretion. Although it also inhibited prolactin when injected into a single portal vessel,[27] evidence that in vivo dopamine inhibited prolactin at the pituitary was lacking, especially as some in vivo studies had shown that dopamine seemed to act on the hypothalamus to release PIF.[28]

Because dopamine receptors were found in the pituitary gland but were absent in the hypothalamus, Brown et al contended that dopamine acted directly on the pituitary as an inhibiting factor rather than indirectly on the hypothalamus.[29] These receptors were found, not only on pituitary membranes but also, specifically, on lactotrophs.[30] Dopamine was also found in hypophysial stalk plasma[31 & 9] in amounts sufficient to inhibit prolactin release.[32]
A major breakthrough occurred when more accurate and sophisticated ways were found of measuring dopamine in stalk blood using liquid chromatography coupled with an electrochemical detector system[31] and by radioenzymatic assay techniques.[9]

Since the initial investigations of the 1960's and 70's, many other studies have now shown that dopamine has an important role in inhibiting prolactin secretion.[6, 10, 33, 34 & 23] Inhibition occurs via specific D2 dopamine receptors which are negatively linked to adenylate cyclase ie. DA reduces adenylate cyclase activity and inhibits cAMP accumulation.[35, 36 & 37]

Other mechanisms invoked at a subcellular level to explain DA effect on the lactrotroph include modulation of the electrical activity of the cells through an interaction of DA with a "receptor-potassium channel" complex located in the cytoplasmic membrane and, by a change of Ca^{++} flux. This mechanism may operate by maintaining a high (Ca^{++}) when DA is absent and the voltage dependent Ca^{++}channels are maximally activated; DA, by causing hyperpolarization of the membrane, may inactivate the membrane Ca^{++} channels which, in

turn, lead to a decrease in the cytosolic Ca^{++} thus inhibiting prolactin release.[38]

Anatomy of the Tuberoinfundibular Dopaminergic System

There are two major DA pathways from the hypothalamus to the pituitary gland. These are the incertohypothalamic and the (TIDA) pathways.[32] There are also projections from the periventricular DA system.[33] It is the TIDA system, however, which is directly involved in prolactin secretion. The perikarya of these TIDA neurons are located in the arcuate and periventricular nuclei of the medial basal hypothalamus and are further subdivided into two groups: the TIDA neurons itself, with terminals in the median eminence and the pituitary stalk, and the tuberohypophysial neurons, with terminals in the neural and the intermediate lobes of the pituitary.[32]

DA axon terminals are especially abundant in the median eminence where they are found close in proximity to other monoaminergic terminals, apendymal cells , and precapillary spaces of the portal vessels.[39] DA projections to the intermediate and neural lobes originate from the anterior and central portions of the arcuate nucleus, respectively.[33] TIDA neurons lack a high affinity transport system for DA and thus more of this monoamine is available for transport by the blood away from the terminals.[40]

There is considerable diversity within the TIDA system and this has been well reviewed by Ben-Jonathan.[33] The implication of this is that axon terminals of the two TIDA branches (to the posterior lobe

and to the median eminence) may have different susceptibilities to extrinsic modulating influences or to genetic defects.

Blood flow within the hypothalamo-hypophysial vessels:

DA is transported along a capillary network called the hypophysial portal capillaries in the median eminence, pituitary stalk and neural lobe which connects the hypothalamus and the pituitary gland. These capillaries are supplied by the superior, middle and inferior hypophysial arteries. Blood is able to flow both antegrade and retrograde, depending on the state of vasoconstriction in these vessels. Substances can thus be transported from the hypothalamus and posterior pituitary and vise-versa.[39]

DA secreted into the portal blood is derived primarily from a newly synthesized pool rather than from a storage pool and depends upon intact hypothalamic storage and monoamine oxidase activity.[41] The control of Dopamine synthesis itself may possibly influence prolactin secretion. In rats it has been shown that a reduced PTERIN cofactor (BH_4) is necessary along with tyrosine and O_2 for the synthesis of DA, and the administration of BH_4 increases DA synthesis in the striatum.[42 & 43]

Other Inhibiting Factors

Although DA is the main inhibitory substance operating on the lactotrophs, it seems it is not the only one because the amount of this catecholamine in the hypophysial blood, significantly reduces the amount of prolactin but does not completely supress its secretion.[10]

It is also possible that Dopamine tonically stimulates the secretion of another factor, PIF.[32 & 22]

Another reason to postulate some other inhibitory factor is that there is a discrepancy in the amount of Dopamine in the hypophysial stalk plasma necessary to inhibit prolactin in male rats as compared to female rats.[10] Although a second source of DA may be contributed to by the posterior pituitary,[33] this contribution is probably very small.[7]

DA and prolactin are both involved in an interplay with other releasing and/or modulating hormones and neurotransmitters.[33]

Factors affecting DA action on Prolactin and other inhibitory factors influencing Prolactin secretion.

1) Neuropeptides and Neurotransmitters: Because of reservations expressed above that DA was the only PIF, the search continued for other neurotransmitters and neuropeptides which may influence prolactin release. Although it now appears that GABA[10, & 33] the TRH metabolite histadyl-proline diketopiperazine[44 & 45] and the catechol oestrogen 2-hydroxyestradiol[46] all inhibit prolactin, it is unlikely

that any are tonic inhibitors of spontaneous prolactin release.[10] However, GABA may play a role in regulating prolactin and LH surges. Employing a push-pull cannula[47] it has been shown that mechanisms regulating prolactin and luteinizing hormone surges reside in the MPO/AH and this may be causally related to and modified by Estradiol (E_2) secretion, which, in turn, may be related to GABA release. GABA may also inhibit the release of either another nondopaminergic PIF, or a substance which tonically inhibits the release of a PRF.[47]

GABA has a dual effect on prolactin secretion, one component of this GABAergic pathway exerts a stimulatory effect on prolactin via inhibition of TIDA neuron function and an inhibitory component probably acts via specific GABAergic receptors.[48 & 49]

The most important nondopaminergic factor inhibiting prolactin release appears to be secreted as a $GnRH^{50}$ associated peptide (GAP) recently isolated by Seeberg et al using genetic recombination techniques to synthesize the fragment which is part of the GnRH precursor and is separated from the GnRH peptide by 3 amino-acids (GlyLys Arg).[42 & 43] It is found in hypothalamic neurons coexisting with $GnRH^{50}$ and both GnRH and GAP are secreted in situations where there is an inverse relationship between prolactin and gonadotrophin secretion and where it has been invoked as a possible explanation for this.[43]

Conversely, reduced (or absent) GnRH and GAP release into the hypophysial portal vessels would lead to high prolactin and low

gonadotrophin concentrations in plasma.[42]

The conclusions postulated about GAP have been critisized for a number of reasons[42] but further research will undoubtedly clarify this issue in due course.

Other neurotransmitters and peptides which have been postulated to inhibit prolactin release from the pituitary include noradrenalin,[10, 51 & 52] acetylcholine[34, 53, 10, 10 & 51] and prolactin itself.[10, 33, 54 & 59]

Histamine seems to have a dual action on prolactin secretion, depending on which receptors, H1 or H2, are operative.[55, 56 & 10] The former stimulates prolactin secretion, perhaps via an interaction with TIDA neurons,[56] while the latter results in inhibition of prolactin secretion, possibly mediated via nor adrenalin.[57]

The effect of noradrenalin seems less consistent than that of DA and its physiological role is uncertain.[10]

This action is probably very complicated as has been highlighted in in-vivo experimentation utilizing intrahypothalamic and intrapituitary perfusion studies using a push-pull cannula and measuring catecholamines (and other neuropeptides) draining the median eminence through portal vessels to the pituitary.[47]

Noradrenalin turnover in different parts of the hypothalamus may differ because the projections of the NE axon terminals are diffuse with little or no topographical orientation.[47] Thus the effect of the catecholamine may even differ in opposing directions ie. stimulating or inhibitory, depending on different structures within the hypothalamus.[47] Modulation by estradiol receptors or receptive neurons may also determine nor adrenalin action. DA itself probably acts on NE release indirectly in the MBH, disinhibiting its release in male rats.[47]

Acetylcholine (AcCh) probably inhibits phasic or induced secretion of prolactin[34] Evidence for this is that when the acetylcholine content in the central nervous system, is increased by physostigmine, prolactin decreases.[58] However, cholinergic agonists or antagonists probably do not affect basal prolactin levels.[59] Muscarinic and nicotinic antagonists block oestrogen induced prolactin release.[34] In other conditions stimulation of nicotinic and muscarinic receptors may give opposite actions[59] and some of these actions may be dependent on the dose of the respective neurotransmitters.[34]

Prolactin itself has a negative feedback on its own secretion.[10, 33, 54, 60, 61 & 63] by causing an increase in the rate of DA synthesis and turnover in the TIDA nerve terminals in the median eminence after a delay of 12-16 hours.[61] This action of prolactin requires protein synthesis as it is blocked by pretreatment with cycloheximide and thus it has been thought that its action on TIDA

neurons may either be a direct one or an indirect action altering the activity of afferent neuronal systems projecting onto the TIDA neurons.[61]

This short feedback loop of prolactin on the TIDA or other neuronal networks is probably the most important feedback mechanism of prolactin as no identifiable target organ hormone(s) have been isolated.[33] Two possible interdependent components of prolactin action exist.[61] The first is a rapid tonic component which mediates short term changes in TIDA neuronal activity in response to acute changes in circulating prolactin. The second component is the induction of a delayed component, which responds to prolonged increases in plasma Prolactin and modifies the capacity of the tonic component.[61]

The physiological significance of this may be to prevent rapid depletion of anterior hypothalamic prolactin stores by acute stimuli to prolactin release eg. suckling where within minutes the prolactin concentration in the blood may rise ten fold.[54]

Prolactin Releasing Substances

There has been a profusion of substances reported as stimulating prolactin release by direct stimulation of the pituitary lactotrophs, the most important of which is TRH which will be discussed below (see page 17) with suckling. It has been pointed out however, that the capacity of any one of these peptides to cause the release of prolactin, does not necessarily mean that they are releasing hormones in the physiological sense, even though some have been measured in hypophysial stalk plasma.[10] Experimental design has focussed on prolactin release in vitro but this has limited verification in the in vivo situation after dopamine restraint.[47]

These substances include VIP,[62, 63, 64, 65, 66, 67] serotonin,[68] neurotensin,[69] bombesin,[70] angiotensin II,[71] vasopressin,[72] substance P,[69] epidermal growth factor,[73] cholecystokinin,[62] estradiol,[74] and GnRH,[4 & 75] B-endorphan,[76] met enkephalin,[76] and leu-encephalin,[77]

The endogenous opioids, the endorphans and the enkephalins, may increase prolactin secretion not only by a decrease in the release and synthesis of DA, but also by increasing the uptake of DA into TIDA neurons, thereby decreasing the availability of DA for transport via portal vessels to the anterior pituitary.[33 & 78]

The main action of the opioids on prolactin secretion is directed at the hypothalamus, but whether they modify prolactin release under normal conditions or only in the stress related rise in prolactin, is uncertain. However, it seems possible/likely, that the stress

related rise in prolactin which overcomes normal DA inhibition, may also be related to an opioid related release of prolactin- releasing hormone.[33] Both μ- and k-opiate receptors mediate the increases in prolactin.[79]

Factors which modify Prolactin release

The action of ovarian steroids on Prolactin release:

Although ovarian steroids modify prolactin release, the exact mechanisms remain controversial[33] mainly due to the fact that these steroids have an intergrated action on brain and pituitary, and prevent a clear distinction between primary and secondary effects. Secondly, they regulate the secretion of other reproductive hormones (e.g.GnRH) which may influence prolactin secretion indirectly. Estrogens (E_2) and progesterone have synergistic or antagonistic actions depending on their relative concentrations in plasma and on their rate of change and are also involved in prolactin synthesis and possibly in regulating prolactin receptors. Short term E_2 treatment increases hypothalamic DA synthesis and turnover rate as well as DA concentration in portal blood.[33] However, administration of β-estradiol acutely suppresses dopamine secretion into portal blood.[80] Estrogen may also antagonise the inhibitory action of dopamine on prolactin secretion by reducing the capacity of the lactotrope to incorporate dopamine into prolactin secretory granules.[81] It has also been postulated that Estrogen may directly affect the release of prolactin from rat lactotrope cells in vitro.[82]

In the human menstrual cycle, there is no clear cyclical change in prolactin secretion but oestrogen may be responsible for the increased prolactin levels in the ovulatory and luteal phases as there is a positive correlation between prolactin and E_2 at these times.[6, 2 & 83]

Diet influences both prolactin and growth hormone release in man. It appears to do so by an action of carbohydrate (high) and fat (low) on REM sleep. Nocturnal release of prolactin seems to be dependent on alternating REM and non-REM sleep and, by increasing the CHO and decreasing the fat in the diet, prolactin secretion is reduced.[84]

Prolactin Releasing Factors and Suckling.

A prolactin releasing factor has been well documented in birds[85] and has been postulated in mammals.[10, 33, 34, 86 & 87] TRH releases prolactin from prolactinoma cells in culture[88] in dissociated pituitary cell preparations[89] and in-vivo in certain conditions.[90 & 91] TRH acts via membrane receptors and its uptake into prolactin cells seems to occur followed, possibly, by binding of the peptide to a nuclear component.[92]

Although TRH may be important in the suckling reflex it is uncertain whether this peptide plays a part in prolactin secretion under physiological conditions.[83, 85 & 53] This controversy arises because most stimuli resulting in an elevated prolactin do not concommitantly result in an increase in TRH (and vise-versa).[53] However, suckling is associated with a concommitant rise of TRH and prolactin secretion in the rat.[7, 10 & 33] Results of various investigations[10] point to the fact that the prime mediator of increase in prolactin responsiveness to TRH remains to be identified, but that one of these factors may be estradiol.[10] Somatostatin may also modify the TRH effect on prolactin secretion.[93]

At a cellular level, it has been strongly suggested that TRH stimulates prolactin secretion via hydrolysis of Phosphotydil bisphosphate ($Ptd(4,5)P_2$) to Inositol triphosphate ($Ins\ P_3$) (possibly by directly stimulating phospholipase C) and thus increasing the intracellular calcium which, in turn, causes

exocytosis of prolactin.[94 & 95] Alternatively, TRH may have a dual effect on the intracelluar Ca^{++} pool - a rapid discharge and then a more sustained, slower onset via diacylglycerol accumulation and protein kinase C activation.[95]

Another receptor ligand stimulated intracellular process for prolactin may be arachadonic acid acting via c-AMP[96] and serine proteases.[97]

SUCKLING:

A comprehensive review of this subject has been dealt with by numerous authors.[7, 10 & 33]

The neuroendocrine reflex induced by suckling gives rise to an acute, massive rise of prolactin.[53] The mechanism explaining this rise is speculative but may either be due to a decrease in DA or an increase in a stimulator (PRH) or both.[33] Stimulators (PRH) which have been implicated include TRH[86, 87 & 91] and VIP[33] but the data is inconclusive.[33, 98, 86, 90, 99, 83, 89, 86 & 87] The evidence that this rise is due to a decrease in dopamine alone is also inconclusive[33, 100 & 101] and the detailed physiological significance of dopamine dynamics occuring in conscious mothers during suckling has not been resolved.[10]

It has been suggested that the brevity and magnitude of this decrease in dopamine alone is not enough to account for the prolactin surge during suckling.[98] The problem of interpretation is

made even more difficult by the fact that other peptide neurotransmitters are released at the same time and luteinizing hormone and follicle stimulating hormone are inhibited.[10] Thus the relative importance of all these factors provide complexity which is difficult to interpret in isolation and may be the result of an interaction of all these different substances presumably acting via releasing factors from the hypothalamus.

Two interdependent routes may govern prolactin secretion during suckling.[33] One route includes the TIDA neurons, PRH and the long portal vessels. The second involves the tuberohypophysial DA neurons, PRH and the short portal vessels. Prolactin and other modulators of prolactin secretion supposedly exert their major effect on the hypothalamus long portal vessels.[33] The synchronous release of prolactin and oxytocin during suckling may be regulated in the posterior pituitary but if this is so then prolactin activity will have to be verified in this area.[33]

The physiological role of serotonin in the suckling induced release of prolactin is well established.[33, 100, 34, 85, 101] Although serotonin itself probably does not increase prolactin secretion, the neuronendocrine reflex which occurs in suckling acts via serotoninergic pathways arising in the midbrain dorsal raphe nucleus in the hypothalamus.[33, 34] The effects of serotonin may be modified by gonadal steroids.[33] It stimulates prolactin release probably via a PRH release but may also involve dopaminergic and opiate mechanisms.[33]

Thus, from this short review it is apparent that the control of prolactin secretion occurs at the level of the pituitary and the hypothalamus and that numerous hormone peptides and neurotransmitters, acting either on their own or in a complicated synergistic or antagonistic fashion with other factors, bring about an overall summation to effect final prolactin secretion and that yet other peptides may modulate this effect. There appears to be much controversy in elucidating which of these factors has particular relevance in the physiological in vivo situation but dopamine restraint of prolactin secretion is of central importance in this control.

PROJECT

To investigate the role of GnRH in releasing synchronous spikes of LH and prolactin in the luteal phase of the menstrual cycle. A potent GnRH antagonist was administered to three women in the luteal phase of their menstrual cycle and the effect that this had on LH and prolactin secretion (and FSH) observed. Initially the dose response curve to the antagonist was established by administering incremental doses of the antagonist to a subject and finding the dose which would completely block LH secretion. Once this had been established, a supraphysiological dose of the antagonist was used in the subsequent experiments as described hereafter.

AIMS

1. To establish the dose of a GnRH antagonist (Ac-D-Nal(2), D-α-Me-4-Cl-Phe^{2}, D-Tryp^{3}, D-Arg^{6}, D-Ala^{10}), which would block GnRH release at the GnRH receptor site in the hypothalamus. This dose was then administered to three normally menstruating women in the luteal phase of the menstrual cycle and luteinizing hormone and prolactin responses were measured at approximately 15 minute intervals over a period of 6-7 hours.

2. To establish whether GnRH releases luteinizing hormone and prolactin from the anterior pituitary in the luteal phase of the menstrual cycle.

SUMMARY

Methods Used

Methods used to investigate the role of GnRH in the luteal phase of the menstrual cycle have focussed on either indirect means of observation or observation of hormone levels after the administration of GnRH. In the experimental design of the protocol which follows, a potent GnRH antagonist was administered in the luteal phase and the effect on luteinizing hormone, prolactin and follicle stimulating hormone observed. The results obtained suggest the GnRH does not control the release of prolactin secretion as was formerly believed.

Introduction

It has been postulated that prolactin and luteinizing hormone share a common releasing factor, possibly GnRH (or LHRH) or that the mechanism producing synchronous spikes which occur in the luteal phase of the menstrual cycle related to a periodic decrease in tuberoinfundibular (TIDA) neuronal activity via inhibitory dopaminergic mechanisms. GnRH may also act indirectly to modulate TIDA neuron activity [4].

Braund et al [4] showed that there was a prolactin response to GnRH in the luteal phase and suggested that the observed synchrony in luteinizing hormone and prolactin secretion at this stage of the menstrual cycle was due to a physiological response of both the gonadotrope and the lactotrope cells to endogenous GnRH.

However, this was based on indirect evidence only. Using a potent GnRH antagonist, -D-Tryp^{3} D-Arg^{6} -, synchronous luteinizing hormone and prolactin secretory spikes in the luteal phase of four normally menstruating women were analysed and the role of GnRH inferred from this method.

The study initially focussed on the dose of GnRH antagonist which would be sufficient to block luteinizing hormone (and perhaps also follicle stimulating hormone) from the pituitary gland and to observe any side effects which occurred on administration of this peptide. Once the blocking dose of the antagonist for luteinizing hormone was established, the effect on prolactin secretion was observed during the luteal phase of the cycle.

Subjects, Materials and Method

Four women with regular, normal menstrual cycles participated in a two day trial after signing informed consent. All had been quite healthy and were not taking any medication, including the oral contraceptive pill. None had a history of allergy or eczyma. Not all subjects were able to spend two consecutive days in hospital and thus the protocol allowed for blood samples for the basal levels of luteinizing hormone and prolactin to be taken in the luteal phase of one cycle and again after the antagonist had been administered during the luteal phase of the next menstrual cycle. Serum progesterone concentrations were assessed to check that the test was performed during the luteal phase. Although the subjects were not confined to bed rest, they were encouraged to rest as much as possible during the test. There was no prohibition on eating or drinking and although cigarette smoking was not proscribed it was discouraged for the period of the test.

Subject 1 C.W. Age 42 years.

Aim: To assess the dose response curve.

The test was performed on Day 18 of a 30 day cycle. An intravenous cannula was inserted via the antecubital vein and was kept patent with a heparin - saline solution.

Samples of blood were drawn at 20 minute intervals and sent for luteinizing hormone, prolactin, and follicle stimulating hormone. At time 0 minutes, 10 μg of the antagonist was given. This was repeated in incremental doses at 60 minutes, 120 minutes and 180 minutes and the doses given were 30μg, 100μg, and 300μg respectively. The serum

progesterone was <20 and it is therefore uncertain whether the test was performed during the luteal phase. The test was repeated a month later, using higher doses of the antagonist (300μg, to 1000 μg) and three and a half hours later 10μg of (GnRH) was given to see whether there was any response or whether the blocking action of the antagonist was still apparent. This amount of GnRH was repeated at hourly intervals (three times).

Subject 2 S.D. Age 24 years.

On Day 18 of a 30 day cycle, blood was collected at 15 minute intervals for luteinizing hormone, follicle stimulating hormone and prolactin, for a total period of 6 hours, 45 minutes. A basal progesterone level was taken. On the subsequent day, basal luteinizing hormone, follicle stimulating hormone and prolactin were again collected for one hour and forty-five minutes at fifteen minute intervals. Thereafter, at time 0, 100μg of the antagonist was given intravenously and 500μg boluses given at time 1 hour, 2 hours and 3 and a half hours.

The results are tabulated below.

Subject 3 P.E. Age 26 years.

The test was performed during the luteal phase of one cycle to obtain basal levels and then repeated in the luteal phase of the subsequent menstrual cycle after administration of the antagonist as described for Subject 2. The results are tabulated below.

Subject 4 H.L. Age 45 years.

Basal levels were performed on Day 22 of a 28 day cycle. The antagonist was administered on Day 17 two cycles after the initial cycle. A similar protocol as that for Subject 2 (above) was used. The results are tabulated below.

Materials: luteinizing hormone and follicle stimulating hormone were measured using Amersham RIA kits. The intra-assay coefficient of variation for luteinizing hormone was 3,7% for the low value (10 mIU/ml) and 3,5% for the high value (59 mIU/l) with 2,2% for 15 mIU/l and 2,8 % for 36 mIU/l. The interassay coefficient of variation was 8,1% for 10 mIU/l, 4,3% for 15 mIU/l, 5,3% for 36 mIU/l and 6,2% for 59 mIU/l.

The intra-assay coefficient of variation for follicle stimulating hormone was 3,2% for the low value (5,9 mIU/ml) and 5% for the high value (47 mIU/l) with 3,1% for 13 mIU/l and 5,1 % for 25 mIU/l. The interassay coefficient of variation was 8,4% for 5,9 mIU/l, 5,4% for 13 mIU/l, 5,1% for 25 mIU/l and 10,3% for 47 mIU/l.

The values for progesterone were:- Intra-assay coefficient of variation: 8,4% for the low value (1,5 mIU/ml) and 5,8% for the high value (18 mIU/l) with 7,5% for 3,2mIU/l.
The interassay coefficient of variation was:
10,0% for 8,4 mIU/l, 6,6% for 3,2mIU/l and 7,2 for 18mIU/l.

The progesterone was measured using a Diagnostic Products

Coat-a-count kit (RIA).

Prolactin was measured using a Serono (RIA) kit. The intraassay coefficient of variation was 6,2%. The intraassay coefficient of variation was not given.

GnRH used for the dose response curve was Relifalt from Hoechst.

The GnRH antagonist was made in the protein synthesis peptide laboratory of the University of Cape Town, Medical School and had been tested for purity, allergic free properties and side effects. The antagonist has the chemical formula:

Ac-D-Nal(2),D-α-Me-4-Cl-Phe^{2}, D-Tryp^{3}, D-Arg^{6}, D-Ala^{10}

Results

Subject 1 (Table 1 and Fig.1): Although incremental doses of the antagonist blocked luteinizing hormone secretion, there was less of a sustained effect on prolactin secretion. With higher doses of the antagonist (Table 2 and Fig.2) this effect was even more pronounced for LH but the effect on prolactin was minimal. Luteinizing hormone secretion is partially blocked after a dose of 300μg of the antagonist and completely inhibited after 500μg. Prolactin and follicle stimulating hormone are not significantly affected. After 1000 g of the antagonist was given at time 2 hours, the luteinizing hormone concentration remained suppressed for at least 1 hour. Subsequent administration of 10μg GnRH caused an increment in luteinizing hormone and prolactin.

From this experiment it was established that 500μg of the antagonist would block luteinizing hormone and that the effect would last for 1 hour at least. It was also found that hourly boluses thereafter of 500μg of the antagonist would also block luteinizing hormone release. 1000μg of the antagonist, i.e. twice maximum, was used in subsequent experiments to be sure that maximum blocking or antagonism occurred.

Although the first set of values (Table 1) was associated with a low progesterone level (i.e. probably not in the luteal phase) it was felt that this would not affect the dose response curve of the antagonist on luteinizing hormone.

NAME	AGE	SEX	LMP	DRUG
C.W.	24 years	F	Luteal	Ant.6

Sample Number	Time	Real time	Dose (antagonist)	LH	FSH	PRL
1	-20 min		-	12		14.9
2	0		10ug	8.6		12.3
3	20		-	8.1		10.7
4	40		-	7.9		11.9
5	60		30ug	7.7		10.4
6	80		-	9.7		9.8
7	100		-	7.7		9.4
8	120		100ug	7.8		8.6
9	140		-	7.2		7.3
10	160		-	7.6		8.6
11	180		300ug	5.8		8.9
12	200		-	6.9		9.2
13	220		-	6.8		9.3
14	240		-	7.0		10.8
15	260		-	6.4		9.3
16	280		-	-		-
17	300		-	5.9		10.7

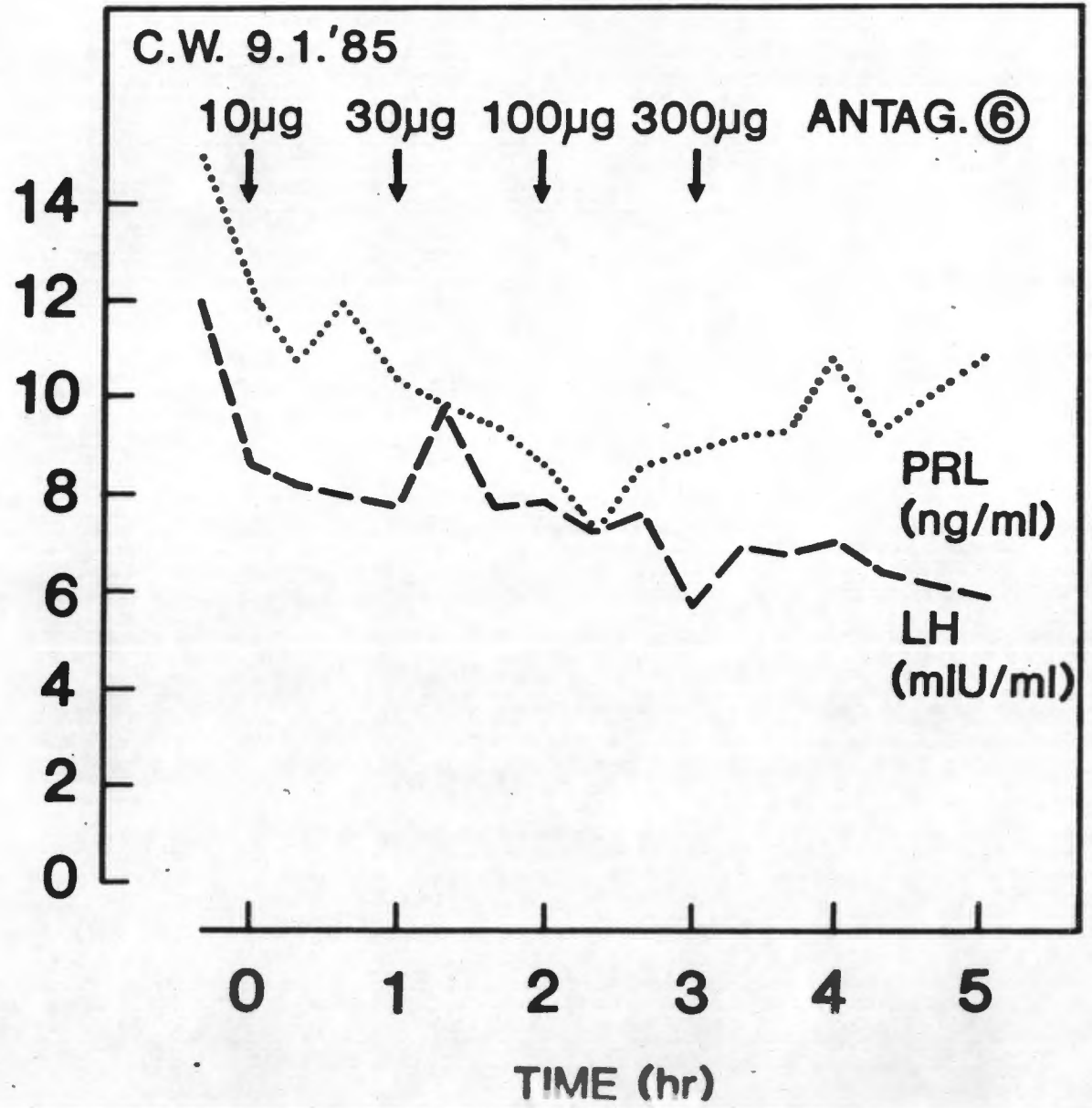
Table 1

Dose-response values of antagonist action on LH and prolactin:
Subject 1

(LH in mIU/ℓ and prolactin in ng/ℓ)

Graph showing dose - response curve of the Antagonist - action on LH and prolactin.

FIG 1



NAME	AGE	SEX	LMP	DRUG
C.W.	24	F	Luteal	Antagonist 6 LH - RH

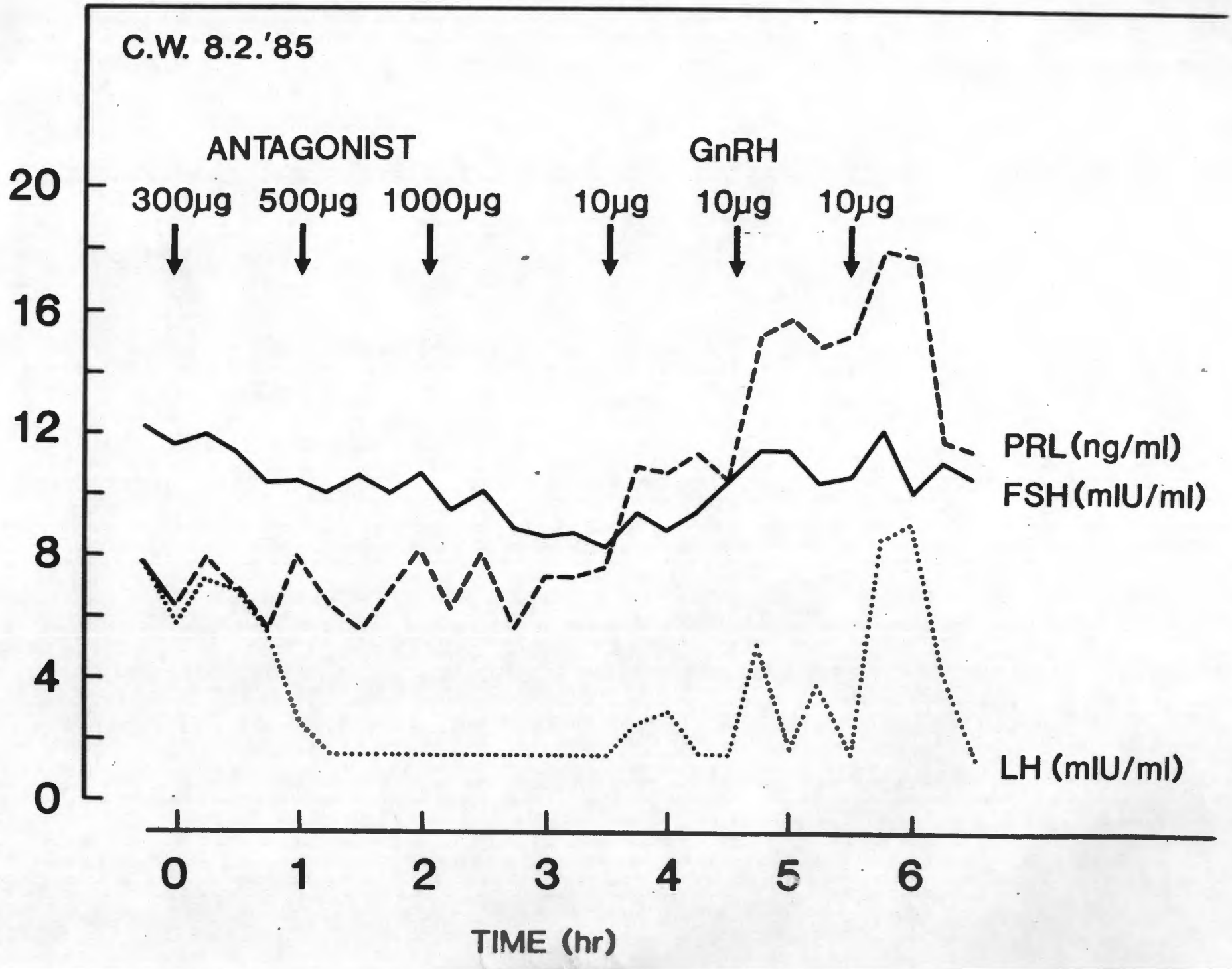
Sample Number	Time 8.2.85	Real Time	Dose	LH	FSH	PRL
1	-20"	9h40	-	7.8	12.2	7.8
2	0	10h00	300ug ant	5.9	11.6	6.3
3	15"	10h15	-	7.2	11.9	7.9
4	30"	10h30	-	6.8	11.4	6.9
5	45"	10h45	-	5.5	10.3	5.6
6	1 hour	11h00	500ug ant	2.6	10.4	7.9
7	1'15"	11h15	-	1.5	10.0	6.5
8	1'30"	11h30	-	1.5	10.6	5.6
9	1'45"	11h45	-	1.5	10.0	6.8
10	2 hours	12h00	1000ug ant	1.5	10.7	8.2
11	2'15"	12h15	-	1.5	9.5	6.3
12	2'30"	12h30	-	1.5	10.1	8.1
13	2'45"	12h45	-	1.5	8.9	5.7
14	3 hours	13h00	-	1.5	8.6	7.3
15	3'15"	13h15	-	1.5	8.7	7.3
16	3'30"	13h30	10ug LH-RH	1.5	8.3	7.6
17	3'45"	13h45	-	2.5	9.5	10.9
18	4 hours	14h00	-	2.9	8.9	10.7
19	4'15"	14h15	-	1.5	9.5	11.4
20	4'30"	14h30	10ug LH-RH	1.5	10.5	10.5
21	4'45"	14h45	-	5.1	16.5	15.2
22	5 hours	15h00	-	1.8	11.5	15.8
23	5'15"	15h15	-	3.8	10.4	14.9
24	5'30"	15h30	10ug LH-RH	1.5	10.6	5.3
25	5'45"	15h45	-	8.5	12.1	18.0
26	6 hours	16h00	-	9.1	10.1	17.8
27	6'15"	16h15	-	4.3	11.1	11.8
28	6'30"	16h30	-	1.5	10.5	11.5

Table 2

Dose - response values obtained for LH, FSH and prolactin after administration of the antagonist: Subject 1

(LH and FSH in mIU/ℓ and prolactin in ηg/mℓ)

Dose - response curve of Antagonist action on LH, FSH and prolactin
FIG 2



Subject 2: The basal luteinizing hormone and prolactin levels showed synchronous peaks occurring at approximately time 0 (beginning of the test) and lasting for fifteen minutes and another at time 6 hours 30 minutes lasting for more than fifteen minutes (see Table 3 and Fig.3 and 4).

The GnRH antagonist appeared to block the initial peak of LH which occurred at minus 1 hour but did not seem to have any significant effect on prolactin or FSH peaks. (Table 4 and Fig 3 & 4)

NAME	AGE	SEX	LMP	DRUG
SD	24	F	now day 18	Antag. 6

Sample Number	Time 11.3.85	Real Time	Dose (I.V.)	LH	FSH	PRL
1	09h30	0		8.9	5.5	7.4
2	09h45	15min		6.3	4.7	7.6
3	10h00	30min		7.0	5.0	6.5
4	10h15	45min		6.0	4.8	6.3
5	10h30	1hr		6.1	4.79	6.4
6	10h45	1'15"		4.7	4.4	7.5
7	11h00	1'30"		4.8	4.0	7.3
8	11h15	1'45"		5.1	4.2	8.7
9	11h30	2hr		4.1	4.1	9.4
10	11h45	2'15"		4.2	3.7	8.6
11	12h00	2'30"		3.8	4.4	6.0
12	12h15	2'45"		3.5	3.4	7.7
13	12h30	3hr		3.1	3.6	7.1
14	12h45	3'15"		6.0	4.4	7.3
15	13h00	3'30"		8.5	3.4	8.2
16	13h15	3'45"		8.1	3.4	11.3
17	13h30	4hr		6.0	4.1	9.3
18	13h45	4'15"		5.3	3.5	7.4
19	14h00	4'30"		6.2	3.9	4.7
20	14h15	4'45"		5.1	3.87	6.4
21	14h30	5hr		4.0	3.5	9.5
22	14h45	5'15"		5.0	3.9	7.0
23	15h00	5'30"		7.7	3.1	8.7
24	15h15	5'45"		5.4	3.3	9.5
25	15h30	6hr		6.5	3.1	9.3
26	15h45	6'15"		3.5	3.8	8.5
27	16h00	6'30"		10.5	3.3	11.6
28	16h15	6'45"		miss*	3.8	12.9

*missing

Progesterone 40.3 nMol/ℓ

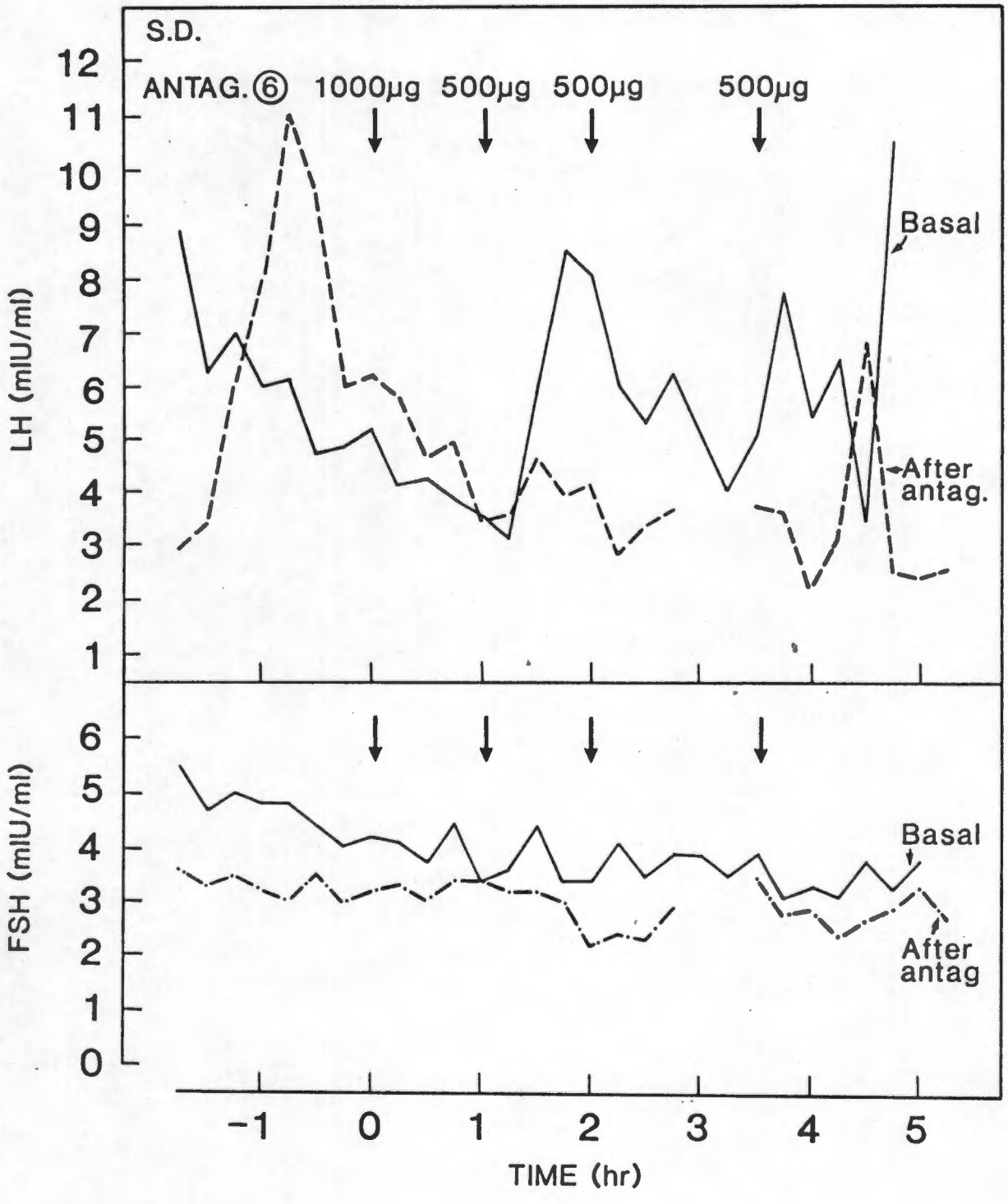
Table 3

Basal measurements (values) of LH, prolactin and FSH in the luteal phase of the menstrual cycle: Subject 2

(LH and FSH in mIU/ℓ and prolactin in ng/mℓ)

Subject 2 : Graphs showing the effect of the GnRH antagonist on LH and FSH

FIG 3



NAME	AGE	SEX	LMP	DRUG
SD	24	F	day 19	Ant. 6

Sample Number	Time 12.3.85	Real Time	Dose	LH	FSH	PRL
1	09h15	-1'45"	-	2.9	3.6	7.3
2	09h30	-1'30"	-	3.4	3.3	7.2
3	09h45	-1'15"	-	6.0	3.5	6.1
4	10h00	-1'00"	-	8.0	3.2	5.7
5	10h15	-45"	-	11.0	3.0	5.8
6	10h30	-30"	-	9.6	3.5	6.8
7	10h45	-15"	-	6.0	3.0	6.2
8	11h00	0	1000ug	6.2	3.2	4.4
9	11h15	15"	-	5.8	3.3	5.4
10	11h30	30"	-	4.6	3.0	5.9
11	11h45	45"	-	4.9	3.4	4.9
12	12h00	1 hour	500ug	3.4	3.4	5.0
13	12h15	1'15"	-	3.5	3.2	6.4
14	12h30	1'30"	-	4.6	3.2	6.4
15	12h45	1'45"	-	3.9	3.0	10.3
16	13h00	2 hours	500ug	4.1	2.2	8.4
17	13h15	2'15"	-	2.8	2.4	7.2
18	13h30	2'30"	-	3.3	2.3	8.5
19	13h45	2'45"	-	3.6	2.9	8.1
22	14h30	3'30"	400ug	3.7	3.5	10.1
23	14h45	3'45"	-	3.6	2.8	8.4
24	15h00	4 hours	-	2.2	2.9	7.3
25	15h15	4'15"	-	3.1	2.4	8.4
26	15h30	4'30"	-	6.8	2.7	6.5
27	15h45	4'45"	-	2.5	2.9	9.0
28	16h00	5 hours	-	2.4	3.3	8.6
29	16h15	5'15"	-	2.5	2.7	7.7

Progesterone 60.8 nMol/ℓ

Luteal phase: progesterone $>$ 20 nMol/ℓ

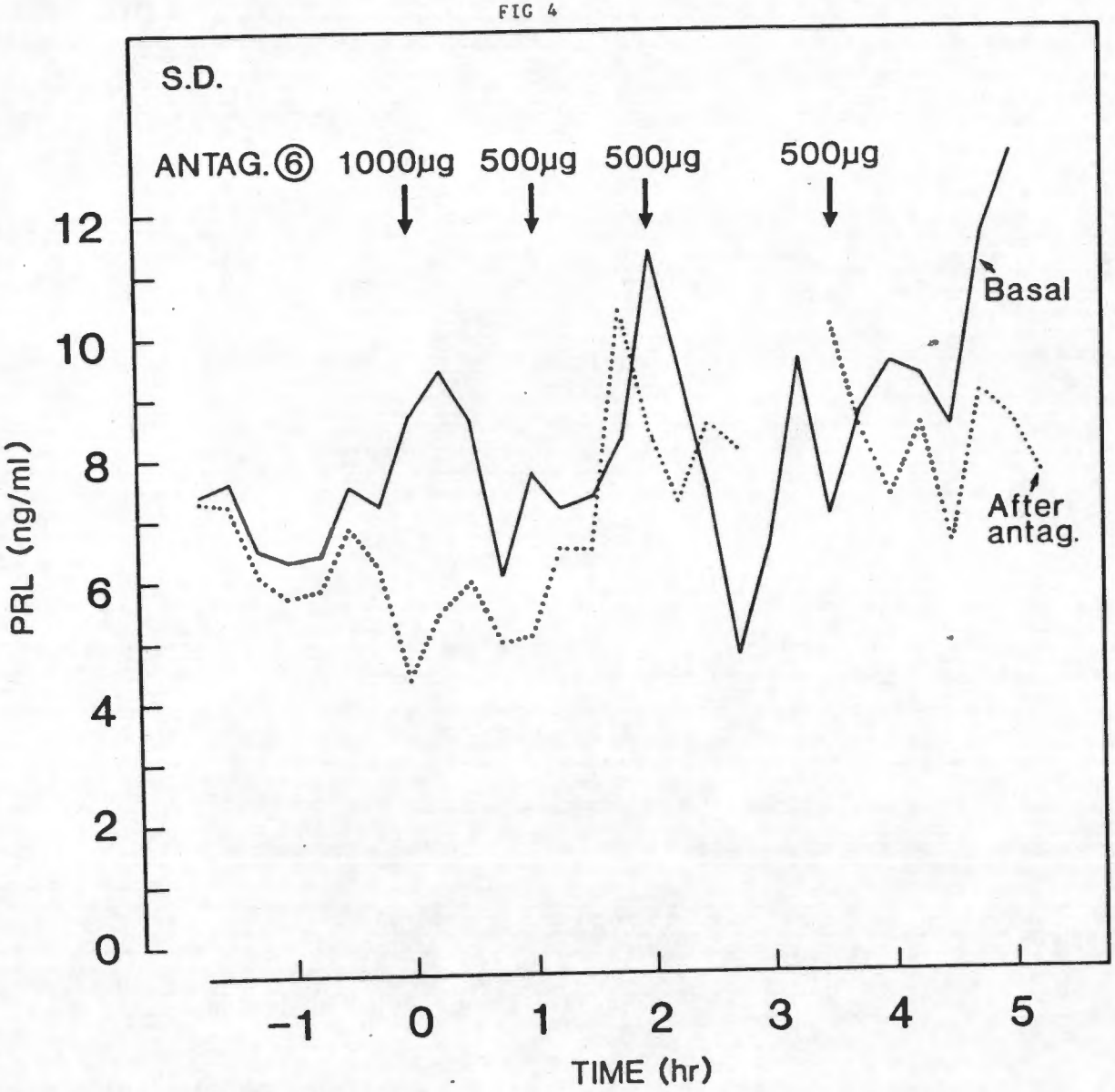
Table 4

Serum LH, prolactin and FSH values in the luteal phase of the menstrual cycle after administration of the GnRH antagonist:

Subject 2

(LH and FSH in mIU/ℓ and prolactin in ng/mℓ)

FIG 4



Subject 2 : Graph showing the effect of the GnRh antagonist on prolactin

Subject 3: (Table 5 and Fig.5 & 6). Basal levels of the hormones showed synchronous peaks of luteinizing hormone peaks and prolactin occurring at time 0 and 2 hours 45 minutes. There was also a luteinizing hormone peak which occurred at 6 hours 30 minutes but this was not accompanied by a prolactin spike.

After administration of the antagonist, luteinizing hormone peaks seemed to be suppressed but there appeared to be little effect on either prolactin or follicle stimulating hormone. (Table 6 and Fig.5 & 6)

NAME	AGE	SEX	LMP 17/28	DRUG
P.E.	26 years	F	Day 17	Nil

Date: 21/5/85

Sample Number	Time	Real Time	Dose	LH	FSH	PRL
1	0	9h30		11.8	7.2	8.1
2	15"	09h45		9.7	7.1	7.0
3	30"	10h00		11.5	7.5	5.4
4	45"	10h15		8.4	7.4	4.9
5	1 hour	10h30		8.0	7.5	4.9
6	1'15"	10h45		8.4	6.9	5.4
7	1'30"	11h00		5.9	7.1	5.4
8	1'45"	11h15		7.8	7.8	4.1
9	2 hours	11h30		7.3	6.9	3.9
10	2'15"	11h45		5.3	6.8	4.3
11	2'30"	12h00		6.2	7.4	4.9
12	2'45"	12h15		13.4	7.5	13.6
13	3 hours	12h30		12.8	7.5	12.1
14	3'15"	12h45		10.3	7.2	10.1
15	3'30"	13h00		7.1	7.6	6.8
16	3'45"	13h15		5.9	6.7	6.3
17	4 hours	13h30		5.4	6.5	5.7
18	4'15"	13h45		5.2	6.2	5.4
19	4'30"	14h00		4.5	6.2	6.2
20	4'45"	14h15		5.4	6.4	5.2
21	5 hours	14h30		5.4	5.9	5.0
22	5'15"	14h45		3.1	6.2	4.7
23	5'30"	15h00		3.6	7.0	5.3
24	5'45"	15h15		3.7	5.8	5.8
25	6 hours	15h30		4.1	5.2	4.9
26	6'15"	15h45		8.4	5.5	4.7
27	6'30"	16h00		9.7	6.5	4.7
28	6'45"	16h15		9.2	5.8	4.8

Progesterone 46.8 nMol/ℓ

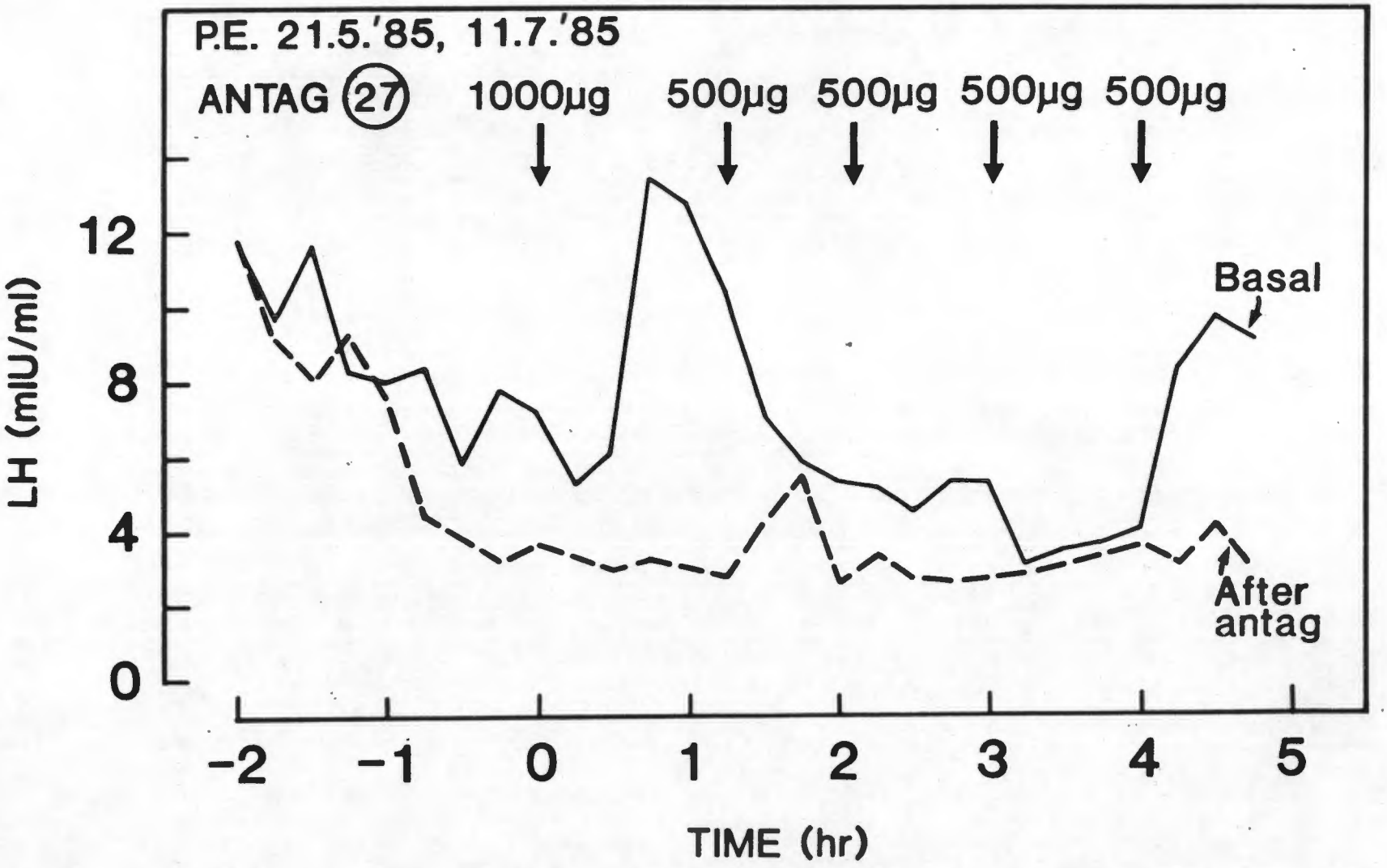
Table 5

Basal measurements (values) of LH, prolactin and FSH in the luteal phase of the menstrual cycle: Subject 3

(LH and FSH in mIU/ℓ and prolactin in ng/mℓ)

Subject 3 : Graph showing the effect of the GnRH antagonist on LH

FIG 5



NAME	AGE	SEX	LMP 18/28	DRUG
Date: 11/7/85	26 years	F		Antag.27

P.E.

Sample Number	Time	Real Time	Dose	LH	FSH	PRL
1	-2 hours	09h30	-	11.7	9.2	13.4
2	-1'45"	09h45	-	9.1	8.2	10.3
3	-1'30"	10h00	-	8.0	8.1	8.9
4	-1'15"	10h15	-	9.3	9.4	8.7
5	-1 hour	10h30	-	7.6	8.2	6.1
6	-45"	10h45	-	4.4	8.3	6.4
7	-30"	11h00	-	3.9	7.7	5.8
8	-15"	11h15	-	5.2	8.9	6.8
9	0	11h30	1000ug	5.7	8.0	7.1
10	15"	11h45	-	3.3	7.9	5.9
11	30"	12h00	-	3.0	8.1	7.1
12	45"	12h15	-	3.4	8.5	13.4
13	60"	12h30	-	-	-	-
14	1'15"	12h45	500ug	2.8	8.0	8.3
15	1'30"	13h00	-	4.3	8.7	7.8
16	1'45"	13h15	-	5.5	7.4	5.8
17	2 hours	13h30	-	2.7	7.2	5.8
18	2'15"	13h45	500ug	3.4	8.0	6.3
19	2'30"	14h00	-	2.8	7.2	5.6
20	2'45"	14h15	-	2.7	8.3	5.1
21	3 hours	14h30	500ug	4.8	7.9	5.0
22	3'15"	14h45	-	2.7	7.4	6.1
23	3'30"	15h00	-	3.1	7.7	6.6
24	3'45"	15h15	-	3.4	7.4	7.1
25	4 hours	15h30	500ug	4.6	8.3	6.2
26	4'15"	15h45	-	3.1	7.3	4.8
27	4'45"	16h15	-	4.2	8.2	6.2
28	5 hours	16h30	-	3.0	7.8	5.8

Progesterone 74.6 nMol/ℓ

Table 6

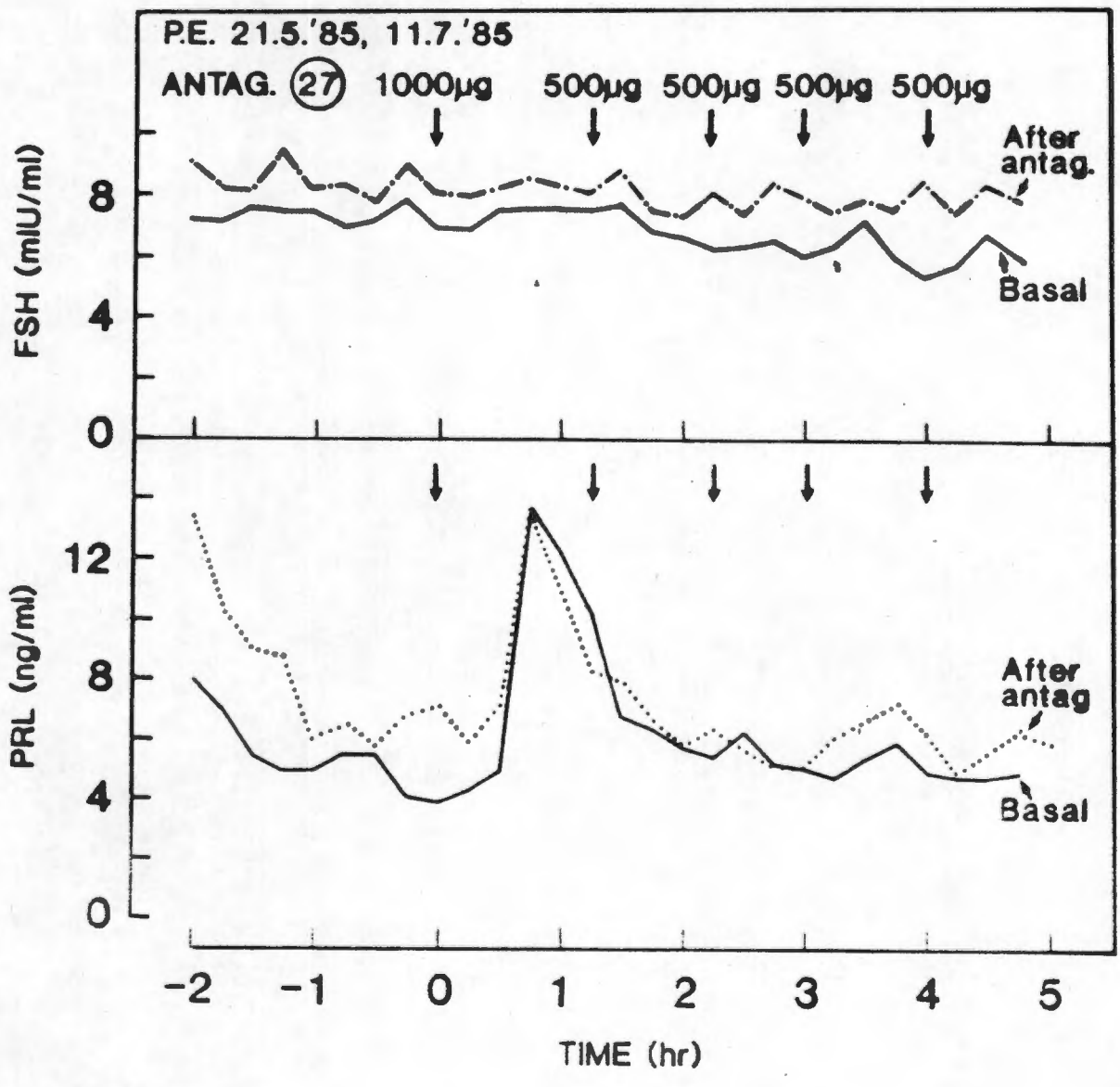
Serum LH, prolactin and FSH values in the luteal phase of the menstrual cycle after administration of the GnRH antagonist:

Subject 3

(LH and FSH in mIU/ℓ and prolactin in ng/mℓ)

Subject 3 : Graphs showing the effect of the GnRH antagonist on FSH and prolactin

FIG 6



Subject 4: (Table 7 and Fig.7 & 8) The peaks occurred in the basal period at time 1 hour 45 minutes and probably at time 6 hours and 15 minutes and the second peak was associated with a prolactin spike.

During the second part of the experiment using the antagonist (Table 8 and Fig.7 & 8) a luteinizing hormone peak occurred at the beginning of the sampling period (Not accompanied by a prolactin peak). The antagonist blocked the luteinizing hormone secretion most noticeably at time 3 hours and 4 hours but does not appear to have suppressed prolactin secretion or follicle stimulating hormone secretion.

NAME	AGE	SEX	LMP 22/28	DRUG
H.L.	45 years	F		Ant. 27

Date 22.8.85

Sample Number	Time	Real Time	Dose	LH	FSH	PRL
1	0	09h30		10.6	11.4	8.1
2	15"	09h45		8.9	10.5	6.9
3	30"	10h00		8.9	10.7	7.5
4	45"	10h15		7.6	9.9	6.3
5	1 hour	10h30		7.6	8.2	5.3
6	1'15"	10h45		9.7	9.8	9.8
7	1'30"	11h00		9.1	8.8	6.0
8	1'45"	11h15		17.8	10.9	7.8
9	2 hours	11h30		18.1	10.8	6.4
10	2'15"	11h45		15.7	10.0	8.3
11	2'30"	12h00		11.9	10.0	9.7
12	2'45"	12h15		12.8	10.7	9.8
13	3 hours	12h30		7.9	10.5	9.3
14	3'15"	12h45		8.5	10.9	8.4
15	3'30"	13h00		7.3	11.0	8.0
16	3'45"	13h15		6.0	9.9	8.0
17	4 hours	13h30		7.4	10.0	8.3
18	4'15"	13h45		6.0	9.0	8.8
19	4'30"	14h00		5.4	8.8	8.6
20	4'45"	14h15		8.3	9.6	9.8
21	5 hours	14h30		7.7	10.4	9.3
22	5'15"	14h45		6.9	9.3	8.6
23	5'30"	15h00		4.6	8.0	8.3
24	5'45"	15h15		3.5	8.6	8.6
25	6 hours	15h30		5.7	9.0	9.9
26	6'15"	15h45		11.9	9.6	12.2
27	6'30"	16h00		14.3	10.5	13.1
28	6'45"	16h15		9.7	10.0	13.7
29	7 hours	16h30		9.1	9.1	14.8

Progesterone 32.7 nMol/ℓ

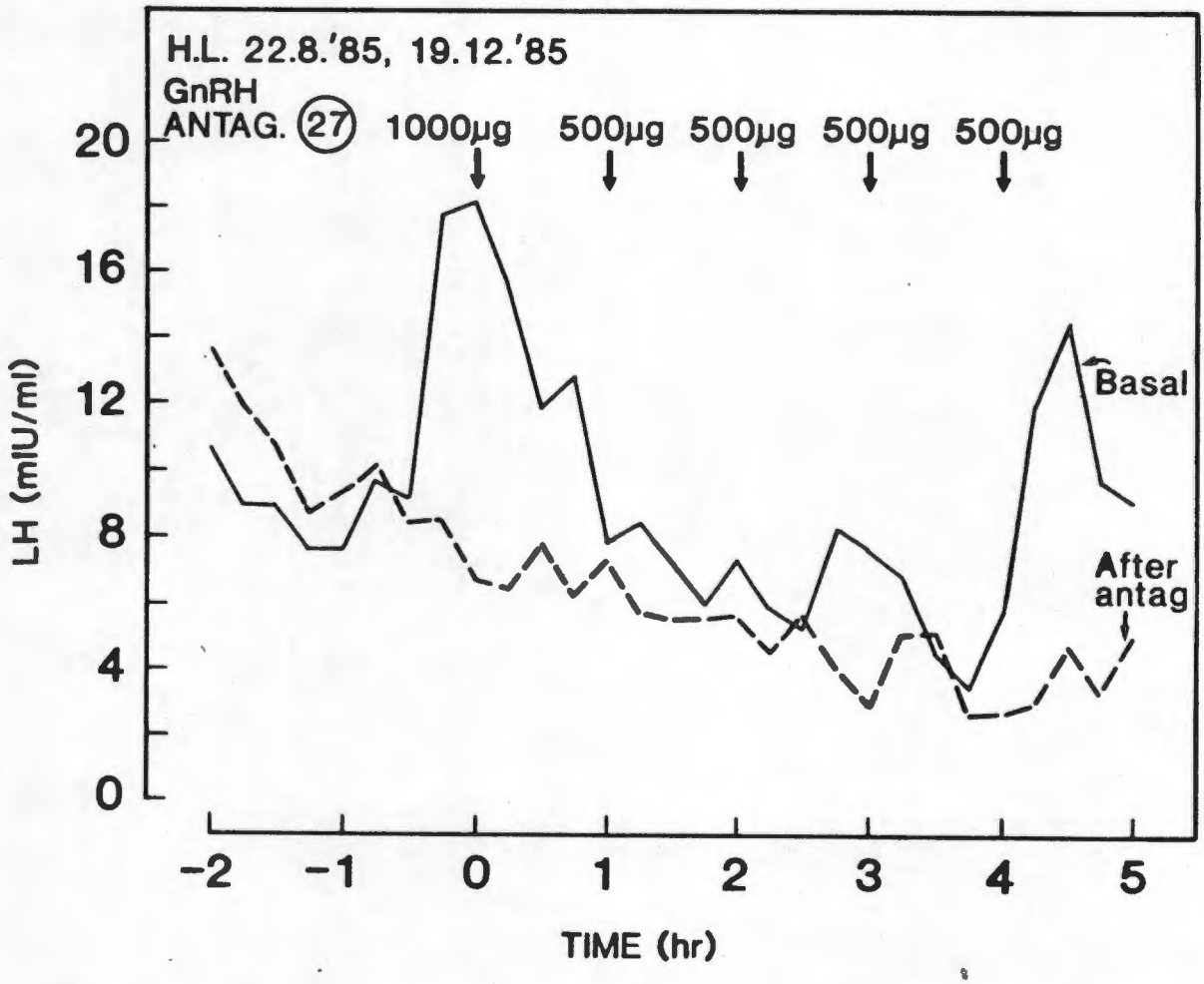
Table 7

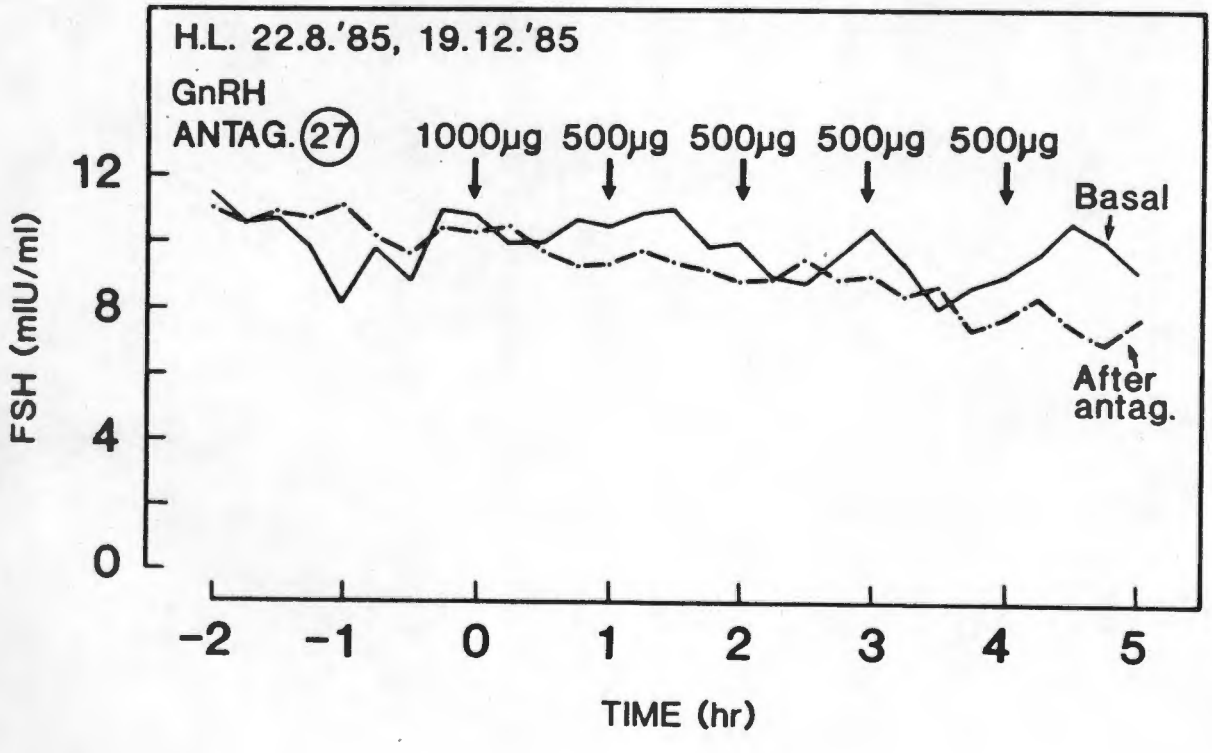
Basal measurements (values) of LH, prolactin and FSH in the luteal phase of the menstrual cycle: Subject 4

(LH and FSH in mIU/ℓ and prolactin in ng/mℓ)

Subject 4 : Graphs showing the effect of the GnRH antagonist on LH and FSH

FIG 7





NAME	AGE	SEX	LMP 17/28	DRUG
H.L.	45 years	F		

Date: 19.12.85

Sample Number	Time	Real Time	Dose	LH	FSH	PRL
1	-2 hours	09h00	-	13.7	11.0	9.7
2	-1'45"	09h15	-	11.8	10.5	8.7
3	-1'30"	09h30	-	10.6	10.8	8.5
4	-1'15"	09h45	-	8.7	10.7	8.5
5	-1 hour	10h00	-	9.3	11.1	7.6
6	-45"	10h15	-	10.1	10.1	6.9
7	-30"	10h30	-	8.4	9.7	7.9
8	-15"	10h45	-	8.5	10.4	7.5
9	0	11h00	1000ug	6.8	10.3	7.3
10	15"	11h15	-	6.5	10.5	7.1
11	30"	11h30	-	7.8	9.7	7.4
12	45"	11h45	-	6.4	9.3	7.9
13	1 hour	12h00	500ug	7.4	9.4	8.7
14	1'15"	12h15	-	5.9	9.8	8.5
15	1'30"	12h30	-	5.6	9.4	8.2
16	1'45"	12h45	-	5.6	9.1	8.7
17	2 hours	13h00	500ug	5.7	8.9	8.8
18	2'15"	13h15	-	4.6	8.9	8.9
19	2'30"	13h30	-	5.7	9.5	10.2
20	2'45"	13h45	-	4.1	8.9	10.6
21	3 hours	14h00	500ug	3.0	9.0	10.3
22	3'15"	14h15	-	5.1	8.4	10.0
23	3'30"	14h30	-	5.2	8.7	9.9
24	3'45"	14h45	-	2.7	7.3	8.9
25	4 hours	15h00	500ug	2.7	7.7	8.8
26	4'15"	15h15	-	3.0	8.3	8.3
27	4'30"	15h30	-	4.8	7.5	8.2
28	4'45"	15h45	-	3.4	7.0	8.3
29	5 hours	16h00	-	5.0	7.6	9.7

Progesterone 51.1 nMol/ℓ

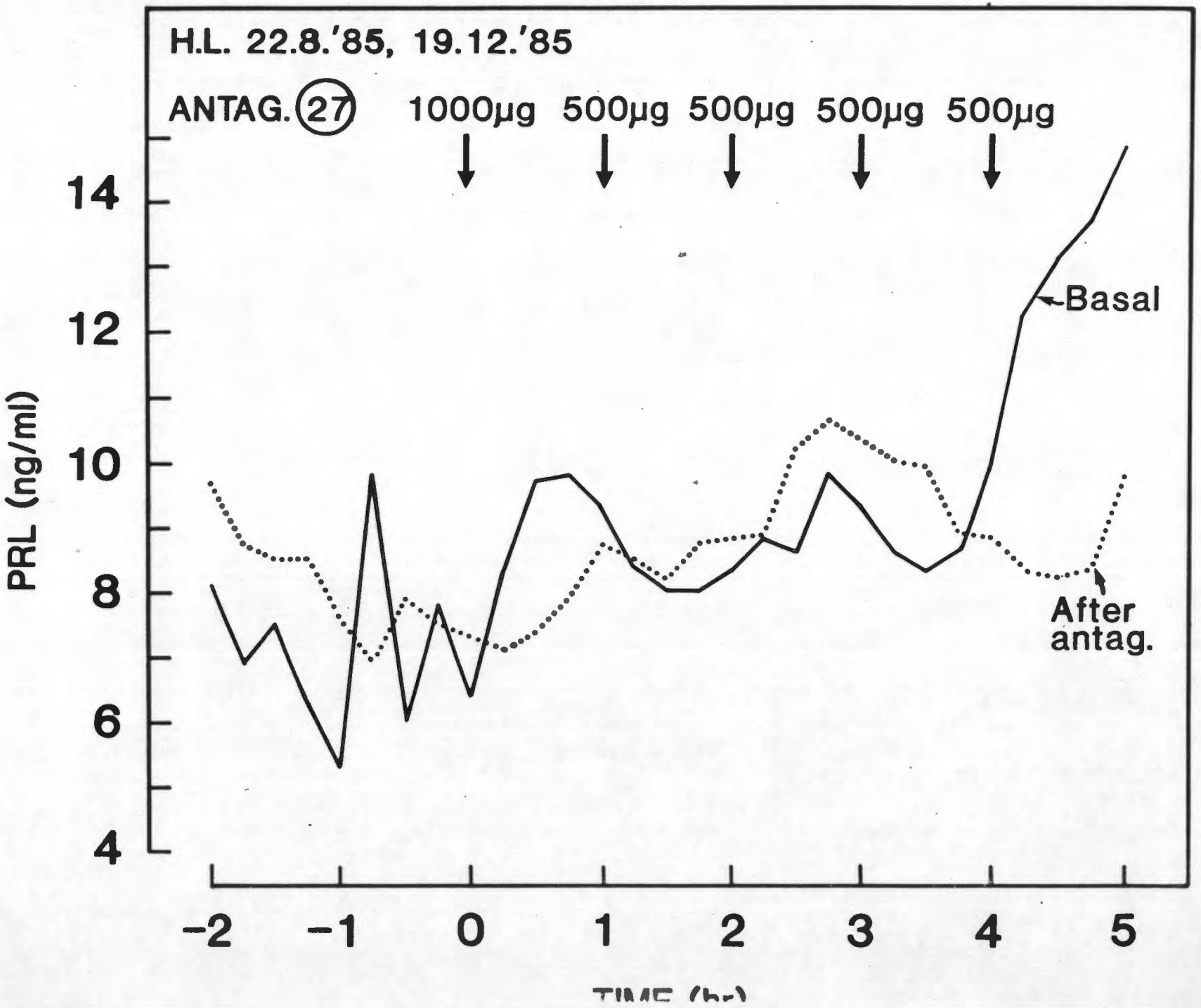
Table 8

Serum LH, prolactin and FSH values in the luteal phase of the menstrual cycle after administration of the GnRH antagonist: Subject 4

(LH and FSH in mIU/ℓ and prolactin in ng/mℓ)

Subject 4 : Graph showing the effect of the GnRH antagonist on prolactin

FIG 8



STATISTICS

Tables 9, 10 and 11 and figures 9, 10 and 11 depict the mean basal and experimental values (i.e. after administration of antagonist) for LH, FSH and prolactin. The serum concentrations of the respective hormones are given on the x-axis and the number of the specimen (i.e. time) on the y-axis. Tables 9, 10 and 11 also show the means and standard error of the means for all the specimens taken over the test period, both basal and after administration of the antagonist. Although there may be limitation in this method by averaging the values of peak hormone secretion occuring at different times for different subjects, thus giving occasional unacceptably high standard deviations and SEM values, the overall pattern for LH is still one of a blocking effect by the antagonist which is not evident in Figure 11 for prolactin (see Table 11) secretion. (Two ways of overcoming this limitation would have been to start all the tests for each case at time 0 with a peak or else to shift all peaks occurring in the course of the test period across so that they coincided. The mean values could then have been confidently determined. The method adopted in the above protocol is acceptable if peaks occur close to one another providing the duration of the peaks were not too long. The means became more inaccurate as peaks occurred further apart.)

Successive maximums and minimums were located on each curve (Fig. 9 to 11) and differences between these means tested using the t-test. The significance of these peak-trough sets were found to be between $p < 0,01$ and $p < 0,1$ for LH (basal). After the administration of the antagonist there were no significant peak-trough differences for LH

i.e. flat curve. This response differed markedly when compared with FSH and prolactin. There were no significant peak-trough sets for FSH either before or after the administration of the antagonist. Prolactin showed significant and consistent peak-trough sets both before and after the administration of the antagonist (p = 0,8 to p = 0,05).

The t-test was used to determine peaks as it uncovered more significant spikes of secretion during the test period that did the 3 CV method. The statistical analysis used was similar to that used by other authors.[104]

Pulse analysis is critical in interpreting the synchronicity of prolactin and luteinizing hormone. Two methods commonly employed are the "t-test" and the 3 CV approach. The former is probably more valid when multiple, frequent samples are obtained as it yields fewer false positive peaks: this is true if samples are taken at e.g. 5 minute intervals.[104] When the sampling interval is reduced from 20 minutes to 5 minutes there is a modest increase (50 - 75%) in luteinizing hormone and follicle stimulating hormone peak frequency using the t-test. However, using the 3 CV method, luteinizing hormone peak frequency increased by 82% and follicle stimulating hormone frequency by 67%.

Because there were so few peaks which occurred with 15 minute sampling periods during the course of the test period in the cases above, the more statistically significant peaks were uncovered using

the "t" test rather than the 3 CV method. The t-test may also be advantageous in indicating the greatest difference between the peak frequency in the patients and that in the noise series.[104] This test also has the advantage of using the actual variance at the nadirs and peaks, rather than a predicted variance calculated from the mean intra assay CV of the assay.[104] One other advantage of this method is that it offers an estimate of the likelihood of false positive (type 1) errors. Had we increased our sampling times to 5 minute intervals this may well have resulted in a 50 - 75% increase in peak frequency using the t-test method i.e. smaller peaks would have been included as statistically important because the samples would have been obtained nearer the peaks and nadirs.[104] More frequent sampling at e.g. 4 minutes or less would have uncovered (more) high frequency low amplitude spikes but probably not high amptitude peaks.[105]

Sample No. (time)	HL	SD	PE	Means	Standard Deviation S.D.	Standard error of the means (S.E.M.)
1	10.6	8.9	11.8	10.43	1.46	0.84
2	8.9	6.3	9.7	8.30	1.78	1.03
3	8.9	7.0	11.5	9.13	2.26	1.30
4	7.6	6.0	8.4	7.33	1.22	0.71
5	7.6	6.1	8.0	7.23	1.00	0.58
6	9.7	4.7	8.4	7.60	2.59	1.50
7	9.1	4.8	5.9	6.60	2.23	1.28
8	17.8	5.1	7.8	10.23	6.69	3.86
9	18.1	4.1	7.3	9.83	7.34	4.24
10	15.7	4.2	5.3	8.40	6.35	3.66
11	11.9	3.8	6.2	7.30	4.16	2.40
12	12.8	3.5	13.4	9.90	5.55	3.20
13	7.9	3.1	12.8	7.93	4.85	2.80
14	8.5	6.0	10.3	8.27	2.16	1.25
15	7.3	8.5	7.1	7.63	0.76	0.44
16	6.0	8.1	5.9	6.67	1.24	0.72
17	7.4	6.0	5.4	6.27	1.03	0.59
18	6.0	5.3	5.2	5.50	0.44	0.25
19	5.4	6.2	4.5	5.37	0.85	0.49
20	8.3	5.1	5.4	6.27	1.77	1.02
21	7.7	4.0	5.4	5.70	1.87	1.08
22	6.9	5.0	3.1	5.00	1.90	1.10
23	4.6	7.7	3.6	5.30	2.14	1.23
24	3.5	5.4	3.7	4.20	1.04	0.60
25	5.7	6.5	4.1	5.43	1.22	0.71
26	11.9	3.5	8.4	7.93	4.22	2.44
27	14.3	10.5	9.7	11.50	2.46	1.42

Table 9 : LH Basal

LH in mIu/ℓ

Means, standard deviations and standard error of the means of LH for the three subjects - Basal measurements (no antagonist)

Sample No. (time)	HL	SD	PE	Means	Standard Deviation S.D.	Standard error of the means (S.E.M.)
1	0.0	0.0	0.0	0.00	0.00	0.00
1	0.0	0.0	0.0	0.00	0.00	0.00
1	0.0	0.0	0.0	0.00	0.00	0.00
1	11.4	5.5	7.2	8.03	3.04	1.75
2	10.5	4.7	7.1	7.43	2.91	1.68
3	10.7	5.0	7.5	7.73	2.86	1.65
4	9.9	4.8	7.4	7.37	2.55	1.47
5	8.2	4.8	7.5	6.83	1.80	1.04
6	9.8	4.4	6.9	7.03	2.70	1.56
7	8.8	4.0	7.1	6.63	2.43	1.41
8	10.1	4.2	7.8	7.37	2.97	1.72
9	10.8	4.1	6.9	7.27	3.37	1.94
10	10.0	3.7	6.8	6.83	3.15	1.82
11	10.0	4.5	7.4	7.28	2.78	1.60
12	10.7	3.4	7.5	7.20	3.66	2.11
13	10.5	3.6	7.5	7.20	3.46	2.00
14	10.9	4.4	7.2	7.50	3.26	1.88
15	11.0	3.4	7.6	7.33	3.81	2.20
16	9.9	3.4	6.7	6.67	3.25	1.88
17	10.0	4.1	6.5	6.87	2.97	1.71
18	9.0	3.5	6.2	6.23	2.75	1.59
19	8.8	3.9	6.2	6.30	2.45	1.42
20	9.6	3.9	6.4	6.64	2.87	1.66
21	10.4	3.5	5.9	6.60	3.50	2.02
22	9.3	3.9	6.2	6.47	2.71	1.56
23	8.0	3.1	7.0	6.03	2.59	1.49
24	8.6	3.3	5.8	5.90	2.65	1.53
25	9.0	3.1	5.2	5.77	2.99	1.73
26	9.6	3.8	5.5	6.30	2.98	1.72
27	10.5	3.3	6.5	6.77	3.61	2.08
28	10.0	3.8	5.8	6.53	3.16	1.83
29	9.1	0.0	0.0	3.03	5.25	3.03

Table 10 : FSH basal

Units in mIu/ℓ

Means, standard deviations and standard error of the means of FSH for the three subjects - Basal measurements (no antagonist)

Sample No. (time)	HL	SD	PE	Means	Standard Deviation S.D.	Standard error of the means (S.E.M.)
1	8.1	7.4	8.1	7.87	0.40	0.23
2	6.9	7.6	7.0	7.17	0.38	0.22
3	7.5	6.5	5.5	6.50	1.00	0.58
4	6.3	6.3	4.9	5.83	0.81	0.47
5	5.3	6.4	4.9	5.53	0.78	0.45
6	9.8	7.5	5.4	7.57	2.20	1.27
7	6.0	7.3	5.4	6.23	0.97	0.56
8	7.8	8.7	4.1	6.87	2.44	1.41
9	6.4	9.4	3.9	6.57	2.75	1.59
10	8.3	8.6	4.3	7.07	2.40	1.39
11	9.7	6.0	4.9	6.87	2.51	1.45
12	9.8	7.7	13.6	10.37	2.99	1.73
13	9.3	7.1	12.1	9.50	2.51	1.45
14	8.4	7.3	10.1	8.60	1.41	0.81
15	8.0	8.2	6.8	7.67	0.76	0.44
16	8.0	11.3	6.3	8.53	2.54	1.47
17	8.3	9.3	5.7	7.77	1.86	1.07
18	8.8	7.4	5.4	7.20	1.71	0.99
19	8.6	4.7	6.2	6.50	1.97	1.14
20	9.8	6.4	5.2	7.13	2.39	1.38
21	9.3	9.5	5.0	7.93	2.54	1.47
22	8.6	7.0	4.7	6.77	1.96	1.13
23	8.3	8.7	5.3	7.43	1.86	1.07
24	8.6	9.5	5.8	7.97	1.93	1.11
25	9.9	9.3	4.9	8.03	2.73	1.58
26	12.2	8.5	4.7	8.47	3.75	2.17
27	13.1	11.6	4.7	9.80	4.48	2.59
28	14.8	12.9	4.8	10.83	5.31	3.07

Table 11 : Prolactin basal

Units in ng/ml

Means, standard deviations and standard error of the means of prolactin for the three subjects - Basal measurements (no antagonist)

Sample No. (time)	HL	SD	PE	Means	Standard Deviation S.D.	Standard error of the means (S.E.M.)
1	13.7	2.9	11.7	9.43	5.75	3.32
2	11.8	3.4	9.1	8.10	4.29	2.48
3	10.6	6.0	8.0	8.20	2.31	1.33
4	8.7	8.0	9.3	8.67	0.65	0.38
5	9.3	11.0	7.6	9.30	1.70	0.98
6	10.1	9.6	4.4	8.03	3.16	1.82
7	8.4	6.0	3.9	6.10	2.25	1.30
8	8.5	6.2	5.2	6.63	1.69	0.98
9	6.8	5.8	5.7	6.10	0.61	0.35
10	6.5	4.6	3.3	4.80	1.61	0.93
11	7.8	4.9	3.0	5.23	2.42	1.40
12	6.4	3.4	3.4	4.40	1.73	1.00
13	7.4	3.5	3.0	4.63	2.41	1.39
14	5.9	4.6	2.8	4.43	1.56	0.90
15	5.6	3.9	4.3	4.60	0.89	0.51
16	5.8	4.1	5.5	5.07	0.84	0.48
17	5.7	2.8	2.7	3.73	1.70	0.98
18	4.6	3.3	3.4	3.77	0.72	0.42
19	5.7	3.6	2.8	4.03	1.50	0.86
20	4.1	3.7	2.7	3.50	0.72	0.42
21	3.0	3.6	4.8	3.80	0.92	0.53
22	5.1	2.2	2.7	3.33	1.55	0.90
23	5.2	3.1	3.1	3.80	1.21	0.70
24	2.7	6.8	3.4	4.30	2.19	1.27
25	2.7	2.5	4.6	3.27	1.16	0.67
26	3.0	2.4	3.1	2.83	0.38	0.22
27	4.8	2.5	4.2	3.83	1.19	0.69

Table 12 : LH experimental

(with antagonist)

LH in mIu/ℓ

Means, standard deviations and standard error of the means of LH for the three subjects - after administration of the GnRH antagonist.

Sample No (time)	HL	SD	PE	Means	Standard Deviation S.D.	Standard error of the means (S.E.M.)
1	9.7	7.3	13.4	10.13	3.07	1.77
2	8.7	7.2	10.3	8.73	1.55	0.90
3	8.5	6.1	8.9	7.83	1.51	0.87
4	8.5	5.7	8.7	7.63	1.68	0.97
5	7.6	5.8	6.1	6.50	0.96	0.56
6	6.9	6.8	6.4	6.70	0.26	0.15
7	7.9	6.2	5.8	6.63	1.12	0.64
8	7.5	4.4	6.8	6.23	1.63	0.94
9	7.3	5.4	7.1	6.80	1.04	0.60
10	7.1	5.9	5.9	6.30	0.69	0.40
11	7.4	4.9	7.1	6.47	1.37	0.79
12	7.9	5.0	13.4	8.77	4.27	2.46
13	8.7	6.4	10.0	8.37	1.82	1.05
14	8.5	9.4	8.3	8.73	0.59	0.34
15	8.2	10.3	7.8	8.77	1.34	0.78
16	8.7	8.4	6.6	7.91	1.15	0.66
17	8.8	7.2	5.8	7.27	1.50	0.87
18	8.9	8.5	6.3	7.90	1.40	0.81
19	10.2	8.1	5.6	7.97	2.30	1.33
20	10.6	10.1	5.1	8.60	3.04	1.76
21	10.3	8.4	5.0	7.90	2.69	1.55
22	10.0	7.3	6.1	7.80	2.00	1.15
23	9.9	8.4	6.6	8.30	1.65	0.95
24	8.9	6.5	7.1	7.50	1.25	0.72
25	8.8	9.0	6.2	8.00	1.56	0.90
26	8.3	8.6	4.8	7.23	2.11	1.22
27	8.2	7.7	6.2	7.37	1.04	0.60
28	8.3	3.4	5.8	5.83	2.45	1.41

Table 13 : Prolactin experimental

after antagonist

Units in ng/mℓ

Means, standard deviations and standard error of the means of prolactin for the three subjects - after administration of the GnRH antagonist.

Sample No. (time)	HL	SD	PE	Means	Standard Deviation S.D.	Standard errer of the means (S.E.M.)
1	11.0	3.6	9.2	7.93	3.86	2.23
2	10.5	3.3	8.2	7.33	3.68	2.12
3	10.8	3.5	8.1	7.47	3.69	2.13
4	10.7	3.2	9.4	7.77	4.01	2.31
5	11.1	3.0	8.2	7.43	4.10	2.37
6	10.1	3.5	8.3	7.30	3.41	1.97
7	9.7	3.0	7.7	6.80	3.44	1.99
8	10.4	3.2	8.9	7.50	3.80	2.19
9	10.3	3.3	8.0	7.20	3.57	2.06
0	10.5	3.0	7.9	7.13	3.81	2.20
1	9.7	3.4	8.1	7.07	3.27	1.89
2	9.3	3.4	8.5	7.07	3.20	1.85
3	9.4	3.2	8.4	7.00	3.33	1.92
4	9.4	3.2	8.0	6.87	3.25	1.88
5	9.4	3.0	8.7	7.03	3.51	2.03
6	9.1	2.2	7.4	6.23	3.59	2.08
7	8.9	2.4	7.2	6.17	3.37	1.95
8	8.9	2.3	8.0	6.40	3.58	2.07
9	9.5	2.9	7.2	6.53	3.35	1.93
0	8.9	3.5	8.3	6.90	2.96	1.71
1	9.0	2.8	7.9	6.57	3.31	1.91

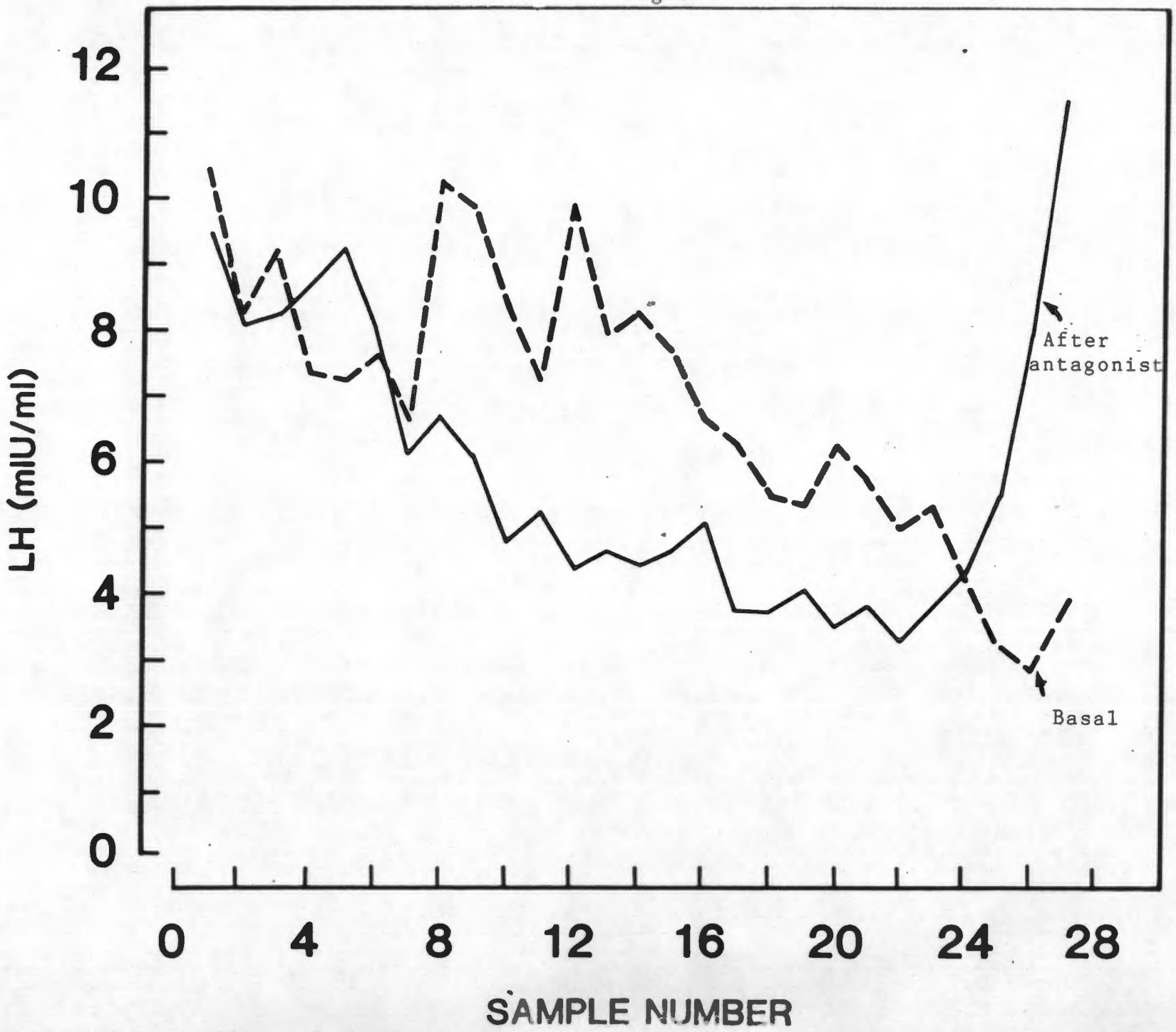
Table 14 : FSH experimental

after antagonist

Units in mIu/ℓ

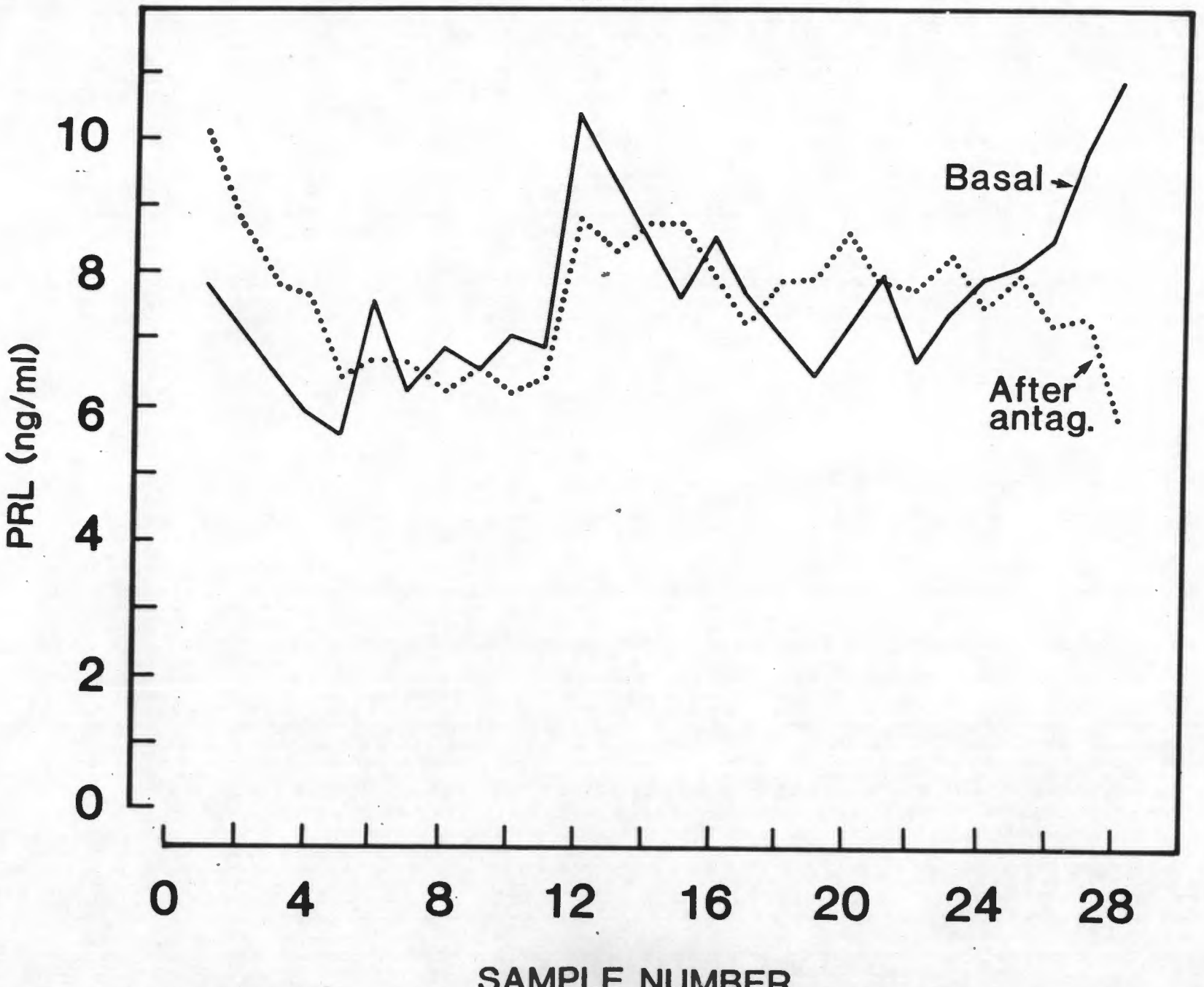
Means, standard deviations and standard error of the means of FSH for the three subjects - after administration of the GnRH antagonist.

Fig 9



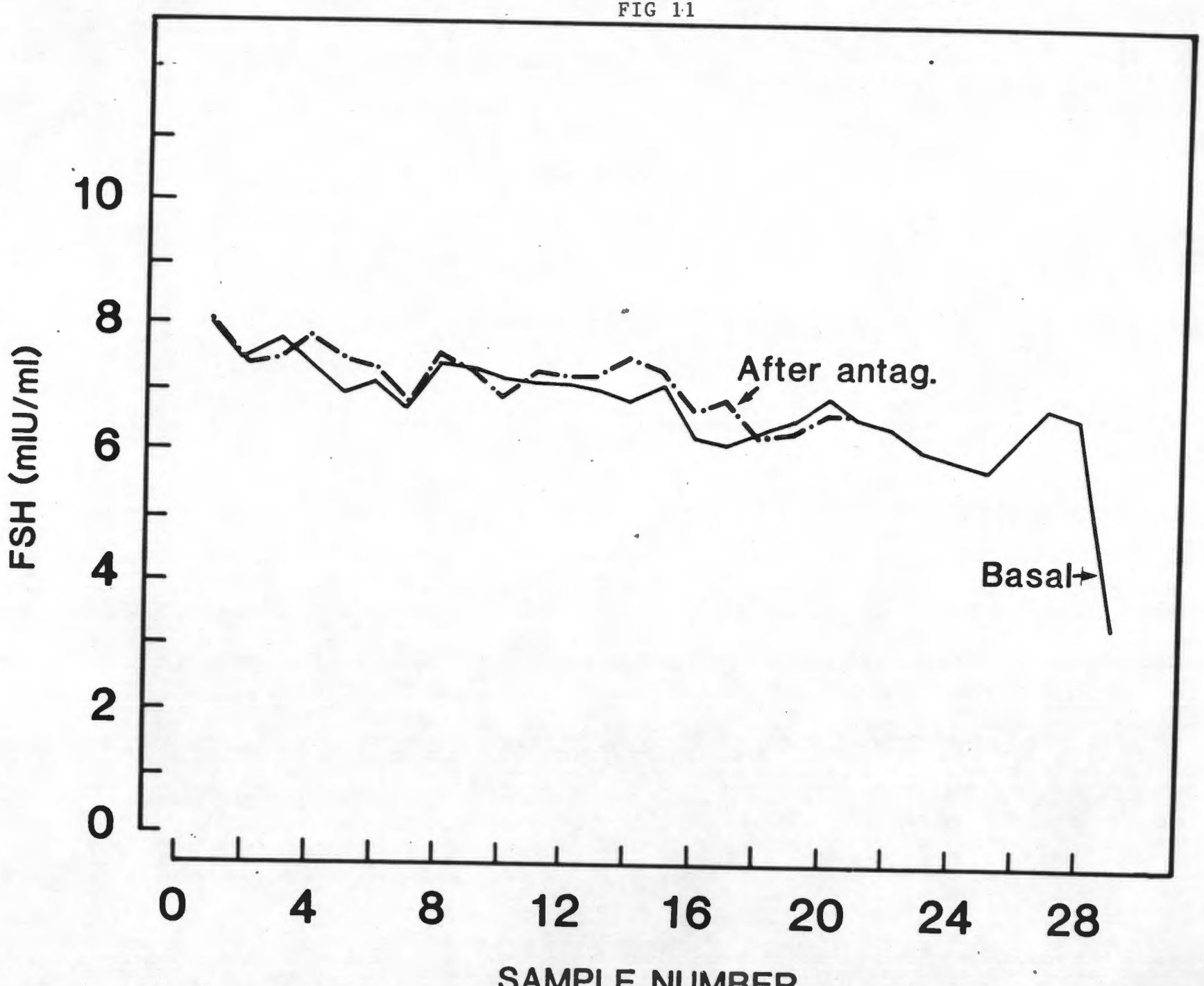
Graph showing the effect of the GnRH antagonist on LH : the means of the three subjects

FIG 10



Graph showing the effect of the GnRH antagonist on prolactin : means of the three subjects

FIG 11



Graph showing the effect of the GnRH antagonist on FSH : means of the three subjects

Discussion

From the data presented above it seems that a supramaximal dose of a potent GnRH antagonist which was able to block luteinizing hormone was not able to block synchronously occuring prolactin secretory peaks in the luteal phase of the menstrual cycle. Another observation was that the antagonist appeared to have very little effect on FSH secretion during this phase of the menstrual cycle.

The luteal phase of the menstrual cycle is typified by a low basal rate of luteinizing hormone secretion punctuated by episodic pulses of substantial magnitude ,and was used to allow more precise characterization of pulse analysis and reduce the probability of associating a prolactin pulse with a random fluctuation of the luteinizing hormone assay.[4] Prolactin pulses are also significantly higher in the luteal phase.[6, 17]

There is concordance of LH, FSH and prolactin secretory spikes during the luteal phase[83 & 4] but the reason for this is unknown.[4] One postulate was that gonadal steroids possibly modulated LH pulses.[107, 108 & 109] Both oestrogen[83, 110, 111 & 112] and progesterone acting either on its own[113] or synergistically with oestrogen[114] may enhance prolactin secretion by possibly modulating the action of dopamine at the lactotrope initially by increasing inhibition via DA and then as a rebound, increasing prolactin levels itself after DA withdrawal.[115] This may involve estradiol indirectly inhibiting dopamine by increasing the receptor sensitivity of the lactotrope to exogenous DA[116 & 80] or by directly modulating DA at

the lactotrope receptor.[117 & 80] It may also stimulate the lactotrope directly to release prolactin.[118] Estradiol affects the numbers of GnRH receptors,[119] the size of the releasable pool of luteinizing hormone and the phasic release of GnRH.[119] However, it should be noted that the administration of oestrogen and progesterone to women with chronic hypothalamic anovulation does not release either LH or prolactin (and thus probably not via GnRH directly).[4]

It is thus apparent that the interplay of estradiol and dopamine on the lactotrope and gonadotrope acting either directly or indirectly via dopamine or GnRH secretion is complex.

Braund et al postulated that GnRH itself was able to increase the secretion of both hormones from the pituitary gland[4] as synchrony of LH and prolactin was retained during naxolone infusion and after the injection of GnRH, and was abolished by administration of the dopamine receptor antagonist metochlopramide.[4] In subject 1 above, GnRH appeared to stimulate both prolactin and LH which would be in keeping Braund's (et al) observation. [4]. These authors felt that it was unlikely that periodic reduction of TIDA neuron activity accounted for LH and prolactin synchrony because MCP treatment abolished the prolactin but not the LH pulsality.[4] However, it is possible that GnRH itself may inhibit the activity of TIDA neurons which may in turn affect LH and prolactin pulses in a synchronous manner. (However, this association may have been fortuitous as the long duration of this peak is perhaps against it being due to a

spontaneous physiological peak.)

Conclusion

From this data a potent GnRH antagonist appears not to have blocked prolactin peaks or FSH surges. It therefore seems unlikely that GnRH is solely responsible for the synchronous secretion of LH and prolactin. An interesting observation was that the GnRH antagonist also failed to suppress FSH secretion.

This pilot study had the limitation of using a small number of subjects in the sample group and further testing using more subjects and employing more frequent blood samples at shorter time intervals between samples would help to validate the the above results.

The GnRH antagonists have thus provided a promising and useful tool for investigating the physiology of the menstrual cycle.

BIBLIOGRAPHY

1 McNeilly,A.S. (1985) Prolactin and the Corpus Luteum in the Luteal Phase ed. Jeffcoate,S.L. 1985

2 Leighton,P.C., Mc Neilly,A.S., Chard,T. (1976) "Short term variation in blood levels of prolactin in women" J.Endocrinol 68: pp 177-178

3 Franchimont,P., Dourcy,C.,et al (1976) "Prolactin levels during the menstrual cycle." Clin.Endocrinol. 5: pp 643-650

4 Braund,W., Roeger,D.C., & Judd,S.J. (1984) "Synchronous Secretion of Luteinizing Hormone and Prolactin in the Human Luteal Phase: Neuroendocrine Mechanisms" J.Clin.Endocrinol Metab. Vol.58 No.2 pp 293-297

5 Archer,R. "Molecular evolution of the polypetide hormones: the neurohypophysial hormone - neurophysin model" Neuropeptides: Biochemical and Physiological Studies ed. R.P.Miller (1981). Publ. Churchill-Livingstone

6 Neill,J.D. (1974) "Prolactin: its secretion and control". Handbook of Physiology. Section 7, Endocrinology. ed.Knobil,E., Sawyer,W.H. 4(2): pp 469-88. Publ. American Physiological Society, Washington D.C.

7 Nicol,C.S. (1978) "Physiological actions of Prolactin." Handbook of Physiology. Section 7, Endocrinology. ed.Knobil,E., Sawyer,W.H. 4(2): pp 469-88. Publ. American Physiological Society, Washington D.C.

8 Nicol,C.S. (1978) "Comparative Aspects of Prolactin Physiology: "Is Prolactin the initial growth hormone in Mammalian species also?" Progress in Prolactin Physiology and Pathology ed. Robyn,C. and Harter,M. Publ. Elsevier/North Holland Biomedical Press))

9 Ben-Jonathan,N., Olivier,C., Weiner,H.J. & Porter,J.C. (1980) "Dopamine in hypophysial portal plasma of the rat during the estrous cycle and throughout pregnancy." J.Reprod.Fertil. 58: pp 501-512

10 Leong,D.A.,Frawley,L.S., Neil,J.D. (1973) "Neuroendocrine Control of Prolactin Secretion" Annu.Rev.Physiol.45: pp 109-27

11 Farkouh,N.H.,Packer,M.G., Frantz,A.G. (1979) "Large molecular size Prolactin with reduced receptor activity in human serum: High proportion in Basal State and Reduction after Thyrotropin-Releasing Hormone". J.Clin.Endocrinol.Metab.: 48: 6 pp 1026-1032

12 Sinha,Y.N. (1980) "Molecular size variants of prolactin and growth hormone in mouse serum: strain differences and alterations of concentrations by physiological and pharmacological stimuli." Endocrinology 107: no.61959-69

13 Suh,H.K., Frantz,A.G. (1974) "Size Heterogeneity of Human Prolactin in Plasma and Pituitary Extracts". J.Clin.Endocrinol.Metab. 39: pp 928-35

14 Walker,A.M., Farquhar,M.G. (1980) "Preferential release of newly synthesized Prolactin granules is the result of Functional Heterogeneity among mammotrophs". Endocrinology 107: pp 1095-1104

15 Nogami,H.& Yoshimura,F. (1982) "Five Structural Criteria of Prolactin Cells Identified Immunohistochemically in the male Rat." Anat.Rec. 202: pp 261-274

16 Muller,E.E., Nistico,G., & Scapagnini,U (ed) (1977) Neurotransmitters and Anterior Pituitary Function Published by Academic Press: New York, San Francisco & London.

17 Vekemans,M., Delvoye,P., L'Hermite, M & Robyn, C (1977) "Serum prolactin levels during the menstrual cycle." J.Clin.Endocrinol.Metab. Vol.44.No.5 pp 989-993

18 Backstrom,C.T., McNeilly,A.S., Leask,R.M. & Baird,D.T. (1982) "Pulsatile secretion of LH, FSH, prolactin, oestrodiol and progesterone during the human menstrual cycle". Clinical Endocrinol 17: pp 29-42

19 Shin,S.H. & Reifel,C.W. (1981) "Adenohypophysis has an inherent property for pulsatile Prolactin secretion." Neuroendocrinology 32: pp 139-44

20 Ducharme,J.A. & Collins,R. (1982) "Pubertal Developments" in Clinics in Endocrinology and Metabolism; Paediatric Endocrinology

21 Everett,J.W. (1954) "Luteotrophic function of autografts of the rat hypophysis." Endocrinology 54: pp 685-690

22 Macleod,R.M. (1976) "Regulation of Prolactin Secretion." Frontiers in Neuroendocrinology ed. Martin,l.,Gauong,W.F. Publ.N.Y. Raven Press pp 169-194

23 Ben-Jonathan,N. (1980) "Catecholamines and Pituitary Prolactin Release." J.Reprod.Fertil. 58: pp 501-512

24 Lu,K-H., Amenomori,Y., Chen,C.L. & Meites,J. (1970) "Effects of Central Acting Drugs on Serum and Pituitary Prolactin Levels in Rats." Endocrinology 87: pp 667-672

25 Hokfelt,T (1967) "The possible ultrastructural identification of tubero-infundibular dopamine- containing nerve endings in the median eminence of the Rat." Brain Res. 5: pp 121-123

26 Macleod,R.M & Lehmayer,J.E (1974) "Studies on the mechanism of the Dopamine-mediated inhibition of Prolactin secretion." Endocrinology 94: p 1077

27 Tokahara,J., Arimura,A., Schally,A.V. (1974) "Suppression of Prolactin release by a purified porcine PIF preparation and catecholamines infused into a hypophysial portal vessel." Endocrinology 95: p 462

28 Kamberi,I.A., Mical,R.S., Porter,J.C. (1971) "Effects of anterior pituitary perfusion and intraventricular injection of catecholamines on prolactin release. Endocrinology 88: p 1012

29 Brown,G.M., Seeman,P., Lee,T. (1976) "Dopamine/ Neuroleptic receptors in Basal Hypothalamus and Pituitary." Endocrinology 99: pp 1407-10.

30 Goldsmith,P.C., Cronin,M.J. Weiner,R.I. (1979) "Dopamine Receptor Sites in the Anterior Pituitary." J.Histochem.Cytochem. 27: pp 1205-7

31 Plotsky,P.M., Gibbs,D.M., Neill,J.D. (1978) "Liquid Chromatographic- Electrochemical measurement of Dopamine in Hypophysial Stalk Blood in Rats." Endocrinology 102: no.6: pp 1887-1894

32 Gibbs,D.M. & Neill,J.D. (1978) "Dopamine levels in Hypophysial Stalk Blood in Rats are sufficient to inhibit Prolactin Secretion in-vivo." Endocrinology 102: pp 1895-1900

33 Ben-Jonathan (1980) "Dopamine: A Prolactin inhibiting Hormone." Endocr.Rev. Vol.6 No.4: pp 564-589

34 Enjalbert,A., Ruberg,M., & Kordon,C. (1978) "Neuroendocrine Control of Prolactin secretion." ed.Robyn Publ.Elsevier/North Holland Biomedical Press. pp 83-94

35 Tam,J.W., Daniels,P.S. (1981) "The role of Adenosine 3',5'-monophosphate in Dopaminergic inhibition of Prolactin release in anterior pituitary cells." Endocrinology 109: p 403

36 Schettini,G., Cronin,M.J., & McCleod,R.M. (1983) "Adenosine 3,5'-monophosphate (cAMP) and calcium-calmodulin interrelation in the control of Prolactin secretion: evidence for Dopamine inhibition of c-Amp accumulation and Prolactin release after Calcium mobilization." Endocrinology 112: p 1801

37 Swenner,L., Denef,C. (1982) "Physiological concentration of Dopamine decrease cAMP levels in cultured rat anterior pituitary cells and enriched populations of lactotrophs: Evidence for a causal relationship to inhibition of prolactin release." Endocrinology 111: p 398

38 Israel,J.M., Jaquett,P., et al (1985) "Electrophysiological effect of Dopamine on prolactinoma cells." Prolactin: Basic and Clinical Correlates. ed. Macleod,R.M., Turner,M.D. et al. Publ. Liviane Press, Padova Sect.111

39 Ajika,K., Hokfelt,T. (1979) "Projections to the median and the arcuate nucleus with special reference to monoamine systems: effects of lesions." Cell Tissue Res. 158: p 15

40 Demarest,K.T., Moore,K.E (1979) "Lack of high affinity transport system for Dopamine in the median eminence and posterior pituitary." Brain Res. 171: p 545

41 Gudelsky,G.A., Porter,J.C. (1979) "Release of newly synthesized Dopamine into the hypophysial portal vasculature of the Rat." Endocrinology 104: p 583

42 Fink,G. (1985) "Has the Prolactin inhibiting peptide at last been found?" Nature vol.316 pp 487-8

43 Nikolics,K., Mason,A.J.,et al (1985) "A Prolactin-inhibiting factor within the precursor for human gonadotrophin-releasing hormone." Nature vol.316: pp 511-516

44 Bauer,K., Graf,K.J.,et al (1978) "Inhibition of Prolactin secrection by histidyl-proline-diketopiperazine." Nature 274: pp 174-5

45 Enjalbert,A., Ruberg,M.,et al (1979) "Inhibition of in-vitro Prolactin secretion by h-p-d, a degredation product of TRH." Eur.J.Pharmacol: 58: pp 97-98

46 Linton,E.A., White,N.,et al (1981) "Hydroxyoestradiol inhibits Prolactin release from the superfused rat pituitary gland." J.Endocrinol. 90: pp 315-22

47 Wuttke,W., Flugge,G.,et al (1985) "In-vitro release rate of neurotransmitters in the control of pituitary Prolactin release." Prolactin: Basic Clinical Correlates Sect.VIII

48 Locatelli,V., Cocchi,D.,et al (1979) "Dual GABA control of Prolactin secretion in the rat." Endocrinology 105: p 778

49 Apuol,J.A., Racagni,G., & Iulliano, E. et al (1981) "Role of Central Nervous System - Derived or Circulating Gamma Aminobutyric Acid on Prolactin Secretion in the rat." J.Clin.Endocrinol.Metab. Vol.108 No.4 pp 1505-1510

50 Phillips,H.S., Nikolics,K., Branton,D. & Seeburg,P.H. (1988) "Immunocytochemical localization in rat brain of a prolactin release - inhibiting sequence of gonadotropin-releasing hormone pro-hormone". Nature: 316 pp 542-545

51 Horrobin,D.F. (1977) "Prolactin" Vol.5. Annual Research Reviews Ch.4. p 38

52 Donoso,A.O., Bishop,W.,et al (1971) "Effects of drugs that modify brain monoamine concentration on plasma gonadotrophin and prolactin levels in the rat" Endocrinology 89: pp 774-784

53 Meites,J. (1973) "Control of Prolactin Secretion." Human Prolactin pp 105-118 Publ. Encepta Medica, Amsterdam & American Elsevier Publ.Co.Inc., New York.

54 Clemens,J.A. & Meites,J. (1968) "Inhibition of hypothalamic prolactin implants of prolactin secretion, mammary growth and luteal function" Endocrinology 82: pp 878-881

55 Arakelian,M.C. & Libertun,C. (1976) "H1 and H2 - Histamine receptor participation in the brain control of prolactin secretion in lactating rats" Endocrinology 100: pp 890-895

56 Knigge,N., Thuesen,B. & Christiansen,P.M. (1986) "Histaminergic Regulation of Prolactin Secretion : Dose Response Relationship and Possible Involvement of the Dopaminergic System." J.Clin.Endocrinol.Metab. Vol.62. No.3. pp 491-496

57 Seltzer,A.M. & Donoso,A.O. (1985) "Participation of Brain Histamine in the Prolactin response to stress." Prolactin: Basic and Clinical correlates. Sect.11 Publ. Liviana Press, Padova

58 Grandison,L.,Gelato,M. et al (1974) "Inhibition of prolactin secretion by cholinergic drug" Proc.Soc Exp.Biol.Med. 145: pp 1236-1239

59 Libertun,C. & McCann,S.M. (1973) "Blockade of the release of gonadotropins and prolactins by subcutaneous or interventricular injection of atropine in male rats" Endocrinology 92: pp 1714-1724

60 Olson,L., Fuxe,K., et al (1968) "The effect of pituitary transplants on the tubero-infundibular dopamine neurons in various endocrine states." Acta Endocrinol 71: pp 233-244

61 Demarest,K.T., Riegle,G.D. & Moore,K.E. (1985) "The Interrelationship between the Rapid "Tonic" and the delayed "Induction" components of the Prolactin - Induced activation of tuberoïnfundibular Dopamine neurons." Prolactin, Basic and Clinical correlates. Sect.viii: pp 533-542 ed.Macleod,R.M., Thorner,M.O.,et al. Publ. Liviana Press, Padova

62 Malarkey,W.B., O'Dorisio,T.M., Kennedy,M. & Cataland,S. (1981) "The influence of vasoactive intestinal polypeptide and cholycystokinin on prolactin release in rat and human monolayer cultures". Life Science 28: pp 2489-2495

63 Gourdji,D., Bataille,D., Vauchin,N., Rosselini,G. & Tixier-Vidal,A. (1979) "Vasoactive intestinal peptide (VIP) stimulates prolactin (PRL) release and c-AMP production in rat pituitary cell line (GH3/B6). Additive effects of VIP and TRH on PRL release." FEBS Lett. 104: pp 165-168

64 Abe,H., Engler,D., Motilch,M.E., Bollinger-Gruber,J. & Reichlin,S. (1985) "Vasoactive intestinal peptide release is a physiological mediator of prolactin release in the rat." Endocrinology 116: p 1383

65 Said,S.I., Porter,J.C. (1979) "Vasoactive intestinal polypeptide; release into hypophysial portal blood. Life Science 24: pp 227-230

66 Ruberg,M., Rotsztein,W.H., Arancibia,S., Besson,J. & Enjalbert,A. (1978) "Stimulation of prolactin release by vasoactive intestinal peptide (VIP)" Eur.J.Pharmacol. 51: pp 319-320

67 Samson,W.K., Said,S.I., Snyder,G. & McCann,S.M (1980) "In vitro stimulation of prolactin release by vasoactive intestinal peptide" Peptides 1: pp 325-332

68 Wehrenberg,W.B., McNicol,D., Frantz,A.G. & Ferin,M. (1980) "The effects of serotonin on prolactin and growth hormone concentrations in normal pituitary stalk-sectioned monkeys." Endocrinology 107: pp 1747-1750

69 Vijayan,E & McCann,S.M (1979) "In vivo and in vitro effects of substance P and neurotensin on gonadotropin and prolactin release". Endocrinology 105: pp 64-68

70 Westendorf,J.M. & Schoubrunn,A (1982) "Bombesin stimulates prolactin and growth hormone release by pituitary cells in culture". Endocrinology 110: 352-358

71 Steele,M.K., Negro-Vilar,A. & McCann,S.M (1981) "Effects of angiotensin II on in vivo and in vitro release of anterior pituitary hormones in the female rat". Endocrinology 109: pp 893-899

72 Shin,S.H (1982) "Vasopressin has a direct effect on prolactin release in male rats". Neuroendocrinology 34: pp 55-58

73 Schoubrunn,A., Krasnoff,M., Westendorf,J.M., & Tashjian,A.H. Jr. (1980) "Epidermal growth factor and thyrotropin-releasing hormone act similarly on a clonal pituitary cell strain: Modulation of hormone production and inhibition of cell proliferation". J.Cell Biochem. 85: 786-796

74 Zyzek,E., Dufy-Borbe,L., Dufy,B. & Vincent,J.D. (1981) "Short-term effect of oestrogen on release of prolactin by pituitary cells in culture". Biochem. Biophys. Res. Commun. 102: pp 1151-1157

75 Denef,C. (1981) "LHRH stimulates Prolactin release from rat pituitary lutotrophs co-cultured with a highly purified population of gonadotrophs." Ann.Endocrinol Paris 42: pp 65-66

76 Enjalbert,A., Ruberg,M., Arancibia,S., Priam,M. & Kordon,C. (1979) "Endogenous opiates block dopamine inhibition of prolactin secretion in-vitro". Nature 270: pp 595-597

77 Lien,E.L., Fenichel,R.L., Garsky,V., Sarantakis,D & Grant,N.H (1976) "Enkephalin-stimulated prolactin release" Life Science 19: 837-840

78 Van Loon,G.R., Appel,N.M.,et al (1982) "Dopiminergic mechanisms regulating Prolactin secretion: Physiology and Pathology" Serono Symposium No.42 "The Gonadotrophins: Basic Science & Clinical Aspects in Females." ed. Flamigni,C. & Givens,J.R. Academic Press, London, N.Y.,

79 Pfeiffer,A., Braun,S., Mann,K et al (1985) "Anterior pituitary hormone responses to a K-opiate antagonist in man" J.Clin.Endocrinol.Metab. Vol 62, No 1 pp 181-185

80 Cramer,O.M., Parker,C.R. Jr, & Porter,J.C. (1979) "Estrogen Inhibition of Dopamine Release into Hypophysial Portal Blood" Endocrinology 104: p 419-421

81 Gudelsky,G.A., Nansel,D.D. & Porter,J.C. (1981) "Role of Estrogen in the Dopaminergic control of Prolactin secretion" Endocrinology 108: p 440

82 Raymond,V., Beaulieu,M., Labrie,F. & Baissier,J. (1978) "Potent antidopaminergic activity of estradiol at the pituitary level of prolactin release". Science 200: pp 1173-1175

83 Franchimont,P., Dourcy,C., Legros,J.J., Reuter,A., Vrindts., Gevaert,Y., Van Cauvenberge,J.R. & Gaspard,U. (1976) "Prolactin levels during the menstrual cycle" Clin.Endocrinol. 5: pp 643-650

84 Kopelman,P.G., White,N. et al (1979) "Impaired hypothalamic control of Prolactin secretion in some massively obese human subjects." Lancet 1: pp 29-42

85 Averill,R.L.W., & Purves,H.D. (1963) "Differential effects of permanent hypothalamic lesions on reproduction and lactation in rats" J.Endocrinol 26: p 463

86 De Greef,W.J., Visser,T.J.,et al (1981) "Evidence for the involvement of hypothalamic Dopamine and Thyrotropin releasing hormone in suckling - induced release of Prolactin." Endocrinology 91: p 213

87 Grosvenor,C.E., Mena,F. "Evidence that thyrotopin releasing hormone and a hypothalamic Prolactin releasing factor may function in the release of Prolactin in the lactating rat." Endocrinology 107: pp 863-68

88 Tashjian,A.H.(Jr), & Jansen,D.K., (1971) "Thyrotropin releasing hormone: direct evidence for stimulation of Prolactin production by pituitary cells in culture," Biochem. Biophys, Res. Commun. 43: pp 516-23

89 Nakano,H., Fawcett,P., & McCann, S.M. (1976) "Enzymatic dissociation and short-term culture of isolated rat anterior pituitary cells for studies on the control of hormone secretion." Endocrinology 98: pp 278-288

90 Koch,Y.,Goldhaber,G. et al (1977) "Suppression of Prolactin and Thyrotropin secretion in the rat by antiserum to thyrotropin-releasing hormone" Endocrinology 100: pp 1476-78

91 Blake,C. (1974) "Stimulation of pituitary prolactin and TSH release in lactating and proestrous rats" Endocrinology 94: p 503

92 Tixier-Vidal,A., Gourdji,D.,et al (1975) Hypothalamic Hormone ed. M.Motta & B.J.Crosignani pp 89-108

93 Enjalbert,A., Epelbaum,J. et al "Reciprocal interactions of Somatostatin with Thyrotropin-releasing hormone and vasoactive intestinal peptide on Prolactin and Growth Hormone secretion in-vitro." Endocrinology Vol.111 No.1 pp 42-47

94 Gershengorn,M.G (1985) "Intracelluar mechanisms of Calcium-mediated stimulation of prolactin secretion by thyrotropin-releasing hormone." Prolactin: basic and Clinical correlates. ed. Macleod, Thorner.,et al Liviana Press, Padova Sect.III.

95 Martin,TFJ. (1985) "Dual intracellular signalling by Ca^{++} and lipids mediates the actions of TRH." Prolactin: basic and Clinical correlates. ed. Macleod, Thorner.,et al Liviana Press, Padova Sect.III.

96 Camoratto,A.M. & Grandison,C. (1985) "Evidence supporting a correlation between arachnidonic acid release and prolactin secretion from GH_3 cells."Endocrinology 116(4): pp 1506-13

97 Nagy,I., Makara,G.B.,Horvath,G. et al (1985) "Tripeptide aldehyde Protease Inhibitors may depress in in-vitro prolactin and growth hormone release." Endocrinology 116(4): pp 1426-32

98 Gautvik,K.M., Tashjian,A.H., Kourides,I.A., et al (1975) "Thyrotropin releasing hormone is not the sole physiological mediator of prolactin release during suckling" N.Engl.J.Med. 290: pp 1162-1165

99 Riskind,P.N., Millard,W.J.,et al (1984) "Evidence that Thyrotropin releasing hormone is not a major Prolactin releasing factor during suckling in the rat. Endocrinology 115: p 312

100 Plotsky,P.M., Neil,J.D. (1982) "Interactions of dopamine and thyrotropin releasing hormone (TRH) in the regulation of prolactin release in lactating rats". Endocrinology 111: pp 168-173

101 Fuxe,K., Hokfelt,T.,et al (1982) "Factors involved in the control of the activity of tubero-infundibular dopamine neurons during pregnancy and lactation." Neuroendocrinology 5: pp 257-70

102 Gallo,R.V., Rabii,J., and Moberg,G.P. (1975) "Effects of Methysergide, a Blocker of Serotonin receptors, on Plasma Prolactin levels in Lactating and Ovierectamized rats." Endocrinology 97: pp 1096-1105

103 Mena,F., Enjalbert,A., Carbonell,L., Priam,M & Kordon, C (1976) "Effect of suckling on plasma prolactin and hypothalamic monoamine levels in the rat". J.Endocrinol. 26: p 463

104 Ross,J Levine, Barnes,K.M., Brody,S., Merriam,G.R., Loriaux,D.L. & Cutler, Jr. G.B. (1984) "A comparison of two methods for detecting hormone peaks: The effects of sampling interval on gonadotropin peak frequency". J.Clin.Endocrinol.Metab. Vol 59. No.6: pp 1159-1163

105 Veldhuis,J.D., Evans,W.S., Rogol,A.D., Drake,C.R., Thorner,M.D., Merriam,G.R. & Johnson, M.L. (1984) "Intensified Rates of Venous Sampling Unmasks the presence of Spontaneous High-Frequency Pulsations of Luteinizing Hormone in Man". J.Clin.Endocrinol.Metab. Vol 59 No.1: pp 96-102

106 Casper,R.F. & Yen,S.C. (1981) "Simultaneous pulsatile release of prolactin and luteinizing hormone releasing factor agonist" J.Clin.Endocrinol.Metab. 52: p 934

107 Santen,R.J. & Bardin,C.W. (1973) "Episodic Luteinizing Hormone Secretion in man: pulse analysis, clinical interpretation, physiologic mechanisms". J.Clin.Invest. Vol 52: pp 2617-2628

108 Fink,G. (1979) "Neuroendocrine Control of Gonadotrophin Secretion". Br.Med.Bull. Vol 35 No.2: pp 155-160

109 Wang,C.F., Lasley,B.L., Lein,A. & Yen,S.C. (1976) "The functional changes of the pituitary gonadotrophs during the luteal phase of the menstrual cycle". J.Clin.Endocrinol.Metab. 42: pp 718-728

110 Nicol,C.S., Meites,J. & Blackwell,C. (1962) "Estrogen stimulation of prolactin production by rat adenohypophysis in vitro". Endocrinology 70: 272-277

111 Schams,D. & Karg (1972) "The immediate response of the plasma prolactin level to estrogen infusion in dairy cows". Acta Endocrinol. 69: pp 47-52

112 Frantz,A.G., Kleinberg,D.L. & Noel,G.L. (1972) "Studies on prolactin in man". Rec.Prog.Horm.Res. 28: p 527

113 Cramer,O.M., Parker,C.R. Jr, & Porter,J.C. (1979) "Stimulation of Dopamine release into Hypophysial Portal Blood by Administration of Progesterone". Endocrinology 105: p 929

114 Williams,R.F., Barber,D.L., Cowan,B.D., Lynch,A.L., Morut,E.L. & Hodgen,G.D. (1981) Hyperprolactinemia in monkeys: induction by an estrogen - progesterone synergy". Steroids 38: p 321

115 Judd,S.J., Rakoff,J.S. & Yen,S.S.C (1978) "Inhibition of gonadotropin and prolactin release by dopamine: Effect of endogenous estradiol levels". J.Clin.Endocrinol.Metab. Vol 47, No.3: pp 494-498

116 Lichtensteiger,W., Karpela,K., Langemann,H. & Keller,P.J. (1969) "The influence of overectomy, estrogen and progesterone on the catecholamine content of hypothalamic nerve cells in the rat". Brain Res 16: p 199

117 MacLeod,R.M. & Lehemeyer,J.E. (1974) "Studies on the mechanism of dopamine-mediated inhibition by prolactin secretion". Endocrinology 94: p 1077

118 Nicol,C.S. & Meites,J. (1962) "Estrogen Stimulation of Prolactin Production by rat adenohypophysis in vitro" Endocrinology .70: p 272

119 Clayton,R.N., Catt,K.J. (1981) Endocr. Rev. 2: p 186