NEUROENDOCRINE REGULATION OF PROLACTIN SECRETION IN THE FEMALE RHESUS MONKEY IN RELATION TO GONADAL STATUS - INVOLVEMENT OF EXCITATORY AMINO ACIDS

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE THESIS REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSPHY

BY

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CERTIFICATE

This thesis by Sarwat Jahan is accepted in its present form by the Department of Biological Sciences as satisfying the thesis requirement for the degree of Doctor of Philosophy in Biology (Reproductive Physiology/Neuroendocrinology).

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Dated: _____

DEDICATED TO MY

MOTHER

&

HUSBAND

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"Acquire knowledge. It enableth its possessor to distinguish right from wrong; it lighteth the way to Heaven; it is our friend in the desert, our society in solitude, our companion when friendless; it guideth us to happiness; it sustaineth us in misery; it is an ornament amongst friends, and an amour against enemies." [Mohammad P.B.U.H]

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SARWAT JAHAN

LIST OF ABBREVIATIONS

3βHSD	3-Beta hydroxy steroid dehydrogenase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionate
AP5	D,L-2 amino 5 phosphonopentanoic acid
CA1	Cerebral aqueduct
CL	Corpus luteum
CNQX	Cyno-3,3-dihydro-7-nitro quinoxaline
CSF	Cerebrospinal fluid
DNQX	Dinitroquinoxline
E_2	Estradiol
EAA	Excitatory amino acid
EDTA	Ethylene di-amino tetra acetic acid
EIA	enzyme immuno assay
ET	Embryo transfer
FSH	Follicle stimulating hormone
FSH-R	Follicle stimulating hormone-Receptor
GCs	Granulosa cells
Glu R	Glutamate receptor 1
GnRH	Gonadotropin releasing hormone
hCG	Human chorionic gonadotropin
IgG	Immuno globulin G antibody
iv	Intravenous injection
IVF	Invitro fertilization
LH	Lutenizing hormone
LH-R	Lutenizing hormone-Receptor
LHRH	Lutenizing hormone releasing hormone
MEIA	Microparticle Enzyme Immuno assay
MK-801	5methyl-10,11-dihydro-5H-dibenzo-cyclohepten-5,10-imine meleate
NaCl	Sodium chloride
NMA	N-methyl-D-L-aspartate
NMDA	N-methyl-D-aspartate

NPY	Neuropeptide Y
P	Progesterone
PCOs	Polycystic Ovarian Syndrome
PIFs	Prolactin inhibiting factors
PMSG	Pregnant mare serum gonadotropin
POA	Pre optic area
PRFs	Prolactin releasing factors
PRL	Prolactin
RF	Resting follicle
TCs	Theca cells
TE	Theca externa
TI	Theca interna
TRH	Thyrotropin releasing hormone
VIP	Vasoactive intestinal peptide
WHO	World Health Organization

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ABSTRACT

ABSTRACT

The secretion of Prolactin (PRL) from the anterior pituitary is under the dual control of two hypothalamic factors; the stimulatory, prolactin releasing factors (PRF) and the inhibitory, prolactin inhibitory factors (PIF). The synthesis and secretion of these hormones is influenced by a variety of neuromodulators, neuropeptides and neurotransmitters, including glutamate and aspartate. N-methyl-D-aspartate (NMDA) and N-methyl D, L-aspartate (NMA) are excitatory amino acid analogues of aspartate which bind to a specific receptors in brain known as NMDA receptor. In both rats and primates, the activation of these receptors has been shown to stimulate the release of PRL, probably through discharge of hypophysiotropic factors.

The present study attempts to examine the role of NMDA receptors in the central regulation of PRL secretion that may have potential involvement in ovarian function and its alteration by glutamate in various phases of menstrual cycle of rhesus monkey (Macaca mulatta).In addition, a possible modulation of PRL secretion by steroids has been assessed in this primate species. The involvement of NMDA receptors in the regulation of PRL secretion in ovariectomized animal has also been investigated.

Immature, immature ovariectomized, and adult intact female monkeys were used in this investigation. NMA (15mg/kg BW) or normal saline was infused through, a teflon cannula implanted in the saphenous vain. Blood samples were collected 20-60 min before and 30-60 min after the injection of the drug. NMA was dissolved in normal saline immediately before use and passed through a 0.22 μm filter at the time of injection. All bleedings were carried under ketamine hydorchloride anesthesia (initial dose 5mg/kg BW, im followed by 2.5 mg/kg at 30 min. intervals). The plasma level of PRL, Estradiol (E_2) and Progesterone (P) were determined by using specific assay systems.

In the first set of experiments, the hypothalamic-lactotrope activity under basal conditions as well as the sensitivity of NMDA receptors to NMA stimulation in immature female rhesus monkey were studied before and after induction of ovulation. Immature (n=5) monkeys were chronically treated with FSH (201U daily for 12 days, im) to initiate follicular development followed by a bolus injection of human chorionic gonadotrpin (hCG; 1250 IU im) to achieve ovulation. Ovarian conditions during FSH/hCG treatment

were examined by ultrosonography, and peripheral estradiol and progesterone were monitored. During the course of FSH/hCG-induced ovarian cycle, all animals were challenged with, NMA at different days of the cycle. The mean basal plasma concentration of PRL during different days of the cycle showed marked differences ($F=8.39$, $P<0.001$). A single iv injection of NMA produced differential effect on PRL secretion during different days of cycle depending upon the level of PRL secretion under basal condition whereas NMA had no demonstrable effect on PRL secretion before the gonadotropins treatment. Maximum increase (213%) in plasma PRL concentration in response to NMA was observed on the 21 day of the induced cycle. Similarly, NMA had no effect on E_2 and P secretion before gonadotropian treatment while on day 12 maximum increase over the basal level ($P<0.0006$) was induced by a single iv injection of NMA on E_2 secretion. NMA has no effect on progesterone secretion over this period except during menstruation ($P<0.04$, 5.55 ± 0.61nmol/L at o min and 8.77 ± 1.20 at 10 min). Ovaries of treated monkey exhibited a marked response to gonadotropin treatment by way of enlargement in size, follicular development and formation of corpous lutem as assessed by ultrasonography.

In another set of experiments, role of FSH and hCG were studied on follicular development and on the induction of normal ovarian cycle in immature female rhesus monkeys. The protocol used was similar as described earlier. Laproscopy was preformed to evaluate the ovarian conditions during different days of the induced cycle. Selected animals were ovariectomized at various time points, before and following ovulation and ovaries were thoroughly studied for follicle/luteal parameters and the tissues were processed for histological observations and for evaluation by light microscopy. Results indicated that before gonadotropin treatment the ovaries showed abundant primordial, primary and preantral follicles. Fluids filled antral follicles were also observed. Ovaries of treated monkey showed a marked response to the gonadotropin treatment by way of enlargement in size, follicular development and corpous luteum formation. Ovarian size was significantly different before and after treatment ($P<0.05$, $P<0.01$, $P<0.001$). Large antral, atretic follicles were observed on day 11, and on day 15 ovulation points on the surface of large follicles were also observed. Abundant luteal tissues were observed in ovaries of animals on day 22 of treatment. Corpora lutea of various sizes (category A

were <6 mm, category B were >6 mm) were also noticed. The ovaries of the monkey examined on day 39 showed regressed corpora lutea.

In the third set of experiments the role of E_2, P and E_2 plus P in influencing the secretion of PRL at the level of hypothalamus was studied by measuring the NMA-induced PRL secretion in ovariectomized immature female monkeys. Treatment of immature monkey with estradiol valerate (500 μg/animal/week) or progesterone (50 μg/animal/week) resulted in a significant ($P<0.03$, $P<0.005$, respectively) increase in PRL concentrations. A single iv injection of NMA (15mg/kg BW) resulted in a rapid and large increase in PRL circulating levels reaching at peak within 15 min of injection (3171.02±472.70mIU/L at o min and 4395.28±242.70 mIU/L at 15 min), whereas a similar dose regimen of NMA failed to induced a significant rise in peripheral concentrations of PRL in ovariectomized animals ($P>0.33$). Combination of these steroids also induced a significant ($P<0.02$) increase in PRL secretion after 15 min of NMA injection but these steroids failed to cause further increase in basal PRL level because of the high level of PRL already present. Steroid replacement in ovariectomized monkeys reestablished the PRL responsiveness to NMA stimulation.

In the final set of experiment, the hypothalamic lactotropes activity under basal condition as well as the sensitivity of NMDA receptors to NMA stimulation in adult normal cycling female rhesus monkey (n=4) were studied. The adult animals were studied during follicular, luteal and menstrual phase of the cycle. The animals were challenged with NMA during these days. Mean basal plasma PRL profile was also studied during these days of the cycle. A single iv injection of NMA (15mg/kg BW) produced differential effect of PRL secretion during different stages of the cycle ($F=4.51$, $P<0.02$). NMA injection resulted in rapid and large increase in PRL circulating levels reaching a peak within 10 min of injection during these days. But the greatest response was observed during the luteal phase (355.87±87mIU/L at o min and 1079.59±189.49 at 10 min) of the cycle. Basal plasma PRL concentrations were also significantly different in the luteal phase as compared to the follicular and menstrual phase of the cycle ($F=11.22$, $P<0.001$).

In conclusion the present study suggests that glutamatergic component of the control system that governs PRL secretion by utilizing NMDA receptor may play an important

role in the regulation of changes in the secretion of PRL in the adult and immature female rhesus monkeys.

1. Present finding are noteworthy in demonstrating the enhancing role of FSH on ovarian morphology and the FSH priming is the primary stimulus for early follicle growth in primates. Moreover, it appears that exogenous FSH administration stimulates aspects of early antral follicle development.
2. Pharmacological doses of FSH could induce a normal ovarian cycle in immature rhesus monkeys with profound gonadotropins deficiency. This study confirms the enhancing role of FSH in ovarian steroidogensis. More importantly, the results highlight the predominant role of gonadotropins in inducing ovulation when negligible amount of gonadotropins are present. Our results demonstrate that FSH is of greater importance in the induction of normal ovarian cycle. In conclusion, it is suggested that sexual precocity observed in these animals is due to an increase in estrogen and progesterone secretions by the ovaries. These steroids in turn may affect the hypothalamo-pituitary axis, thus bringing about an alteration of normal process leading to puberty.
3. NMDA driven PRL-release is dramatically influenced by the steroid milieu, in the immature non-human primates. Hypothalamic NMA induced PRL release may be regulated by either estrogen or progesterone but combination of these steroids failed to cause any further increase in PRL levels. This inhibition in further release of PRL may be attributed to the high levels of PRL itself.
4. Prolactin response to NMA in adult female rhesus monkeys during the luteal phase of the menstrual cycle is different from that observed in follicular and menstrual phases and that the steroids may overtly influence the NMDA dependent drive to prolactin release.

Results of the present study indicate that NMA involvement in central regulation of PRL secretion may occurs through activation of PRL stimulating system depending upon the physiological state or steroids milieu. It is possible therefore, that the NMA induced release of PRF and PRL is enhanced in the presence of ovarian feedback.

CHAPTER 1

PROLACTIN RESPONSE TO NMDA RECEPTORS ACTIVATION DURING DIFFERENT PHASES OF AN INDUCED CYCLE IN IMMATURE FEMALE RHESUS MONKEY USING FOLLICLE STIMULATING HORMONE

INTRODUCTION

Ovarian follicular growth may begin at any time of the female's reproductive life, as early as infancy, and continues throughout puberty, during the ovarian cycle and pregnancy, until the end of the reproductive period (Peter et al., 1969). The precise time at which follicular growth actually starts is difficult to establish. Follicles begin to form during the fourth month of fetal life in the human ovary (Baker et al., 1963). Some of the newly formed follicles start to grow immediately and most of them remain in a resting stage until they either degenerate or some signal(s) activate(s) them to enter the growth phase. In the immature and adult primates the follicular growth is a continuous process. (Koering et al., 1969; 1991; Van Wagenen et al., 1973). The early follicular growth is independent of gonadotropin support in the immature monkey since the hypothalamic-pituitary axis is subdued during this interval (Dierschke et al., 1974; Wildt et al., 1980) but the ovaries are sensitive to Follicle stimulating hormone (FSH) stimulation at this age (Kenigsberg, 1984). For ovulation large estrogenic follicles can transfer their gonadotropin dependence to Luteinization hormone (LH).

Pituitary gonadotropins, FSH and LH, play a central role in the ovarian follicular development and steroidogenesis. LH causes ovulation and stimulates steroidogenesis within the ovary. For normal gonadal function in primates, a pulsatile secretary pattern of LH is required (Dierschke et al., 1970). FSH induces the development of multiple follicles in monkey and women (Hodgen et al., 1986; Jones et al., 1984) and subsequently a dominant follicle is ovulated in response to LH.

Pre-ovulatory LH peak is the central endocrinological event in the primate ovarian cycle, which precedes ovulation by 24-40 hours (Kirton et al., 1970; Monroe et al., 1970). The pre-ovulatory surge of LH causes formation of corpus luteum by inducing changes in thecal and granulosa cells of the ovulating follicle (Baird et al., 1979). In women and rhesus monkeys, the frequency of LH pulses does not appear to change in the follicular phase and during the pre-ovulatory LH surge (Reame et al., 1984; Norman et al., 1984). There is, however, general agreement that there is an increase in the amplitude of the LH pulses during the LH surge (Yen et al., 1972; Norman et al., 1984). During the luteal phase of the cycle, there is a marked reduction in the frequency of LH pulses (Filicori et al., 1982). In contrast to the follicular phase, the ovarian response to these intermittent stimuli is clearly evident in the

form of large pulses of progesterone following each LH pulse (Backstrom et al., 1982). As the corpus luteum wanes and progesterone levels decline, the follicular phase frequency of pulsatile LH secretion is re-established (Filicori et al., 1982).

Prolactin (PRL) has long been known as a luteotropic hormone especially in rodents. It is involved in initiating luteinization of granulosa cells, in maintaining their level of progesterone synthesis in luteal cells, and in inhibiting the activity of progesterone categorizing enzymes 20hydroxysteroid dehydrogenase particularly in rodents (Rothchild, 1981). PRL has been demonstrated to enhance progesterone production in cultured granulosa cells of rats (Crisp et al., 1977) and porcine (Veldhuis et al., 1980) pre-ovulatory follicles. The appearance of specific receptors in granulosa cells late in follicular development and their induction by FSH in culture indicate the likelihood that PRL may exert a physiological action on granulosa cells at the stage of terminal differentiation when they are transformed into luteal cells. PRL injections (Advis et al., 1981) or hyperprolactinemia induced by in vivo administration of dopaminergic receptors blocker (Siegal et al., 1976; Gay et al., 1970) have been found to induce precocious puberty, as well as to increase ovarian responsiveness to LH in immature rats. In contrast to the stimulatory action of PRL or progesterone (P) secretion, progesterone production by granulosa cells from small immature porcine follicle was markedly inhibited by physiological concentration of PRL (Bex et al., 1975) and can be reversed by estradiol (E_2) exposure (De Paolo et al., 1979). Another inhibitory effect of PRL on estradiol secretion was reported for cultured rat granulosa cells obtained from follicles at both pre-antral and pre-ovulatory stages (Fujii et al., 1983; Sauder et al., 1984). Decreased estradiol secretion in vitro appears to be due, at least in part, to an inhibiting action of PRL on FSH induction of aromatase activity (Welschen et al., 1980; Chappel et al., 1979). PRL has been reported to suppress basal and gonadotropin-stimulated E_2 secretions by human ovaries perfused in vitro (Lee et al., 1983).

The evidence that the central nervous system plays a limiting role in the control of gonadotropin secretion in higher primates was based mainly on experiment performed in the rhesus monkey. This role is mediated by the production and release of gonadotropin-releasing hormone (GnRH). Increased GnRH levels are found in the portal blood during the LH surge (Eskay et al., 1975), immunization against GnRH blocks ovulation (Clark et al.,

1978) and also blocks the post ovariectomy rise in LH and FSH (Arimura et al., 1974). The treatment with GnRH antagonist also decreases LH secretion (Norman et al., 1983).

The ovarian steroids, estrogens and progesterone modulate effects of GnRH on pituitary LH release. These steroids have repeatedly been shown to suppress GnRH induced LH release from pituitary gonadotropes both in vivo (Kalra et al., 1982) and in vitro (Kamel et al., 1987). However, the patterns of circulating ovarian steroids during reproductive cycle are associated with basal episodic LH discharge indicating a stimulatory effect of estradiol on gonadotropin secretion. Additionally, there is a growing evidence to show that rising titers of estradiol accelerate the frequency of GnRH and LH discharge during the follicular phase of the menstrual cycle (Sollenberger et al., 1990). The stimulatory effect of estrogens on LH secretion is not exerted directly on GnRH neurons, since GnRH neurons are deficient in estrogen receptors (Watson et al., 1992).

Estrogens stimulate gonadotropin secretion by regulating the effectiveness of the episodic GnRH and neuropeptideY (NPY) signals on pituitary LH output. Thus estrogen, either alone or in conjunction with progesterone enhances the interactive actions of GnRH and NPY (O'Conner et al., 1993).

In primate, as in other species, progesterone induces an LH surge in ovariectomized and estrogen-primed monkeys (Terasawa et al, 1982 and 1987) and in ovarian-intact monkeys treated with estradiol during the follicular phase (Terasawa et al., 1980). The available evidence indicates that the sites of facilitatory action of progesterone are in the hypothalamus: Pentobarbital anesthesia blocked the progesterone-induced LH surge (Terasawa et al., 1980) and stalk section, but not complete hypothalamic deafferentation, blocked the progesterone-induced LH surge (Yeoman et al., 1984); and increases in the electrical activity of hypothalamic neurons (Yeoman et al., 1984) and lutenizing hormone releasing hormone (LHRH) release (Terasawa et al., 1985) were observed during the progesterone-induced LH surge. Nevertheless, the mechanism of progesterone action on LHRH release is unknown. It is quite possible that neuropeptide Y (NPY) neurons mediate steroid actions, because steroid receptors were found in NPY neurons (Sar et al., 1990), but not in LHRH neurons (Fox et al., 1990). Androgens have also been shown to enhance the

stimulation of progesterone secretion by FSH in rat granulosa cell is in vitro (Nimrod et al., 1976).

A variety of neurotransmitters take part in central regulation of GnRH/LH. Recent evidence suggests that N-methyl-D-aspartate (NMDA), a potent agonist of excitatory amino acid neurotransmitters, induces LH secretion through stimulating GnRH release at the hypothalamic level. This excitatory amino acid has been implicated in regulation of the pre-ovulatory surges of LH and GnRH. Systemic administration of this drug elicits acute elevation of plasma LH levels in prepubertal and adult rats. (Ondo et al., 1976; Pohl et al., 1989). The period of sexual quiescence in the prepubertal rhesus monkey is characterized by the inactive hypothalamic-pituitary-gonadal axis and regressed gonadal function. However, pulsatile administration of NMDA to juvenile female rats (Urbanski et al., 1987) and juvenile monkeys (Plant et al., 1989) can induce transient puberty hence, pituitary still retains the capacity to respond to GnRH that may be provoked from the hypothalamus of prepubertal monkeys by an iv injection of N-methyl-D-aspartate. In the adult female rhesus monkey intravenous injection of NMDA causes rapid release of both LH and FSH (Wilson and Knobil, 1982). Photoperiodic animals such as the hamster have also been shown to respond with elevated LH levels to NMDA administration (Urbanski, 1992). Administration of NMDA antagonist on the days preceding the onset of puberty caused a delay in the onset of puberty in both males and female rats (Bourguignin et al., 1990; Urbanski and Ojeda, 1990). Current evidence demonstrates that NMDA antagonists block both spontaneous and steroid-induced LH surges (Urbanski and Ojeda, 1990; Roelof-Meijs et al., 1991). More recently release of glutamate itself has been observed to peak at the time of LH surge (Ping et al., 1994). These findings suggest that NMDA neurotransmission may be an integral component of the neurotransmission line that mediates steroid induced surges of gonadotropin in the female rat (Brann and Mahesh, 1991) and may play an important role on pro-estrous in regulating the pre-ovulatory gonadotropin and prolactin surge. Excitatory amino acid neurotransmitters also appear to be potent modulator of PRL secretion in rodents and primates (Wilson and Knobil, 1982; Gay and Plant 1987; Olney and Price, 1980; Pohl et al., 1989). NMDA elicits PRL secretion in adult rats (Olney and Price, 1980; Arslan et al., 1988) and monkeys (Wilson and Knobil, 1982; Arslan et al., 1991). Administration of NMDA antagonist, MK-801 significantly attenuates the pro-estrous gonadotropin and PRL

surge in immature and the adult cycling female rat. There is increasing evidence that the estradiol is an important determinant of the effect of excitatory amino acid on LH secretion in the female. The fact that estradiol markedly increases neuronal responses to excitatory amino acid (EAA), may provide a biochemical explanation for the steroid facilitation of EAA action on LH secretion (Smith et al., 1989). NMDA administration inhibits LH secretion in ovariectomized monkey (Reyes et al., 1990), whereas it stimulates LH release in ovariectomized rats after estradiol replacement therapy.

The purpose of the present investigation was to examine the effect of exogenous FSH and human chorionic gonadotropin (hCG) on follicular development, induction of ovulation and menstruation in a juvenile primate. The immature monkey was used as a model since ovaries at this age lack endogenous gonadotropin support but is capable of responding to exogenous hormonal stimulation (Keingsberg et al., 1984). In addition, the pituitary gland receives virtually no GnRH stimulation and is under these conditions lacks responsiveness to estrogen feed back (Dierschke et al., 1974).

Limited data are available regarding the role of excitatory amino acids in controlling secretion of PRL during the menstrual cycle in the rhesus monkey. Functional role of PRL in follicular and luteal development in primates is not established and data on secretary dynamics of PRL during the menstrual cycle are scant. During the course of menstrual cycle marked changes occur in steroid milieu, which provides an interesting paradigm to study EAA-steroid interaction in controlling pituitary hormone secretion. Therefore the present investigation was under taken to study some aspects of regulation of prolactin secretion under different physiological state using neuroexcitatory amino acid, NMA, as a neuropharmacological probe.

MATERIALS AND METHODS

ANIMALS

Five immature female monkeys (*Macaca mulatta*), 12-18 months of age, were utilized in this study. The age of the animals was calculated using a dental formula described by Haigh and Scott (1965). The body weight of the animals at the beginning of the experiment ranged from 1.8 - 2.5 kg. The animals housed in individual cages, were maintained under standard colony conditions at the Primate Facility of the Quaid-i-Azam University, Islamabad. They were provided with standard monkey food supplemented with fresh fruits and vegetables. Water was available ad-libitum.

EXPERIMENTAL PROTOCOL

GONADOTROPIN STIMULATION

The monkeys received intramuscularly 20 IU FSH daily for 12 days, dissolved in physiological saline (0.9% NaCl). The protocol used for induction of ovulation was similar to that described by Schenken et al (1984). A bolus dose of 1250 IU hCG was administered intramuscularly on 13^{th} day to induce ovulation. Throughout the treatment, day 1 of the first injection was considered as day 1 of the cycle. All animals were checked daily for menstruation, and any uterine bleeding recorded for 2 or more consecutive days were regarded as menstruation (Fig.1). Changes in the sex skin colour were evaluated and graded + to +++ by using a series of colour standards.

ULTRASONIC IMAGING

Follicular development was monitored by ultrasonography (Echo camera SSD 500,Aloka) with the use of 5-MHZ on day 9 of an induced cycle. On day 21, animals were re-examined by ultrasonography to determine the formation of the corpora lutea.

CATHETERIZATION

Before handling, the animals were anaesthetized with ketamine hydrochloride (5 mg/kg; Ketavet, Parke-Davis, Freiburg, FRG) and while under sedation, a Teflon cannula

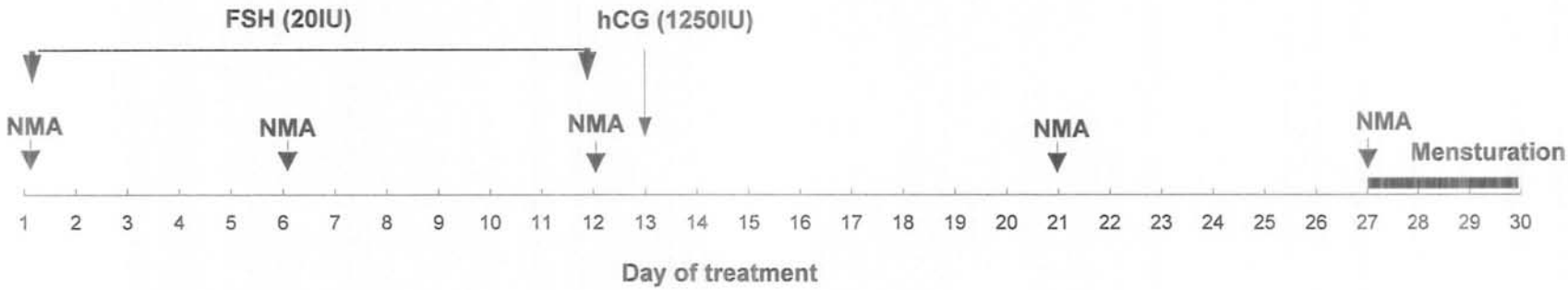


Fig.1.Protocol for immature female rhesus monkeys showing the FSH and hCG treatment in each animal. Monkeys received FSH (20IU) for 12 days, hCG(1250 IU) was administered on day 13 and luteal phase followed until the onset of menstruation.

(Vasocan Brannule 0.8 mm/22 G.O.D., B. Braun, Melsangen AG, Belgium) was inserted in the sephanous vein for blood sampling and drug infusion. Ketamine hydrochloride anesthesia (2.5 mg/kg, im) was given at 30 min intervals. The dose of ketamine used was not enough to induce narcosis but was sufficient to immobilize the animals.

BLEEDINGS

Sequential blood samples (2.0 ml) were obtained at 10 min intervals in heparinized syringes. Following withdrawal of each sample, an equal volume of heparinized (5 1U/ml) saline was injected into the tubing. All bleedings were carried out between 10:00-14:00 h to minimize diurnal variations. Blood samples were immediately centrifuged at 3000 rpm for 10 min. Plasma was separated and stored at –15°C until analyzed.

Effect of Single Intravenous Injection of NMA on Plasma PRL, E_2, P Secretion in Monkeys during different Phases of the Induced Cycle

NMA was dissolved in normal saline immediately before use and passed through a 0.22μm filter at the time of injection. A single iv injection of NMA (15mg/kg BW) was administered to the immature monkeys via the cannula during different days of the induced cycle (1,6,12,21,27 day). Blood samples (~2 ml) were collected at –20, -10, 0, 10, 20, and 30 min, relative to NMA injection at 0 min.

Effect of Gonadotropin Stimulation on Plasma PRL, E_2 and P Secretion in Monkeys during the Induced Cycle

Single blood sample was obtained after every two days of the induced cycle in order to determine the plasma PRL, E_2 and P secretion during the gonadotropin treatment until the onset of menstruation. Plasma was separated and stored at –15°C until analyzed.

HORAMONE DETERMINATIONS

Plasma levels of Prolactin (PRL), Progesterone (P), Estradiol (E_2) were determined in duplicate using specific assay systems.

PROLACTIN

The enzymeimmunoassay (EIA) system, presently employed, was developed for the Special Programme of Research in Human Reproduction of the World Health Organization and is intended for the measurement of PRL in plasma. The time required to complete an assay is approximately 5 hrs. The concentration range covered by the standards was 0-2500 mIU/L (WHO IPR 84/500). The sensitivity of the assay was 20 mIU/L.

The assay is of an immunometric ("sandwich") design, utilizing two anti-Prolactin antibodies. The first is a polyclonal antibody and is attached to a magnetic particle. The second is a monoclonal antibody and is labeled with alkaline phosphatase. The assay has three main stages.

Immunoextraction

Sample is incubated with magnetic anti-Prolactin for 30 minutes at 37°C. Prolactin in the sample binds to the magnetic particles. Other serum components are removed by decantation following a magnetic separation that includes one wash step.

Labeled Antibody Reaction, The solid phase is incubated with alkaline phosphatase labeled anti-Prolactin for 2 hours at 37°C. The labeled antibody reacts with any PRL bound to the magnetic particles after immunoextraction. Excess-labeled antibody is removed by decantation following a magnetic separation that includes two wash steps.

Colour Development, The solid phase is incubated with a coloured enzyme substrate for 1 hour at 37°C. The presence of alkaline phosphatase causes a colour change from yellow to pink. The intensity of the pink colour produced is a measure of the amount of alkaline phosphatase labeled antibody and hence PRL bound to the magnetic particles. The

reaction is terminated by addition of Stop Buffer and the optical density of all tubes is measured. The intensity of the colour formed by the enzyme reaction is directly proportional, within the working range of the assay, to the concentration of PRL in the sample. The concentration of PRL in a sample or control can then be determined directly by interpolation from the standard curve. Results were calculated according to the WHO Immunoassay processing programme. The intra- and inter-assay coefficients of variation were 7% and 11%, respectively.

PROGESTERONE

The plasma concentration of progesterone was determined by using enzymeimmunoassay (EIA) system developed for the Special Programme of Research in Human Reproduction of the World Health Organization and is intended for the measurement of progesterone in serum or plasma.

The assay is a direct 2-step serum EIA with no pre-extraction of samples. The concentration range covered by the standards is approximately 0-100 nmol/L with a minimum detectable dose of approximately 0.4 nmol/L. The assay takes two days to complete.

Progesterone in serum occurs largely bound to corticosteroid binding protein (CBG) and albumin. This protein bound progesterone is unavailable for antibody binding as antibodies can only bind to unbound or "free" progesterone. In extraction this problem is avoided as steroids are removed from the binding proteins by the extraction process. In direct P assay, a blocking reagent (which binds to the serum binding proteins, but not to the antibody) is used to displace P from serum binding proteins, thus making it available for antibody binding.

The P assay is a direct 2-step assay of a limited reagent ("competitive") design. The assay consists of the following main stages.

Reaction of antibody with serum progesterone: Monoclonal anti-proprogesterone antibody (raised to a progesterone-11α-hemisuccinate protein conjugate), blocking

reagent (30μmol/L Danazol) and magnetic anti-mouse IgG antibody are incubated with the sample overnight at 4.8°C in a refrigerator. Progesterone, which binds to the solid phase antibody, is isolated by means of a magnetic separation including a wash step. Other serum components, including binding proteins, are removed ensuring they do not bind progesterone tracer in the next step.

Reaction of antibody with labeled progesterone: The solid phase is incubated for 2 hours at 4-8°C in a refrigerator with labeled progesterone tracer (progesterone-11α hemisuccinate-alkaline phosphatase). Any P tracer, which binds to the solid phase, is isolated by a magnetic separation including two wash steps.

Colour Development: The solid phase is incubated with a coloured enzyme substrate for 1 hour at 37°C. The reaction is terminated by the addition of a Stop Buffer and the optical density of all tubes is measured. The intensity of the colour formed by the enzyme reaction is inversely proportional, within the working range of the assay, to the concentration of P in the sample. The concentration of P in a sample or control can then be determined directly by interpolation from the standard curve. The sensitivity of the assay is less than 0.4 nmol/L. Results were calculated according to the WHO Immunoassay processing programme. The intra- and inter-assay coefficients of variation were 6% and 9%, respectively.

ESTRADIOL

The Abbott IMX® Estradiol assay is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative measurement of E_2 in serum and plasma (EDTA and heparin). The E_2 assay is based on the Microparticle Enzyme Immunoassay (MEIA) technology. The reagents and sample are added to the reaction cell in the following sequence:

The probe/electrode assembly delivers the sample, Anti-Estradiol (Rabbit, polyclonal) Coated Microparticles and buffer containing surfactant to the incubation well of the reaction cell forming an Antibody-Estradiol complex.

An aliquot of the reaction mixture containing E_2 bound to the Anti-Estradiol Coated Microparticles is transferred to the glass fiber matrix. The microparticles bind irreversibly to the glass fiber matrix. The matrix is washed to remove unbound materials. The Estrogen-Alkaline Phosphatase Conjugate is dispensed onto the matrix and binds to the unoccupied antibody binding sites. The matrix is washed to remove unbound materials. The substrate, 4-Methylumbelliferyl Phosphate, is added to the matrix and the MEIA optical assembly measures the fluorescent product. Sensitivity, which represents the lowest measurable concentration of E_2 that can be distinguished from zero, was determined through statistical evaluation of assay runs. The sensitivity of the IMX E_2 was calculated to be 25 pg/ml.

PHARMACOLOGIC AGENTS

The following drugs were used in the present study:

1. Ketamine hydrochloride (ketavat; park Davis , Berlin, FRG).
2. N-methyl -D,L -aspartic acid (NMA, Sigma Chemical Co. St Louis, MO, USA).
3. Metrodin Urofolliotropin 75 IU.I.F.(Serono S.P.A. Rome, Italy).
4. Profasi (Serono S.p.A. Rome, Italy).

STATISTICAL ANALYSIS

For comparison of baseline PRL, E_2 and P secretion during the five days of the induced cycle, hormone levels were calculated by averaging all the concentrations obtained before NMA treatment. On the other hand, PRL, E_2, P responsiveness to NMA stimulation was determined by comparing basal levels of these hormones calculated by averaging the concentrations immediately before the injection at 0 min and the levels worked out by averaging the concentrations of hormones 10 min after the NMA injection. Student's t-test was used to determine differences between the means of basal and stimulated levels. The data were also subjected to analysis of variance and Duncan's multiple range tests. P values are mentioned for t-test applied. Where analysis of variance is carried out both values for F and P are given.

RESULTS

BODY WEIGHT

The body weight of immature female rhesus monkeys (*Macaca mulatta*) recorded over a period of treatment is presented in Table 1. The mean body weights at the beginning and at the end of the experiment were, 2.30±0.03 kg and 2.18±0.06 kg, respectively. The changes in body weight of animals during the course of experimental period were not significantly different (P>0.05).

GONADOTROPIN STIMULATION

Changes in sex skin colour

In the animals, prior to gonadotropins treatment, the sex skin was of pale colour and was scored as (–). A marked change in the sex skin colour occurred following 12 days of FSH treatment. The pattern of sex skin changes following initiation of treatment was almost similar in all the animals (Fig.2.). A discernible increase in intensity of the sex skin colour was observed on day 3 of treatment. The colour of the sex skin ranged between (+) and (+++) in all of the treated monkeys. The gonadotropin induced sex skin response persisted from day 3 to 22. Increase in the intensity of the sex skin colour was invariably accompanied by swelling of the vulva and the perineal skin. Subsequently there was a gradual waning of the sex skin colour and by day 27-30, the colour had regressed to (-) The decline in the colour intensity was followed by withdrawal bleeding. Subsequent to gonadotropin treatment, uterine bleeding was observed in all the monkeys while the menstruation occurred on day 27. The uterine bleeding lasted 3-4 consecutive days.

Follicular development

The effects of FSH and hCG treatment on ovarian size, follicular development and lutenization assessed by ultrasonography are shown in Fig.3 and 4. Before the treatment, ovaries of monkeys were examined by laproscopy as described in table 2 of chapter 2. Ovaries of treated monkeys exhibited a marked response to gonadotropin treatment by way of enlargement in size, follicular development (Fig. 3) and formation of corpus luteum (Fig.4). In all these monkeys, the ovaries (12.5-14.2 mm in diameter) were enlarged primarily due to the presence of large follicles of various sizes, as assessed by

TABLE 1

Mean body weight (kg)of immature female rhesus monkeys(n=5) before and after the FSH and hCG treatment

Animal No	Body weight(kg)	
	Before treatment	After treatment
991	2.3	2.3
992	2.2	2.1
993	2.3	2.1
994	2.5	2.3
995	2.2	2.1
Mean±SEM	2.3 ± 0.03	2.18 ± 0.06

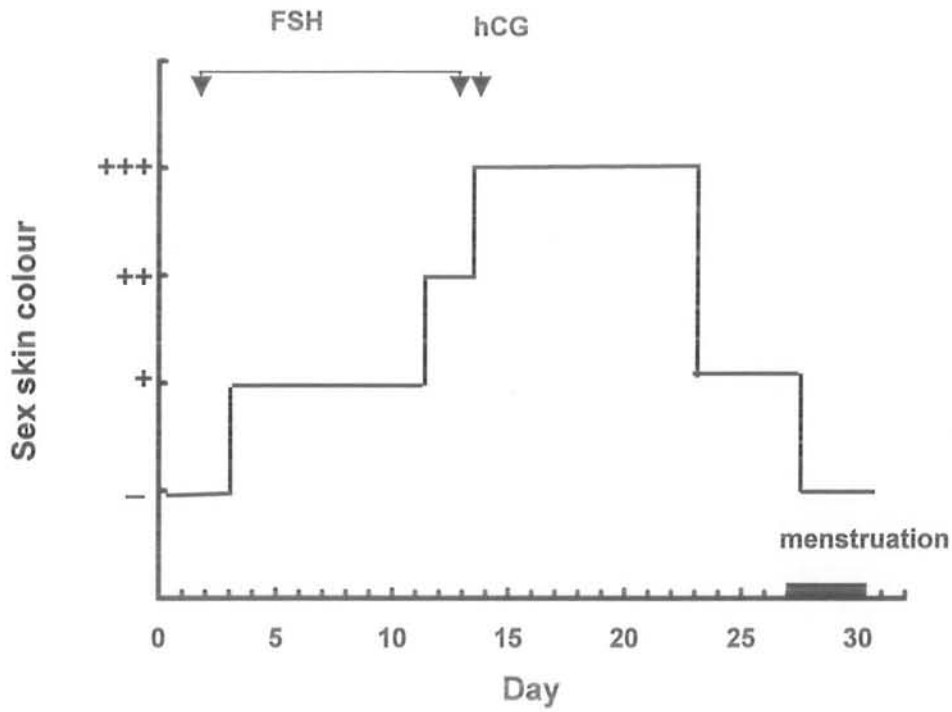
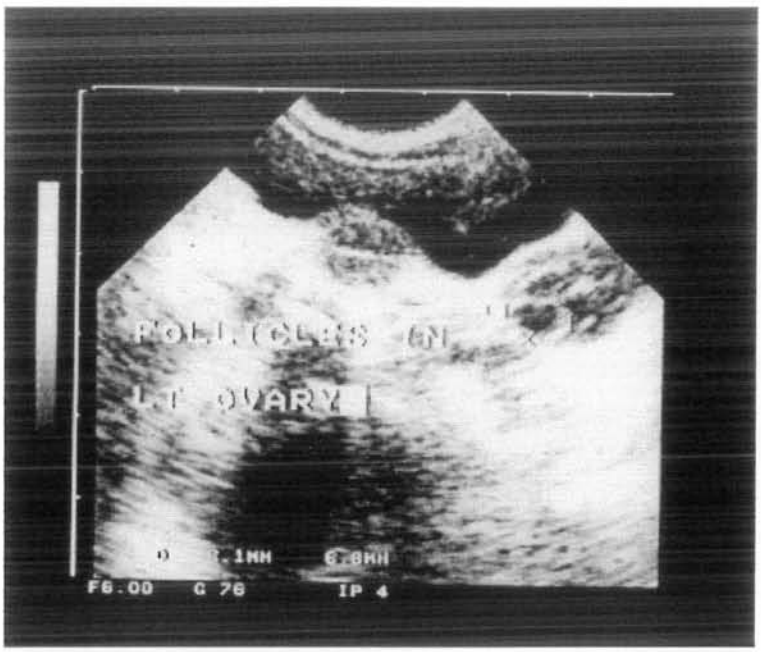
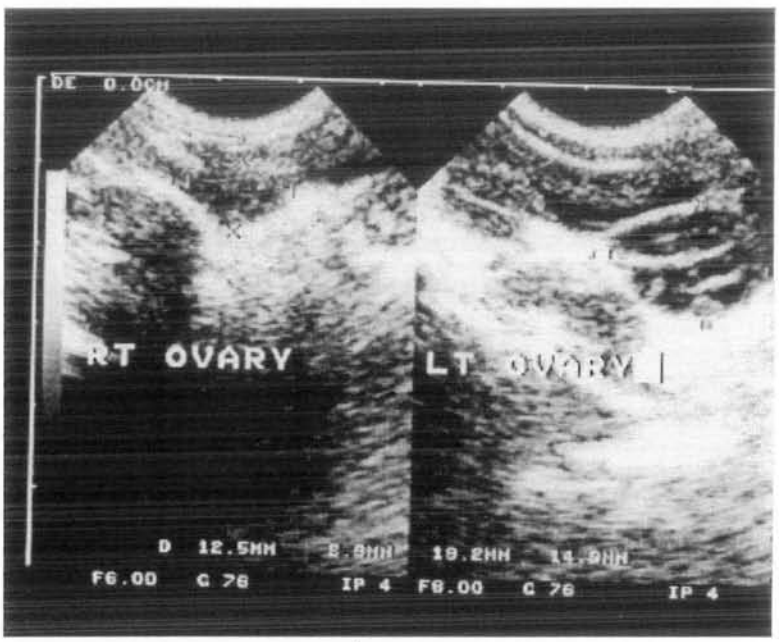


Fig.2. Changes in sex skin colour and onset and duration of uterine bleeding in immature female rhesus monkey after gonadotropin treatment.

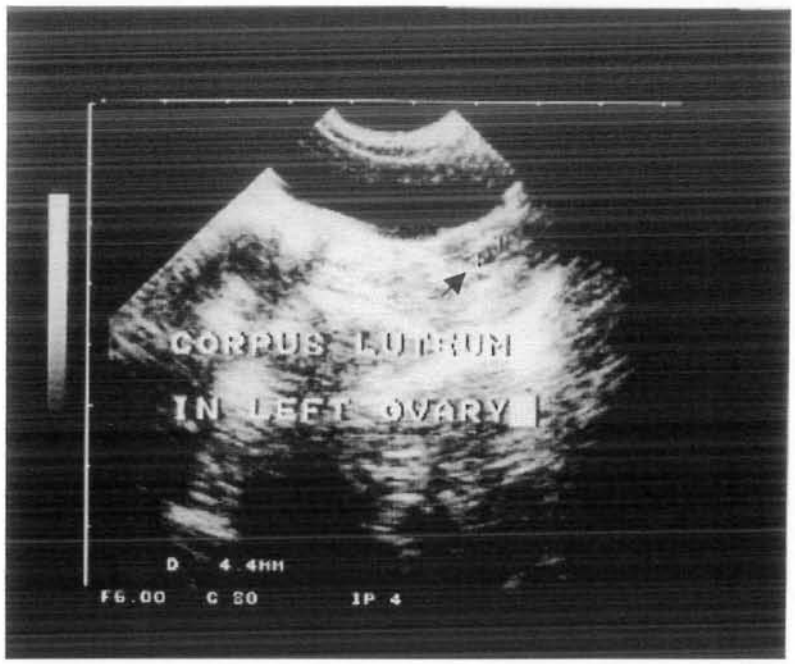


a

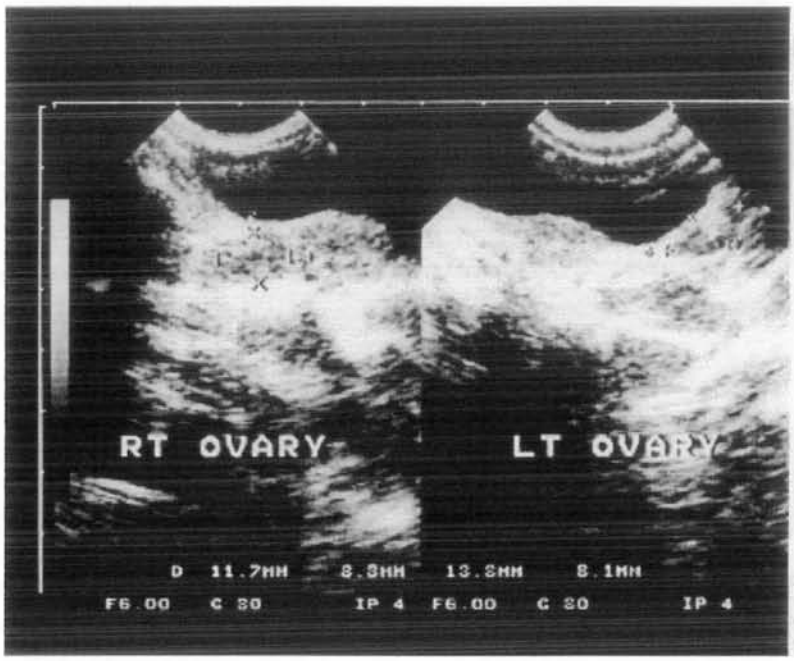


b

Fig.3 . The ultrasonic image from the left ovary showing the size and number of the developing follicles on day 9 of the FSH treatment (a). Photograph b) represent the size of the ovaries.



a



b

Fig.4. Representative ultrasonic images from the left and right ovaries of a monkey (# 983). Photograph a) shows the size and status of corus luteum after FSH and hCG treatment. Photograph b)shows the size of the ovaries.

ultrasonography. Ovaries of animals examined on day 9 of FSH treatment, possessed large developing follicles (~7 in number per ovary) ranging from 6-8 mm in diameter. Ultrasonography performed on day 21 of FSH treatment, revealed a well-developed corpus luteum in the left ovary (4.4 mm in diameter Fig.4).

EFFECT OF SINGLE IV INJECTION OF NMA ON PLASMA PRL SECRETION IN MONKEYS DURING DIFFERENT PHASES OF THE INDUCED CYCLE

The individual and mean plasma PRL profile before and after NMA administration in five immature monkeys during different days of the induced cycle are given in Table 2 to 6 and in Fig.5 and 6. In these monkeys no significant increase ($P>0.05$) in mean plasma PRL concentration was observed following NMA injection on day 1 of the cycle. On the other hand the significant increase in the PRL concentration ($P<0.05$) was noted after NMA injection, on days 6, 12, 21 and on menstruation. PRL concentration before 0 min and after 10 min, following administration of single iv injection of NMA, were 263.66±12.02 mIU/L and 207.58±22.23 mIU/L, respectively, on day 1 of the induced cycle (Table 2). The mean plasma PRL concentrations were 261.56±31.11 mIU/L at 0 min and 407.62±50.79 mIU/L at 10 min on 6^{th} day of the cycle. The plasma PRL concentration on the day 12 was 240.38±19.04 mIU/L and 463.76±36.78 mIU/L at 0 min and 10 min of NMA injection. There was a discernable increase in PRL concentration over the basal levels at 10 min following NMA administration on day 21 of the cycle. Prolactin concentration increased significantly ($P>0.006$) from 327.48±38.69 mIU/L at 0 min to 1024.36±189.29mIU/L at 10 min. A marked rise in mean PRL concentration was also observed on the first day of menstruation, being 265.48±19.62 mIU/L at 0 min and 537.10±62.67 mIU/L at 10 min ($P<0.03$). Mean plasma PRL concentration during these days remained high till 30 min of NMA injection. Maximum increase in plasma PRL concentration in response to NMA was observed on the 21 day of the induced cycle. During this period an increase of 213% over the basal levels was induced by a single iv injection of NMA, whereas on day 6,12 and on menstruation an increase of 55%, 92% and 102%, respectively, over the basal levels was observed after the administration of the drug ($F=5.08$, $P<0.002$). Mean basal plasma PRL concentration were also significantly different during these days ($F=8.39$, $P<0.0001$)

TABLE 2

Plasma PRL concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at day one(before FSH injection) of the cycle.

Time (min)	Plasma PRL (mIU/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	233.2	167.2	182.5	293.1	228.3	220.86 ± 22.11
-10	209.4	176.3	261.4	272.4	272.2	238.34 ± 19.40
0	238.6	239.3	264.3	272.6	303.5	263.66 ± 12.02
10	225.1	215.6	257.3	215.8	124.1	207.58 ± 22.23
20	247.4	268.4	292.1	323.3	291.4	284.52 ± 12.74
30	292.3	219.3	293.4	280.3	303.2	277.70 ± 15.05

TABLE 3

Plasma PRL concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at 6th day of the induced cycle.

Time (min)	Plasma PRL (mIU/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	211.3	226.4	148.4	214.3	312.4	222.56 ± 26.10
-10	223.0	276.4	182.7	214.6	310.1	241.36 ± 22.41
0	200.6	274.6	214.4	227.9	390.3	261.56 ± 31.11
10	365.0	336.3	569.2	325.4	442.2	*407.62 ± 50.79
20	277.3	514.7	637.1	355.6	416.3	440.20 ± 48.34
30	396.7	475.2	646.8	309.4	334.2	432.46 ± 60.13

Values significantly different from those at 0 min

* $P<0.03$

TABLE 4

Plasma PRL concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at 12th day of the induced cycle.

Time (min)	Plasma PRL (mIU/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	291.1	215.2	251.1	259.3	247.1	252.76 ± 12.17
-10	310.3	228.2	211.6	177.3	327.2	250.92 ± 29.04
0	280.3	214.2	219.3	196.4	291.7	240.38 ± 19.04
10	443.2	423.2	553.3	358.8	540.3	*463.76 ± 36.78
20	480.2	470.3	558.4	292.2	472.1	454.64 ± 43.77
30	541.1	631.8	698.5	328.4	517.4	543.44 ± 62.72

Values significantly different from those at 0 min
* P<0.0006

TABLE 5

Plasma PRL concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at 21 day of the induced cycle.

Time (min)	Plasma PRL (mIU/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	292.1	471.2	471.4	247.4	258.3	348.08 ± 50.84
-10	405.3	425.3	349.7	288.1	387.3	371.14 ± 24.19
0	285.2	454.1	346.3	219.4	332.4	327.48 ± 38.69
10	1473.3	968.5	1447.4	622.3	610.3	*1024.36 ± 189.29
20	2072.2	933.4	1569.3	407.4	344.2	1065.30 ± 334.54
30	3191.1	1026.3	1920.2	441.2	300.2	1375.80 ± 535.94

Values significantly different from those at 0 min
* P<0.006

TABLE 6

Plasma PRL concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at menstural day of the induced cycle.

Time (min)	Plasma PRL (mIU/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	250.2	290.5	280.2	354.1	252.3	285.46 ± 18.85
-10	275.3	271.3	256.3	323.1	211.2	267.44 ± 17.97
0	263.6	218.2	277.1	332.3	236.2	265.48 ± 19.62
10	673.6	489.3	585.3	620.2	317.1	*537.10 ± 62.67
20	628.4	779.2	528.2	619.5	290.6	569.18 ± 80.45
30	555.1	681.3	791.1	583.2	293.3	580.80 ± 82.96

Values significantly different from those at 0 min
* P<0.03

TABLE 7

Plasma PRL concentrations in immature female monkeys throughout the FSH and hCG treatment .

Day	Plasma PRL (mIU/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
0	209.1	167.4	182.4	217.2	228.2	200.86 ± 11.33
3	254.2	156.3	192.4	265.4	146.3	202.92 ± 24.52
6	223.4	226.2	214.3	272.4	312.3	249.72 ± 18.61
9	306.3	272.1	232.2	221.5	178.3	242.08 ± 21.95
12	310.3	228.3	251.2	257.5	247.4	258.94 ± 13.74
15	514.2	703.4	772.3	442.1	581.5	*602.70 ± 60.36
18	608.2	881.7	637.4	844.4	822.3	*758.80 ± 56.46
21	405.4	471.8	471.6	288.3	387.3	404.88 ± 33.71
24	250.6	583.5	215.4	253.2	302.2	320.98 ± 67.05
27	250.2	290.4	256.3	257.2	236.4	258.10 ± 8.87

Significantly different from the other days of the cycle (* P<0.05).

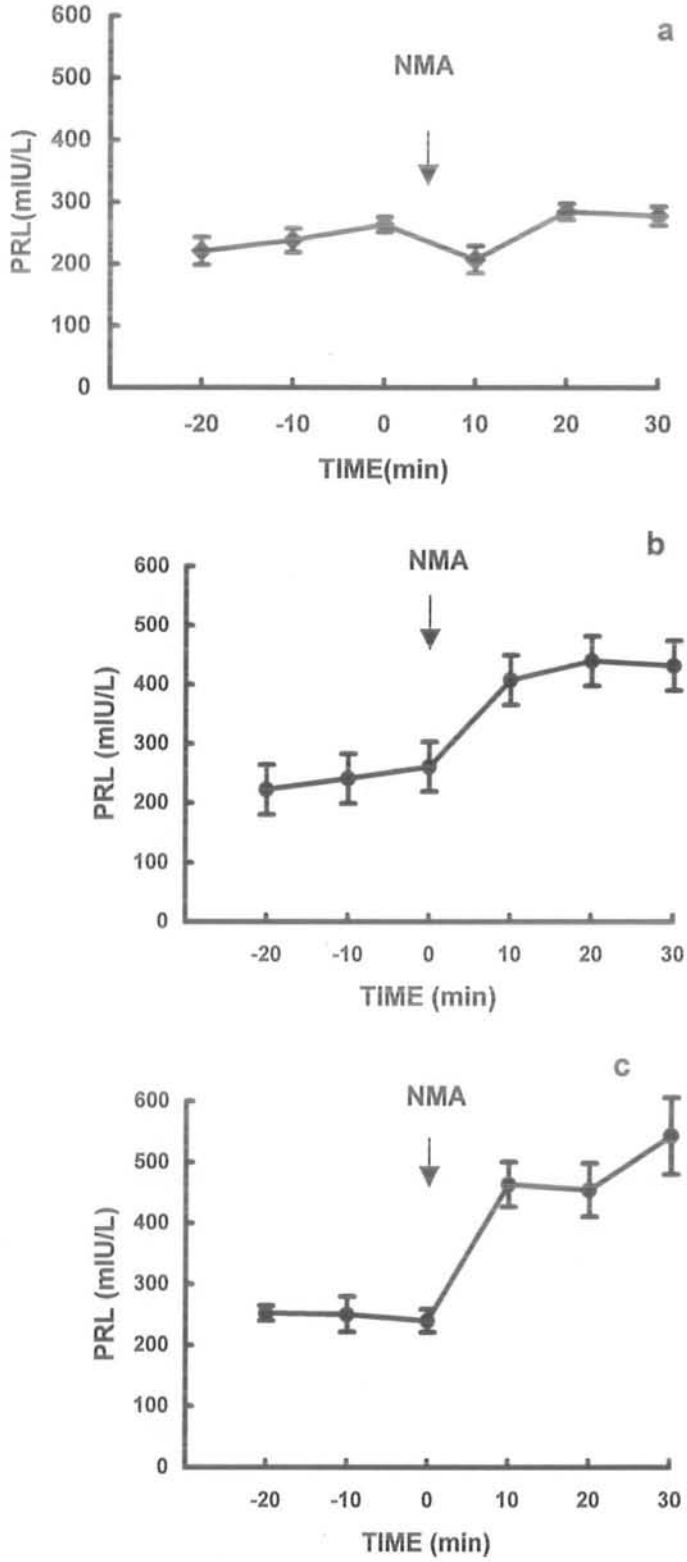


Fig.5. Mean plasma PRL concentrations in immature female rhesus monkeys (n=5) before and after the administration of NMA at day 1(a) day 6 (b) and 12th day (c) of the induced cycle

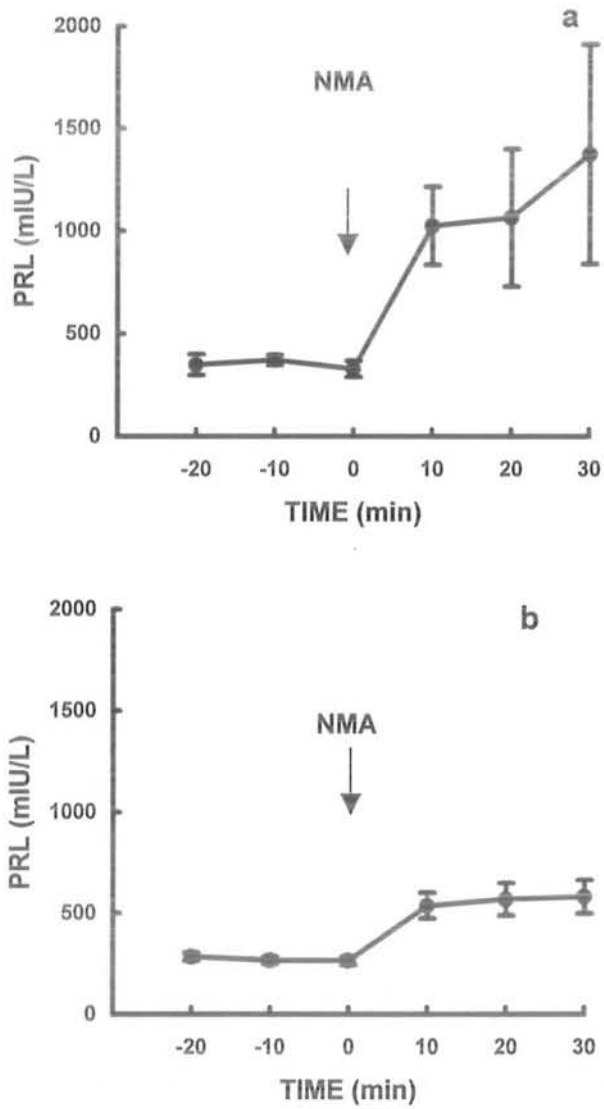


Fig.6. Mean plasma PRL concentrations in immature female rhesus monkeys (n=5) before and after the administration of NMA at day 21(a)and menstural day (b) of the induced cycle.

EFFECT OF GONADOTROPINS STIMULATION ON PLASMA PRL SECRETION IN MONKEYS DURING THE INDUCED CYCLE

The mean basal plasma PRL profile in these monkeys during the gonadotropin treatment (FSH and hCG) period is shown in Table 7 and Fig.7. The mean plasma PRL levels were low before treatment, being 200.86 ± 11.33 mIU/L and remained low up to 12th day of the treatment. The levels ranged from 200.86±11.33 mIU/L to 258.94±13.74 mIU/L during these days without any significant differences (P>0.05). The levels began to rise after hCG treatment on 13th day to reach 602.70±60.36 mIU/L on 15th day. The highest levels were observed on day 18 of the cycle (758.80±56.46 mIU/L) which were significantly different from other days of the cycle (F=24.79, P<0.001). The plasma PRL levels showed a decline on day 21 to 27 which ranged from 404.88±33.71 mIU/L to 258.10±8.87 mIU/L, respectively.

EFFECT OF SINGLE IV INJECTION OF NMA ON PLASMA E2 SECRETION IN MONKYS DURING DIFFERENT PHASES OF THE INDUCED CYCLE

Mean ± SEM, plasma E_2 concentration in response to NMA injections are presented in Tables 8-12 (Fig. 8-9). Mean plasma E2 did not increase significantly (P>0.05) before gonadotropin treatment. The administration of NMA resulted in significant stimulation of E_2 secretion (P<0.04) on day 6 (Fig. 8-b), after 30 min of challenge, and the E_2 concentration were 211.40±5.30 pg/ml at 0 min. and 371.50±41.38 pg/ml at 30 min. A significant (P<0.0006) increase in mean E_2 concentration over the basal level in response to amino acid injection was observed on day 12 (Fig. 8 c). The mean plasma E_2 concentration before (0min) and after (20 min) NMA injection were 253.98±21.31 pg/ml and 627.50±66.25 pg/ml respectively. Maximum increase in plasma E_2 concentration in response to NMA was observed on the day 12 of the induced cycle. Administration of NMA increased mean plasma E_2 concentration in all animals on day 21. The levels at 0 min and 30 min were 89.02±9.79 and 121.40±9.89 pg/ml, respectively. The increase in levels of plasma E_2 concentrations varies significantly (P<0.04). Similar response was observed at the time of menstruation in all the monkeys and administration of NMA significantly increased mean plasma E_2 concentration (P<0.01) after 30 min of challenge

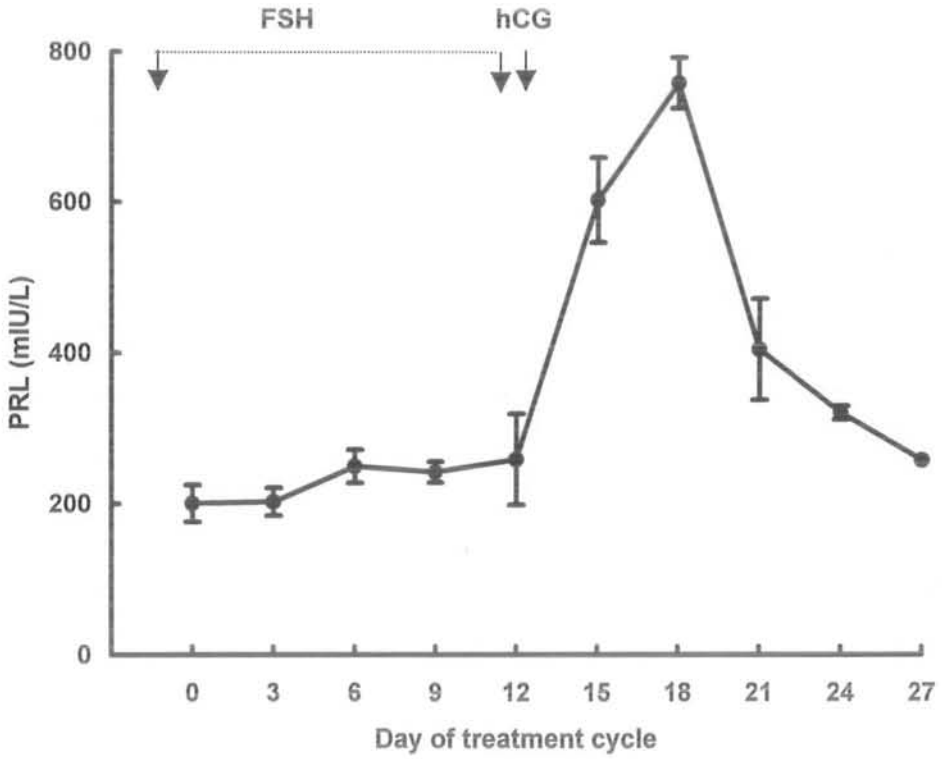


Fig.7. Mean plasma PRL profile throughout the treatment period in immature female rhesus monkeys (n=5) treated with FSH and hCG

TABLE 8

Plasma Estradiol concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at day one(before FSH injection) of the cycle.

Time (min)	Plasma E_2 (pg/ml) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	25.3	26.6	19.3	30.3	27.4	25.78 ± 1.82
-10	29.0	13.3	25.3	35.2	28.3	26.22 ± 3.61
0	27.3	18.2	27.4	29.4	31.2	26.70 ± 2.24
10	34.4	21.4	39.6	48.3	40.1	36.76 ± 4.44
20	24.4	28.3	40.4	18.4	36.4	29.58 ± 3.98
30	29.2	21.4	31.1	21.6	32.3	27.12 ± 2.35

TABLE 9

Plasma Estradiol concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at 6th day of the induced cycle.

Time (min)	Plasma E_2 (pg/ml) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	155.4	198.3	210.4	178.4	185.5	185.60 ± 9.33
-10	139.4	212.2	256.6	195.3	208.4	202.38 ± 18.80
0	199.1	226.2	216.3	199.1	216.3	211.40 ± 5.30
10	316.4	264.2	390.2	289.5	321.2	316.30 ± 21.14
20	411.6	293.3	378.3	245.5	268.3	319.40 ± 32.17
30	516.3	329.1	410.2	299.4	302.5	*371.50 ± 41.38

Values significantly different from those at 0 min
*** P<0.04**

TABLE 10

Plasma Estradiol concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at 12th day of the induced cycle.

Time (min)	Plasma E_2 (pg/ml) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	216.4	210.5	256.4	325.4	290.2	259.78 ± 21.88
-10	207.4	241.4	325.5	298.3	197.5	254.02 ± 25.10
0	189.5	253.3	231.5	314.3	281.3	253.98 ± 21.31
10	234.3	428.2	325.5	413.1	396.4	*359.50 ± 35.93
20	480.4	874.3	594.3	563.2	625.3	***627.50 ± 66.25
30	414.5	887.2	633.2	502.2	478.2	**583.06 ± 83.98

Values significantly different from those at 0 min

* P<0.03

* P<0.005

* P<0.0006

TABLE11

Plasma Estradiol concentrations in immature female monkeys before and aftera single iv injection of NMA (15 mg/kg) at 21 day of the induced cycle.

Time (min)	Plasma E_2 (pg/ml)concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	91.4	61.2	87.7	75.1	85.2	80.12 ± 5.39
-10	98.3	64.4	74.5	82.3	69.2	77.74 ± 5.95
0	105.4	51.6	89.3	96.4	102.4	89.02 ± 9.79
10	116.3	53.5	123.3	104.4	189.2	117.34 ± 21.78
20	126.2	64.6	156.2	99.4	156.2	120.52 ± 17.61
30	139.2	89.7	109.2	126.5	142.4	*121.40 ± 9.89

Values significantly different from those at 0 min

* P<0.04

TABLE 12

Plasma Estadiol concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at menstural day of the induced cycle.

Time (min)	Plasma E_2 (pg/ml) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	14.2	24.5	19.5	21.4	23.5	20.62 ± 1.82
-10	17.3	28.3	21.3	20.3	19.4	21.32 ± 1.87
0	28.4	34.3	26.3	30.3	21.5	28.16 ± 2.12
10	22.5	58.2	59.4	62.4	45.8	*49.66 ± 7.36
20	25.6	84.1	72.7	54.5	78.7	*63.12 ± 10.62
30	35.2	131.2	85.2	75.4	64.5	**78.30 ± 15.66

Values significantly different from those at 0 min
* P<0.02
** P<0.01

TABLE 13

Plasma Estradiol concentrations in immature female monkeys throughout the treatment period .

Day	Plasma E_2 (pg/ml) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
0	24.5	26.3	26.3	30.7	27.4	27.04 ± 0.98
3	46.4	31.2	45.4	35.5	29.4	37.58 ± 3.53
6	199.3	198.7	210.5	195.4	208.3	202.44 ± 2.95
9	358.4	162.5	230.3	298.3	326.2	275.14 ± 35.22
12	216.3	210.4	312.6	325.2	290.1	270.92 ± 24.19
15	215.3	608.3	416.7	510.3	541.3	*458.38 ± 68.16
18	374.3	762.3	562.5	451.2	481.3	*526.32 ± 66.24
21	90.6	61.2	96.4	96.4	85.2	85.96 ± 6.49
24	27.3	92.5	75.3	65.4	96.4	71.38 ± 12.36
27	17.2	24.4	26.3	21.4	23.3	22.52 ± 1.53

Significantly different from the other days of the cycle (* P<0.05).

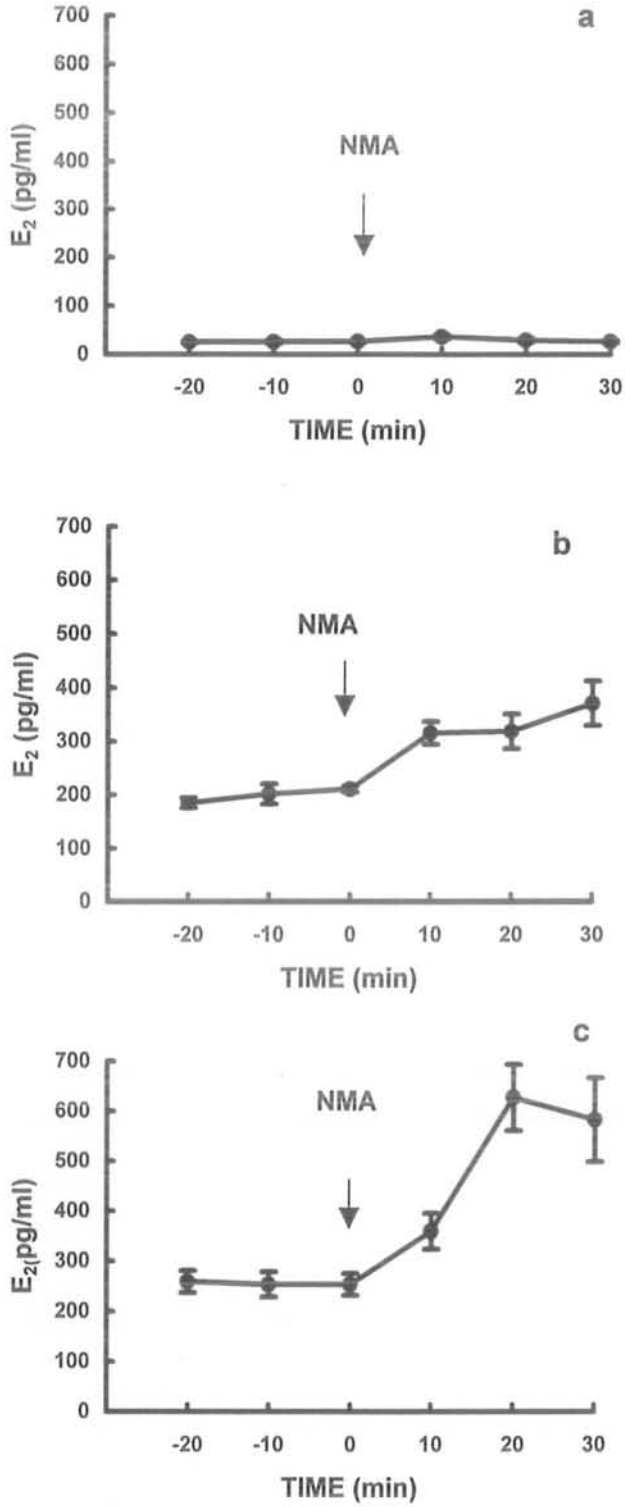


Fig.8. Mean plasma Estradiol concentrations in immature female rhesus monkeys (n=5) before and after the administration of NMA at day 1(a) day 6 (b) and 12th day (c) of the induced cycle.

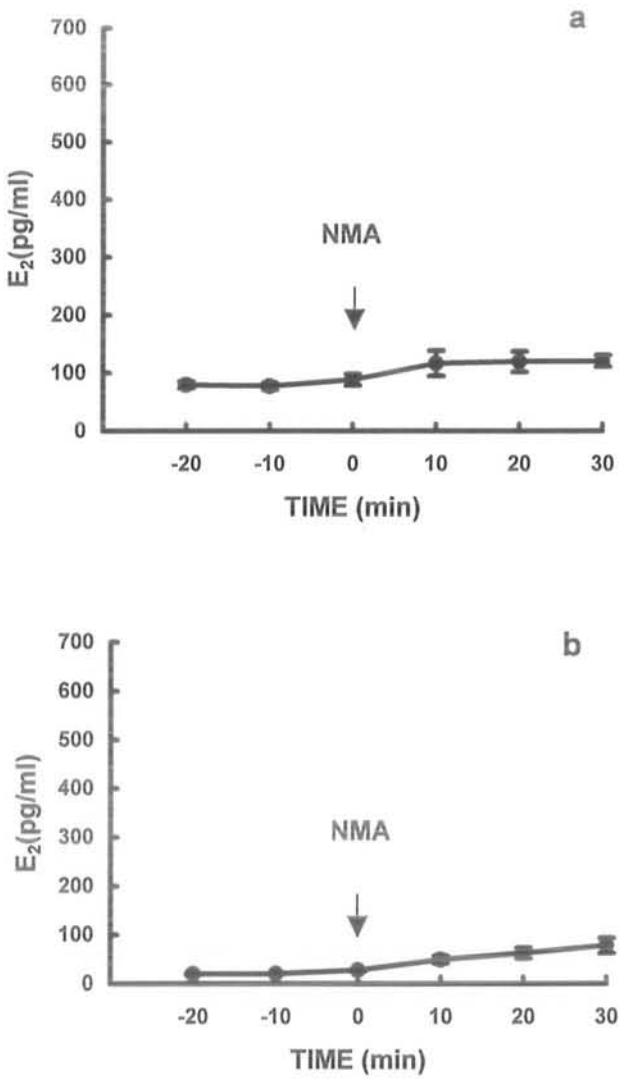


Fig.9. Mean plasma Estradiol concentrations in immature female rhesus monkeys (n=5) before and after the administration of NMA at day 21(a)menstural day (b) of the induced cycle.

(28.16±2.12 to 78.30±15.66 pg/ml). On day 12 an increase of 147% over the basal levels was induced by a single iv injection of NMA, whereas on day 6, 21 and on the first day of menstruation, an increase of 75%, 36% and 81% over the basal levels was observed after the administration of the drug (F=4.11, P<0.01).

EFFECT OF GONADOTROPINS STIMULATION ON PLASMA E2 SECRETION IN MONKEYS DURING THE INDUCED CYCLE

The mean plasma E2 levels were low before the gonadotropins treatment (27.04±0.98 pg/ml, Table 13.and Fig. 12). A prompt increase (F=30.30, P<0.001) in plasma E_2 concentration with mean levels 202.44±2.95 pg/ml was observed on day 6. The highest levels of E_2 during the cycle were found on day 15 and 18, which were 458.38±68.16 pg/ml and 526.32±66.24 pg/ml, respectively. A rapid decline in circulating levels was recorded on day 21 which continued to day 27 (22.52±1.53 pg/ml).

EFFECT OF SINGLE IV INJECTION OF NMA ON PLASMA P SECRETION IN MONKEYS DURING DIFFERENT PHASES OF THE INDUCED CYCLE

The mean plasma P profile before and after signal iv injection of neuroexcitatory, NMA, in these monkeys are presented in Tables 14-18 (Fig. 10-11). No significant difference in mean basal and mean plasma P concentration on the 0,6,12,21 days of the induced cycle (P>0.05), following single iv injection of NMA, was observed. On the other hand a significant increase (P<0.04, Table 18) was noticed on the day of menstruation. Mean P concentration was 5.55±0.61 nmol/L at 0 min and 8.77±1.20 nmol/L at 10 min, respectively.

EFFECT OF GONADOTROPINS STIMULATION ON PLASMA P SECRETION IN MONKEYS DURING THE INDUCED CYCLE

Estradiol levels increased in response to FSH administration and the injection of hCG induced probable ovulation and formation of corpora lutea as judged by elevated progesterone concentration and ultrasonography. The mean plasma P level was low (2.55±0.31 –7.18±0.65 nmol/L) before hCG administration, and remained at basal levels up to day 12. It began to rise after hCG treatment on day 13 reaching 86.36±9.35 and 123.86±7.11 nmol/L on days 15 and 18, being significantly different from the other days of the cycle (F=96.51,P<0.001) shown in Table 19 (Fig. 12). After hCG treatment the mean P levels increased significantly (p<0.05) and no further increase was noticed after day 18 and the P levels declined rapidly.

TABLE 14

Plasma P concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at day one(before FSH injection) of the cycle.

Time (min)	Plasma P (nmol/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	2.3	2.3	2.1	2.4	3.8	2.55 ± 0.31
-10	4.6	2.7	3.0	2.6	3.2	3.22 ± 0.36
0	3.3	5.5	1.8	2.3	2.4	3.04 ± 0.66
10	1.8	2.1	2.3	4.7	4.8	3.16 ± 0.66
20	1.4	3.8	3.2	2.2	2.6	2.66 ± 0.42
30	2.3	4.1	3.0	3.2	4.1	3.34 ± 0.34

TABLE 15

Plasma P concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at 6th day of the induced cycle.

Time (min)	Plasma P (nmol/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	4.2	4.3	3.6	3.6	3.1	3.77 ± 0.22
-10	2.1	5.2	3.3	3.2	3.6	3.47 ± 0.50
0	3.7	4.4	2.3	3.9	7.0	4.25 ± 0.76
10	4.1	2.9	3.7	4.9	3.9	3.90 ± 0.32
20	4.8	4.1	3.6	4.3	7.6	4.86 ± 0.71
30	6.1	5.9	3.4	4.7	8.8	5.78 ± 0.91

TABLE 16

Plasma P concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at 12th day of the induced cycle.

Time (min)	Plasma P (nmol/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	3.0	6.5	3.1	3.5	3.9	3.99 ± 0.66
-10	2.3	4.6	3.4	7.7	6.5	4.90 ± 0.98
0	5.0	5.8	3.4	9.3	3.7	5.43 ± 1.06
10	1.7	5.2	5.1	9.7	5.8	5.49 ± 1.27
20	3.7	7.4	2.7	8.5	7.9	6.03 ± 1.18
30	2.8	7.3	4.3	5.6	8.2	5.64 ± 0.98

TABLE 17

Plasma P concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at 21 day of the induced cycle.

Time (min)	Plasma P (nmol/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	29.1	29.5	25.2	26.0	52.4	32.43 ± 5.06
-10	21.4	29.7	32.2	26.0	44.2	30.70 ± 3.83
0	28.8	24.6	28.3	23.7	46.2	30.31 ± 4.09
10	25.3	30.1	32.5	26.7	72.2	37.36 ± 8.80
20	27.2	30.1	31.0	20.4	53.0	32.33 ± 5.49
30	24.2	32.7	30.3	23.7	31.1	28.36 ± 1.86

TABLE 18

Plasma P concentrations in immature female monkeys before and after a single iv injection of NMA (15 mg/kg) at menstural day of the induced cycle.

Time (min)	Plasma P (nmol/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
-20	3.8	6.6	6.8	7.0	5.6	5.96 ± 0.59
-10	2.6	6.1	7.6	6.7	4.3	5.46 ± 0.89
0	6.7	5.4	7.2	4.3	4.2	5.55 ± 0.61
10	8.4	6.4	10.0	12.7	6.3	*8.76 ± 1.20
20	4.4	5.6	7.5	7.2	4.5	5.82 ± 0.65
30	14.2	5.1	8.2	7.5	8.1	8.61 ± 1.50

Values significantly different from those at 0 min
*** P<0.05**

TABLE19

Plasma P concentrations in immature female monkeys throughout the treatment period .

Day	Plasma P (nmol/L) concentrations Animal No. 991	992	993	994	995	Mean ± SEM
0	2.3	2.3	2.1	2.4	3.8	2.55 ± 0.31
3	4.9	4.7	4.5	6.0	4.8	4.98 ± 0.25
6	4.2	4.3	3.6	3.6	3.1	3.77 ± 0.22
9	7.3	5.3	6.6	7.6	9.2	7.18 ± 0.65
12	3.0	2.5	3.1	3.5	3.9	3.19 ± 0.24
15	85.5	120.0	77.7	85.5	63.1	*86.36 ± 9.35
18	115.0	125.0	106.3	148.9	124.1	*123.86 ± 7.11
21	29.1	29.5	25.2	26.0	52.4	32.43 ± 5.06
24	5.7	13.6	25.7	24.3	24.1	18.66 ± 3.91
27	3.8	6.6	6.8	7.0	5.6	5.96 ± 0.59

Significantly different from the other days of the cycle (* P<0.05).

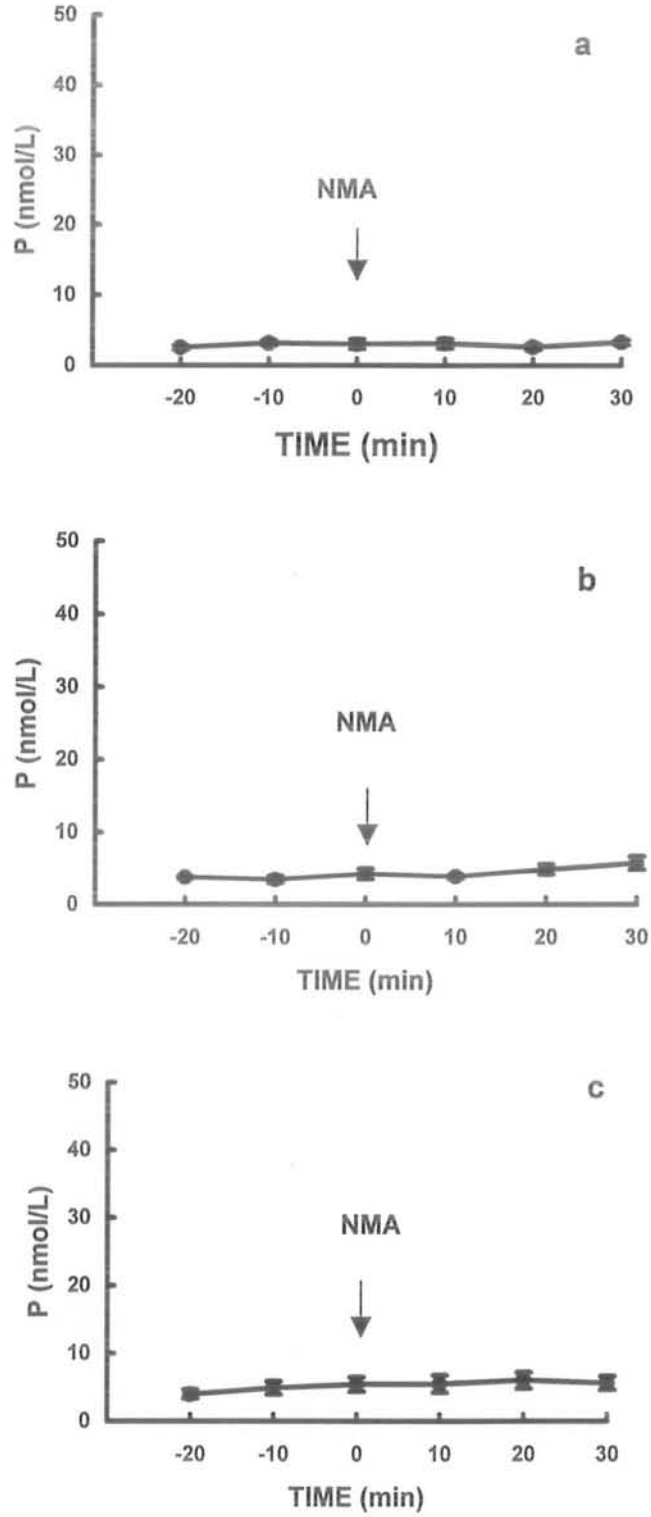


Fig.10. Mean plasma Progesterone concentrations in immature female rhesus monkeys (n=5) before and after the administration of NMA at day 1(a) day 6 (b) and 12th day (c) of the induced cycle .

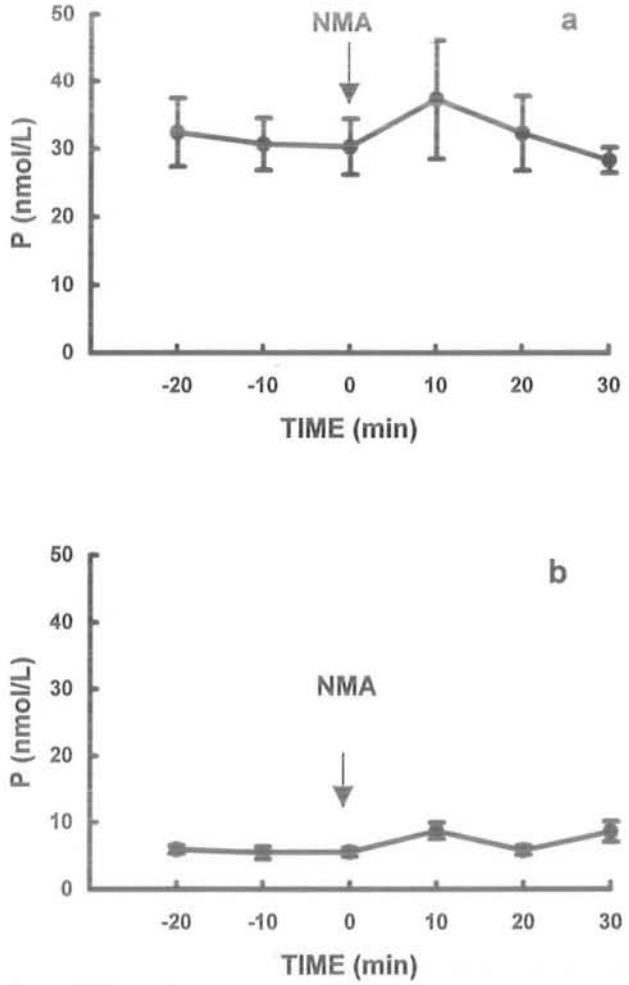


Fig.11. Mean plasma Progesterone concentrations in immature female rhesus monkeys (n=5) before and after the administration of NMA at day 21(a)and menstural day (b) of the induced cycle.

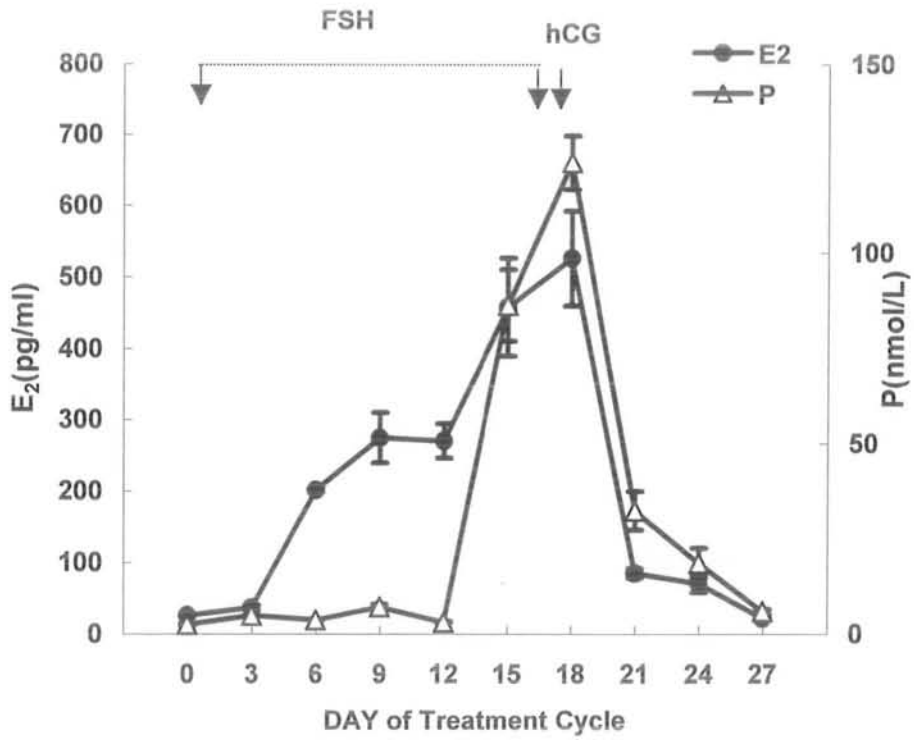


Fig.12. Mean plasma P(nmol/L) and E_2(pg/ml) profile throughout the treatment period in immature female rhesus monkeys (n=5) treated with FSH and hCG.

DISCUSSION

The immature monkey is an excellent model to determine the role of FSH in primate ovaries, because of the inactive hypothalamic pituitary axis at this age permits no release of endogenous gonadotropins (Wildt et al., 1980; Koering et al., 1991), resulting in an FSH free environment, and yet the ovary is responsive to FSH stimulation (Kenigsberg et al., 1984).

The present study was designed to study the role of gonadotropins in follicular maturation and on induction of ovulation using immature female rhesus monkey as a model. This model shows complete gonadotropin deficiency and, therefore, provides a better paradigm for study of gonadotropins.

The role of FSH in the selection of dominant follicle is well documented in adult monkeys (Goodman et al., 1983) and humans (Couzinet et al., 1988). In addition, when FSH release is initiated during the peri-menarchial period in monkeys, follicles begin to develop beyond the 1-mm stage, and cyclic activity is induced (Williams et al., 1983). Present study shows that developing follicles just under 1.0 mm in diameter are capable of being stimulated by FSH. This suggests that follicles of this size must express FSH receptors as they are recruited into the pool from which the dominant follicles are ultimately selected (Marilyn et al., 1994). In these monkeys follicular development was induced and some of the follicles attain a size of approaching preovulatory stage. In vivo studies have demonstrated that the follicles of the female rhesus monkeys show an increased sensitivity to FSH with preovulatory follicular development (Zelezink et al., 1986).

In the present study, prepubertal monkeys were chosen in preference to cyclic animals as a source of small pre antral (immature) follicles because the follicles were not previously exposed to preovulatory levels of estradiol (E_2).

The FSH preparation used in the present study was capable of inducing the development of multiple ovarian follicles that were responsive to hCG. All the monkeys given FSH throughout the follicular phase and hCG on day 13, ovulated few follicles and had functional corpora lutea with the elevated serum progesterone levels observed in their luteal phase. This is in agreement with Schenken et al, 1984. FSH treatment for 12 days

caused the maturation of apparently competent follicles. Our findings imply that FSH together with hCG induced the natural ovarian cycle in the immature female monkeys.

Pulsatile administration of LHRH results in premature initiation of reproductive cyclicity in the female rhesus monkeys (Wildt et al., 1980), guinae pig (Loose et al., 1985) and female rats to advance the onset of first ovulation (Urbanaski et al., 1987). Administration of FSH to immature animals may cause an alteration in the normal chronology of puberty (Koering et al., 1994). The usual age at which the first menstruation occurs in female rhesus monkey is three years (Wilen et al., 1976) and circulating concentration of estradiol rises between 2 $^{1/2}$ to 3 years associated with increase in perineal swelling and coloration (Terasawa et al., 1983;Wilson et al., 1986). A well developed sexual skin is present in adult monkeys (*Macaca mulatta*) only during adolescence. In the present study the first menstruation occurred at the age of 1.5-2 years after the FSH and hCG treatment. Sex skin colour was greatly stimulated by the FSH and hCG treatment and may be regarded as positive, indirect evidence of estrogen production by the ovaries. Prior to gonadotropin stimulation, the sex skin was of pale colour. A marked change in the sex skin colour occurred following 12 days of FSH treatment. Increase in the intensity of the sex skin colour was invariably accompanied by swelling of the vulva and the perineal skin. Present findings suggest that these monkeys also exhibit evidence of estrogen production as indicated by the marked stimulation of sex skin colour and subsequent uterine bleeding. FSH treatment resulted in the maturation of the sexual skin, the condition characteristic of the adult is same as established by Zuckerman et al., 1938. The genital area loses its edema and develops a brilliant red color, which was retained as long as FSH was administered. Once this mature condition was established the response of sexual skin to subsequent FSH treatment is limited to a change in colour.

The present data demonstrate significant changes in prolactin secretion during the induced reproductive cycle in the immature female rhesus monkeys, after the administration of NMA, as it does in estrous rat (Pohl et al., 1989; Luderer et al., 1993), male rat (Strobl et al., 1993) and in adult female rhesus monkeys (Wilson et al., 1982). This action of NMA is probably mediated through the release of hypothalamic PRL releasing factors (PRF). Substance that stimulate PRL secretion and are candidate prolactin releasing factors (PRF's) include thyrotropin releasing hormone (TRH),

vasoactiveintestinal peptide (VIP), and a number of other endogenously occurring peptide (Neill et al., 1988).

In this investigation, NMDA receptors stimulation produces differential effects on PRL release depending upon the different stages of the induced cycle. The present study demonstrates no significant difference in the response of pituitary lactotrophs to exogenous NMA challenge on PRL before gondotropin treatment. Whereas significant rise in plasma PRL levels in response to NMA was observed on day 6 ($P<0.03$), 12 ($P<0.0006$) and menstrual day of the cycle after the gonadotropin treatment. In contrast, NMA induced a several fold increase in plasma PRL concentration on day 21($P<0.006$) of the induced cycle. It is interesting to note that when the animals are challenged with NMA they exhibit an average of 213 % increase in mean plasma PRL concentration during the luteal phase of the cycle (day 21). These findings are consistent with the results earlier observations in the rhesus monkey (Herbert., 1978) in which pituitary lactotropes have been reported to be less numerous and considerably lower concentration of prolactin in the juvenile than in the pituitary gland of the adult animals (Herbert et al., 1974). Systemic administration of NMA to diestrus rats' stimulated PRL secretion (Rula Abbud et al 1993) suggests that activation of PRF_S predominated over the activation of PRL inhibitory neurons in the arcuate nucleus.

In the present experiment, an i.v bolus injection of N-methyl-DL- aspartate, NMA, induced rise in plasma PRL concentration in immature female rhesus monkeys during different phases of the induced cycle. NMA can elicit a several fold increase of plasma prolactin levels in adult female rhesus monkeys (Wilson and Knobil., 1982) and normal cycling female (Pohl et al., 1989) and intact male rats (Arslan et al., 1992) .The failure of NMA to induce an acute release of prolactin in the immature female rhesus monkeys before treatment could possibly be due to a functional immaturity of the pituitary lactotropes in terms of prolactin synthesis and /or insufficient amounts of the releasable hormone. Alternatively, it may be suggested that responsiveness of lactotropes to the hypothalamic inhibiting factors, dopamine, is greater during immaturity. After gonadtropin treatment when the PRL levels increased significantly on day 15-18, the response of NMA was greater during the luteal phase of the induced cycle. Administration of NMA stimulates secretion of PRL in metestrous and proestrus rats

when the steroids levels were greater (Luderer et al., 1993) as observed presently. This action of NMA is probably mediated through the release of hypothalamic PRL releasing factor. Substance that stimulates PRL secretion includes thyrotropin releasing hormone, vasointestinal peptide, and number of other endogenously occurring compound (Neill et al., 1988). Interestingly, post gonadectomy difference in PRL release, the PRL response to NMA was significantly blunted by ovariectomy (Luderer et al., 1993) this suggests that the NMA induced release of PRF and PRL is enhanced in the presence of ovarian feedback. A stimulatory role of steroids on PRL secretion has been previously described (Ajika et al 1972). Brann and Mahesh (1992) have shown that administration of NMDA antagonists MK801, blocks the proestrous prolactin surge in the female rat. Likewise, Brann (1993b) has shown that treatment with non NMDA receptors antagonists dinitroquinoxaline (DNQX) significantly attenuated the preovulatory prolactin surge in the pregnant mare serum gonadotropin (PMSG) primed ovariectomized immature rat.

It is interesting to note that in a previous study acute administration of NMA was promptly followed by a marked increase in plasma PRL concentration in prepubertal male rhesus monkeys (*Macaca mulatta*) in which responsiveness of pituitary was enhanced by chronic intermittent iv infusion of GnRH (Gay and Plant, 1987). FSH in prepubertal monkeys may induce a functional differentiation of the lactotropes as GnRH does in these animals (Gay and Plant, 1987). In the present study, gonadotropin treatment produced a parallel increase in plasma prolactin and progesterone concentrations in the monkeys during the luteal phase of the induced cycle. In several laboratory rodents sharp rise in prolactin secretion has been recorded in peripubertal phase of development (Barkley et al., 1979). Gonadal secretions have been shown to affect peripheral prolactin concentrations and development of lactotropes. An increase in size and number of prolactin cells or lactotropes has been documented in the rat (Pasteels, 1972) following administration of steroids. It is possible, therefore, that the significant rise in the plasma prolactin concentrations in the FSH and hCG treated monkeys, as observed in the present study, could partly be due to an increase in estradiol and progesterone secretion.

The organising effects of steroids on the hypothalamo-pituitary axis have been emphasized by numerous workers (Fleischmann et al., 1990;Leigh et al., 1990). Both the

pituitaries and the ovaries can be prematurely activated to generate a mature pattern of hormone secretion (Wildt et al., 1980). More recent studies indicate that progesterone may play a positive role in the follicle growth, since progesterone receptors have been identified on the theca interna cells of antral follicles in monkeys at all stages of the cycle (Hild-Petito et al., 1988). Furthermore, in the present investigation, treatment of rhesus monkeys with hCG after 12 days of FSH treatment has been shown to evoke an immediate and sustained elevation in serum progesterone levels in association with the formation of corpus luteum, indicating that preantral follicles were fully capable of advancing to the preovulatory stage when provided with an adequate gonadotropin stimulus. These findings are also supported by data revealing that gonadotropin can stimulate follicle maturation in the presence of progesterone (Zelezinik et al., 1980). In the present findings the high levels of progesterone were typical for FSH induced ovarian stimulation in rhesus monkeys as investigated by Schenken et al., 1984. The progesterone levels were low before treatment. In contrast hCG administration resulted in rapid and large increase in P levels on day 15 to 18 of the cycle.

Ovarian steroids are responsible for inducing the preovulatory LH surge and the effect of excitatory amino acids on stimulating LH release requires an estrogen background. The estrogen is needed to enhance anterior pituitary sensitivity to GnRH as excitatory amino acid have been reported to stimulate GnRH both in castrated versus castrated plus steroid treated animals (Arias et al., 1993). Progesterone also enhances excitatory amino acid effects on LH release, possibly by increasing GnRH stores in the hypothalamus, increasing pituitary sensitivity, and enhancing the levels of excitatory amino acid receptors in the hypothalamus (Brann and Maheash, 1994). In the present finding, NMA failed to elicit an increase in progesterone secretion during different days of the cycle. On the other hand, administration of NMA on the day of menstruation caused a significant increase in P levels.

The failure of the hypothalamic pituitary ovarian cell axis to respond to NMDA stimulation is probably a reflection of the low gonadotropin content. The moderate circulating levels of progesterone, inhibiting the response of NMA on LH and hence no significant response were noticed. On the day of menstruation when the levels were low, NMA was able to stimulate the progesterone secretion and we get a significant response.

Onset of puberty results from the removal of, or escape from, inhibitory control. Alternatively, the establishment of excitatory input upto the GnRH neurons may play a more fundamental role in initiating puberty (Ruf et al., 1979; Ojeda et al., 1986). NMA is believed to affect LH secretion by acting through central nervous system located specific receptors (Johnson et al., 1985; Monaglan et al., 1986) and not by directly stimulating the pituitary (Tal et al., 1983).

Recent evidence from several laboratories suggests that steroid hormones are important in physiological regulation of excitatory amino acid release in the hypothalamus (Segrillo et al., 1997; Jiang et al., 1997). Progesterone significantly enhances the release rate of glutamate in the preoptic area (POA) of the hypothalamus just before the LH peak and an increased concentration of amino acid in the POA could act upon GnRH neurons and induce LH release (Ping et al., 1994). Similar to these results, Jarry et al (1992) have reported that POA release rate of aspartate and glutamate are also increased during the estrogen induced LH surge. Release rate of aspartate and glutamate increased in preoptic area at the time of puberty in the female rat (Carbon et al., 1992; Gorell et al., 1993). Since puberty involved significant changes in steroids levels so it is possible that the excitatory amino acid release in the hypothalamus during puberty are due to the changes in steroid milieu. In appropriately estrogen-primed animals, progesterone appears to significantly enhance the effect of NMDA on stimulating LH release in both the female rat and the adult rhesus monkey (Reyes et al., 1991; Carbon et al., 1992). NMDA administration induced plasma elevations of LH in 30-day-old female rats treated with estrogen plus progesterone (Carbon et al., 1992). Similarly, Reyes et al (1991) reported that the LH response to NMDA was highest during the luteal phase of rhesus monkey when serum progesterone levels were the highest.

NMDA treatment on postnatal days (26-29) has been shown by a number of investigators to advance puberty in the female rat (Urbanaski et al., 1990;Venaroni et al., 1990; Brann et al., 1993; Urbanaski et al., 1994). Similarly, prolonged intermittent treatment with NMDA has also been shown to induce precocious puberty in male monkeys (Plant et al .,1989). NMDA is capable of inducing a remarkable degree of synchronization of the day of vaginal opening with the majority of the animals displaying vaginal opening on a single day (Urbanaski et al., 1987; Brann et al., 1993). In agreement with the agonist

studies, NMDA receptor antagonist treatment delays puberty in female rats (Urbanaski et al., 1990;Mejis et al., 1991). The advancement of puberty by NMDA appears to be due to an activation of GnRH neurons as administration of a GnRH antagonist has been shown to block the puberty-advancing effect of NMDA (Plant et al., 1989). Since NMDA has been shown to be important in controlling the frequency and amplitude of GnRH and LH pulses in adult male and female rats (Medhamurthy et al., 1992; Donoso et al., 1990; Mahachoklertwattana et al., 1994; Ping et al., 1994; Mejis et al., 1991), it is possible that this factor underlies the ability of NMDA to advance and synchronize puberty. In present investigation, NMDA receptors stimulation produced differential effects on estradiol release depending upon the days of the induced cycle. In these monkeys no significant increase in plasma E_2 levels in response to NMA was observed before gonadotropin treatment. In contrast, NMA induced a significant increase in plasma E_2 concentration on day 6 of the cycle. Maximum increase in plasma E_2 levels was observed on day 12 of the cycle. An increase of 147% over the basal levels was induced by a single iv injection of NMA. The circulating plasma E_2 levels were low before gonadotropin treatment (Schenken et al., 1984;Koering et al., 1994). With the treatment of FSH a moderate E_2 levels in blood attain which prime the gonadotropes. A prompt increase was noticed on day 6. The highest levels were observed on day 15 and 18 and NMA challenged on day 12 produces a significant increase in E_2 levels.

Estrogen induced LH surge could be blocked by the administration of NMDA and non-NMDA antagonists in immature and ovariectomised rats (Urbanaski and Ojeda 1990;Lopez et al., 1992). Likewise the progesterone induced LH surge also appears to involve excitatory amino acid neurotransmission as Brann and Mahesh (1992) have shown MK801 prior to progesterone induced LH and FSH surge in the estrogen primed ovariectomized immature rat. Using the PMSG treated immature rat Brann et al (1993b) also provided evidence that non-NMDA receptors antagonist dinitroquinoxaline (DNQX) significantly attenuated the preovulatory LH surge (Lee et al., 1993).

Present findings are noteworthy in demonstrating the pharmacological doses of FSH could induce a normal ovarian cycle in immature rhesus monkey with profound gonadotropins deficiency. This study confirms the enhancing role of FSH in ovarian steroidogenesis. More importantly, the results highlight the predominant role of FSH in

inducing estradiol and its ability to induce ovulation when negligible amount of gonadotropins are present. Our results demonstrate that FSH is of greater importance in the induction of ovarian cycle. In conclusion, it is suggested that sexual precocity observed in these animals is due to an increase in estrogen and progesterone by the ovaries. These steroids in turn may affect the hypothalamo-pituitary axis, thus bringing about an alteration of normal process leading to puberty. Results of the present study and above reports indicate that NMA involvement in central nervous system regulation of PRL secretion may occur through activation of PRL stimulating system depending upon the physiological state or steroids milieu. It is possible therefore that the significant rise in the plasma prolactin concentrations in the gonadotropin treated monkey could partly be due to an increase in estradiol and progesterone secretion. This suggests that the NMA induced release of PRF and PRL is enhanced in the presence of ovarian feedback.

CHAPTER 2

EFFECT OF GONADOTROPIN TREATMENT ON FOLLICULAR DEVELOPMENT, OVULATION, AND CORPOUS LUTEUM FORMATION IN THE JUVENILE MACACA MONKEY OVARY

INTRODUCTION

The primate ovary is characterized by having one dominant follicle per menstrual cycle, resulting in a single ovulation each month (Koering et al., 1983). The antral follicles originate as primordial follicles, sequentially recruited, and the majority of them develop up to 1 mm in diameter before degenerating. This follicular growth pattern occurs not only in adult macaque cycling female (Koering et al., 1969) but also in the immature rhesus monkey and human (Van Wagenen., et al.,1973).

The precise time at which the follicular growth actually starts is difficult to establish. The follicles begin to form during the fourth month of fetal life in the human ovary (Baker et al., 1963). Some of the newly formed follicles start to grow immediately and most of them remain in a resting stage until they either degenerate or some signal(s) activate(s) them to enter the growth phase. Primordial and intermediary follicles constitute the stock of resting follicles (RF) which are seen in similar numbers in the right and left ovaries in both humans (Gougeon et al., 1987) and monkeys (Koering et al., 1983) termed as RF (Gougeon et al., 1994) or growing follicles (Faddy et al., 1995).

Folliculogenesis starts when follicles leave the pool of RF to enter the growth phase in the adult ovary. Then the early growing follicle undergoes a developmental process including a cellular proliferation and differentiation. While in primates, only one follicle reaches the preovulatory stage in the reproductive cycle, where as most of the follicles fail and become atretic (Gougeon et al.,1996).

Primordial, intermediary, and small primary follicles differ in their diameter, due to differences in the number and size of granulosa cells (GCs). But they do not differ in mean diameter of their oocyte and its nucleus. Morphometric parameters of RF and early growing follicles in humans show that the oocyte nucleus attain diameter of 19 μm, active follicular growth starts, when approximately 15 GCs are present in the cross-section (Gougeon et al., 1987). In women a strong correlation exists between the RF stock size and the number of growing follicles (Gougeon et al., 1994). The larger the stock, the greater the number of follicles beginning to grow. As follicles continuously leave the RF stock, the number of growing follicles decrease with aging. But in primates the proportion of primary and early growing follicles increases. In the initiation of growth

phase data concerning a possible stimulatory role for gonadotropins in immature primates appear confusing. In contrast to the hypophysectomized monkey fetus in which initiation of follicular growth does not appear blocked (Gulyas et al., 1977), there are no early growing follicles in the anencephalic human fetus (Baker et al., 1980). FSH-R and LH receptor (LH-R) only appear in the ovary from day 5 to 11 (Sokka et al., 1990), whereas immunostaining for FSH and LH to rat RF was shown only from day 8 of age (Mulheron et al., 1989). Thus, data from immature rodents suggest that initiation of follicular growth may be independent of a gonadotropic effect. A GnRH agonist (GnRH-a) blocked the transition between intermediary and primary follicles in monkeys (Gougeon et al., 1992), suggesting that gonadotropins may act on the transformation of flat gonadotropins may act on RF maturation by transforming their GCs, making it possible for these follicles to enter the growth phase in response to an unknown signal. Some of the genes coding for certain proteins, growth factors and their receptors (Zhou et al., 1991;Chegini et al 1992), or protooncogenes (Motro et al., 1993), which are present in GCs and/or the involved in the transition of RF to growing follicles. It has been suggested that pituitary hormones such as TSH may act synergistically with FSH to enhance the entry of RF in the growth phase (Howe et al., 1978).

Morphological studies have shown that definitive theca layers appear when follicles contain three layers of GCs (follicle diameter; 100-125 μm) in monkeys, (Koering,unpublished data), and three to six layers of GCs (follicle diameter; 103-163 μm) in women (Gougeon, unpublished data). The smallest early growing follicles lack an independent blood supply, but secondary follicles 80-100 μm in diameter are served by one or two arterioles, terminating in an anastomotic network just outside the basal lamina (Bassett et al., 1943). The physiological importance of this event is emphasized by the fact that the follicle becomes directly exposed to factors circulating in the blood. In humans and monkeys, follicles pass from the preantral (class 1) to the early antral stage (class 2) at a follicular diameter between 180 and 250 μm (Koering et al., 1983;Gougeon et al., 1979). Healthy follicles measuring 2-5 mm (class 5), referred to as selectable follicles and those follicles present during the late luteal phase constitute the population from which the follicle will be selected for ovulation. In macaque monkeys, follicles of about 1 mm constitute the population of selectable follicles (Koering et al., 1994). During

the early follicular phase, the newly selected follicles belong to this class, and their diameter is between 5.5 and 8.2 mm (Gougeon et al., 1983). The size of the follicle destined for ovulation increases greatly during the follicular phase by cellular multiplication and accumulation of fluid in the antrum until the ovulatory gonadotropin surge, and then only by the later process until ovulation. In humans, the diameter of the preovulatory follicle increases from 6.9 ± 0.5 mm (early follicular phase; range, 5.5 - 8.2 mm) to 13.3 ± 1.2 mm (midfollicular phase; range, 7.8-15.6 mm) and then to 18.8 ± 0.5 mm (late follicular phase; range 15.2-26.9 mm) (Gougeon et al., 1983;Pache et al., 1990). From the mid follicular phase, the primate preovulatory follicle becomes a highly vascularized structure with the area of the theca interna occupied by blood vessels being 2 times larger than in other follicles within the same or contra lateral ovary (DiZerega et al., 1980).

The basic function of the ovulatory follicle is to produce a fertilizable oocyte and to function as an endocrine gland during maturation and transformed into a functional corpus luteum. From a proliferative to a differentiated status preovulatory follicular cells, and especially GCs, become able to assume their full endocrine functions at the completion of a highly regulated process. This process is under the primary control of FSH and LH. Whereas the role of gonadotropins in initiating follicular growth remains unclear. Many studies have demonstrated their tropic action on follicle cells. Women suffering from Kallman's syndrome (Santen et al., 1973) can be induced to ovulate with exogenous gonadotropins while in polycystic ovarian syndrome (PCOs), circulating levels of FSH are very low, can be induced to ovulate by treatment with high doses of FSH (Van Weissenbruch et al., 1993; Sungurtekin et al., 1995). In normal women undergoing in vitro fertilization and embryo transfer (IVF&ET) respectively; treatment with exogenous gonadotropins overrides the normal ovulatory quota by sustaining preovulatory growth of a large number of follicles (Hodgen et al., 1986). The data suggest exclusive role for FSH in sustaining follicular development. FSH alone is capable of stimulating ovarian follicular growth in monkeys treated either with high doses of GnRH antagonist (Karnitis et al., 1994) or with a LH antiserum (Selvaraj et al., 1994), leading to LH-deficient animals.

According to the "two-cell, two gonadotropins" theory (Armstrong et al., 1979), theca interna cells are stimulated by LH to produce aromatizable androgens that are transported to GCs where they are converted to estrogens by aromatizing enzymes, which are induced by FSH. The human thecal steroidogenesis and its control by LH have been extensively documented (Karnitis et al., 1994; Erickson et al., 1993; Hiller et al., 1994), and the recent use of recombinant LH has confirmed previous observations (Nahum et al., 1995). Before the mid cycle gonadotropin surge, the steroidogenic pathways in GCs are organized principally for the metabolism of androgens to estrogens and for de novo synthesis of P and its metabolites after the mid cycle LH surge (Hillier et al., 1985).

The role of FSH in the induction of GC aromatase activity has been demonstrated in rats in vivo by using recombinant human FSH (Smyth et al., 1995). During the late preovulatory maturation, acquisition of LH receptivity transforms the ability of GCs to respond to gonadotropins since aromatase can be stimulated by hCG (Dennefors et al., 1983). After the midcycle gonadotropin surge, 3 beta hydroxy steroid dehydrogenase (3βHSD) is expressed, and GCs turn from a predominantly estrogen-producing to a progestin-producing tissue (Bomsel et al., 1979).

Follicles having diameter of 2- to 6-mm are observed in the ovaries, throughout human infancy, when the circulating levels of gonadotropins are lower than those recorded during puberty and adulthood and when circulating gonadotropins levels increase after 6 yr. of age (Faiman et al., 1971) some of them can become 6 mm (Peters et al., 1979). The growth of follicles from the preantral to the selectable stage only requires tonic levels of gonadotropins(Govan et al., 1975). This could explain why healthy selectable follicles 2-5 mm in diameter are always present from infancy to menopause in humans, even when plasma levels of gonadotropins are low. During basal growth, primate follicles exhibit slight steroidogenic activity. In vitro studies demonstrated that the small and large human preantral follicles produce very low amounts of progesterone and androgen whereas no estradiol production (Roy et al., 1993).

During basal growth, the development of human follicles requires only small levels of gonadotropins. These follicles are faintly responsive to cyclic gonadotropins changes, and their GCs exhibit low proliferative activity, weakly stimulated by gonadotropins. In

addition, except for 17HSD (Sawetawan et al., 1994), steroidogenic enzymes are weakly or not expressed. Follicles become more dependent on FSH when they attain a size of 2 mm and when FSH increases their percentage of atresia decreases (Gougeon et al., 1984). From the mid-to the late luteal phase, granulosa of selectable follicles exhibit a significant increase in the rate of cell proliferation (Gougeon et al., 1984), paralleling the increase in circulating levels of FSH. In addition, when stimulated with human menopausal gonadotropin (hMG) during both the late luteal and early follicular phase, selectable follicles can grow more quickly than non-stimulated selectable follicles (Gougeon et al., 1990).

The full responsiveness of preovulatory GCs to FSH is also confirmed by progressive acquisition during preovulatory maturation of other FSH-induced functions such as the expression of LH-R, which is induced under the primary control of FSH in most species (Richards et al., 1993). An increase in LH binding occurs in human GCs during preovulatory maturation (Kobayashi et al., 1990; Yamoto et al., 1992). Preovulatory GCs becomes able to respond to the mid cycle gonadotropin surge once they have synthesized LH-R. After completion of folliculogenesis, just before ovulation, GCs are differentiated in the preovulatory follicle and start producing high levels of steroids.

This objective of present study was to obtain further insight into the mechanism of follicular development and to study the morphological changes in the follicles. The immature rhesus monkeys (*Macaca mulatta*) was used as primate model because the ovary can respond to exogenous hormonal stimulation (Keinsberg et al., 1984) in a mileu free of meaning full gonadotropin secretion.

Another purpose of the present investigation was to examine the effect of exogenous FSH treatment on follicular development, using human choronic gonadotropin (hCG) to induce ovulation and corpus luteum function in immature rhesus monkeys.

MATERIALS AND METHODS

ANIMALS

Four immature female monkeys (12-18 months) were utilized in this study. The age of the animals was calculated using a dental formula described by Haigh and Scott (1965). Mean body weight of the animals at the beginning of the experiment was 2.3 ±0.11 kg. The animals were housed in individual cages, under standard colony conditions and were offered standard monkey food supplemented with fresh fruits and vegetables. Water was available ad libitum.

EXPERIMENTAL PROTOCOL

The monkeys received intramuscularly 20 IU FSH, daily for 12 days dissolved in physiological saline (0.9% NaCl). The protocol used for induction of ovulation was similar to that described in chapter 1. A bolus dose of 1200 IU hCG was administered intramuscularly on 13th day to induce ovulation. All animals were checked daily for menstruation and any uterine bleeding recorded for 2 or more consecutive days were regarded as menstruation. Laprotomies were performed under aseptic conditions (Fig.1). At the time of laprotomy each monkey was anesthetized with 15 mg/kg body weight ketamine hydrochloride. The ovaries were accessed through a mid-line abdominal incision. The approximate size and the number of follicles and corpora lutea were noted by transillumination of the ovary. The length, width and thickness of the ovaries were determined with the help of a pair of fine vernier callipers. To examine ovarian condition, laprotomies were performed prior, during and following treatment in monkeys. In certain cases one or both ovaries were removed for histological examination at different stages of the induced reproductive cycle. On removal, the ovary was fixed in bouins fluid. Serial paraffin sections were cut at 8 μm and stained with Harris haematoxylin and counter stained with eosin (Mc Manns and Mowry, 1964) and photographed. Micrometery was performed using ocular micrometer.

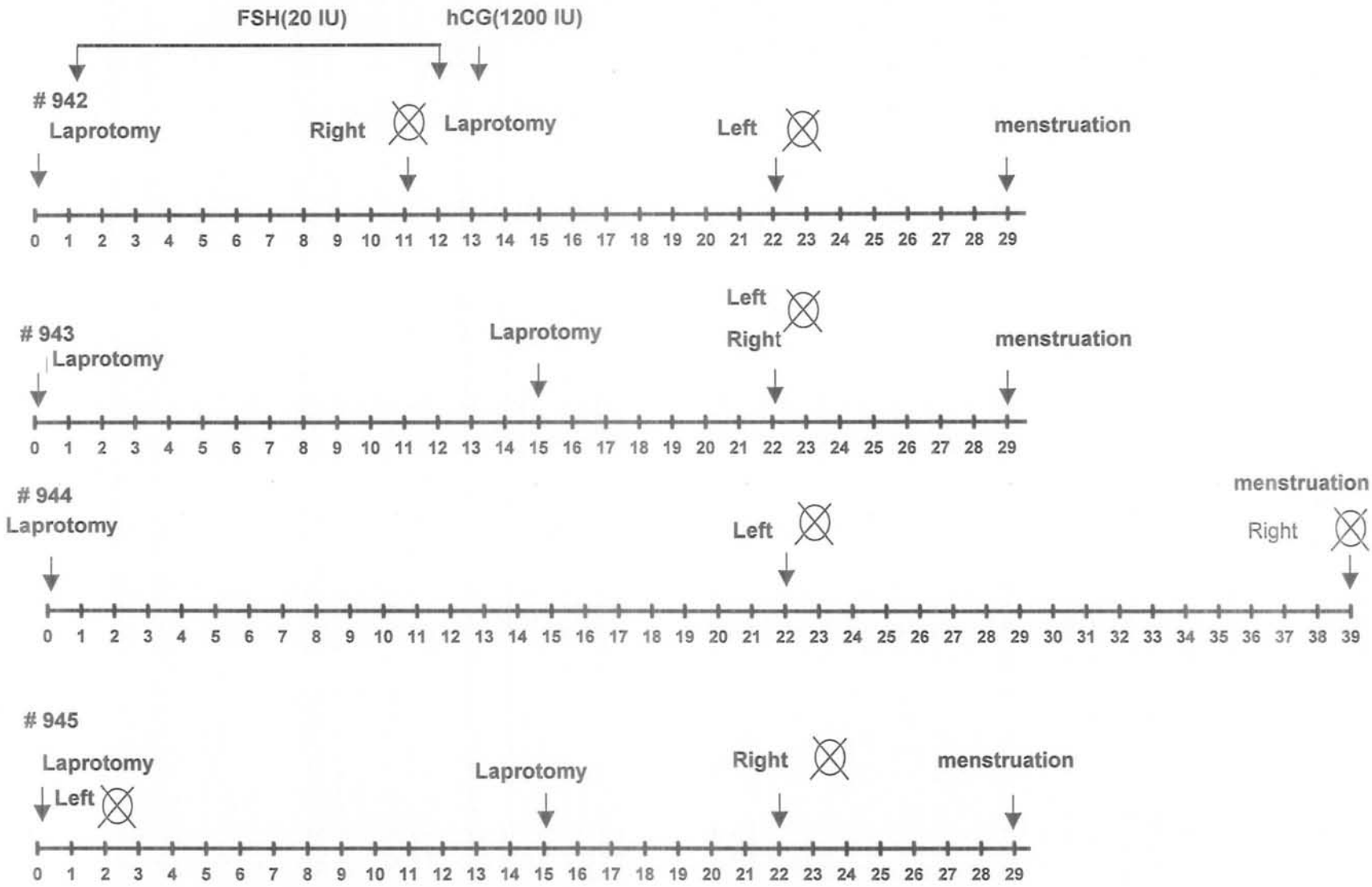


Fig.1. The protocol showing the time of ovariectomy (⊗) and laprotomy performed for each monkey

RESULTS

BODY WEIGHT

The body weight of immature female rhesus monkeys recorded over a period of treatment is presented in Table 1. The mean body weight at the beginning and at the end of the experiment was 2.3 ±0.11 kg and 2.2±0.05 kg, respectively. The changes in body weight of animals during the experiment were not significantly different (P>0.05).

GONADOTROPIN STIMULATION

In monkey Nos. 942, 943 and 945 menstruation occurred on day 29 whereas in monkey No 944, withdrawal bleeding was delayed until day 39. The uterine bleeding lasted 3-4 consecutive days.

FOLLICULAR DEVELOPMENT

The effects of FSH and hCG treatment on ovarian size, follicular development and lutenization are shown in Table 2, 3 and 4. Laprotomies performed in these monkeys before the initiation of treatment demonstrated that the ovaries were fairly consistent in size and contained follicles mostly1-2 mm in diameter. Mean length, width and thickness of right ovary were 0.85±0.08, 0.48+0.02 cm and 0.4±0.04 cm respectively, whereas the left ovary were 0.81+0.1 cm in length, 0.47±0.04 cm in width and 0.38±0.01 cm in thickness (Table 2.) Gross examination by transillumination of ovaries revealed 11-28 follicles (Table 3). Histological examination of the ovaries showed abundant primordial (32.75±0.69 μm in diameter) and primary follicles (43±0.67 μm in diameter) as shown in Fig 2 and Fig 3 in the outer cortical region and secondary and preantral follicles were seen towards the inner peripheral region of the cortex (Fig 3). Preantral follicle with 3-4 layers of granulosa cells with theca interna and externa were present in the inner region. Fluid filled antral follicles, 2 mm in diameter were also present in the inner cortical region (Fig 4). Preantral and antral follicles, 180 μm and 210 μm in diameter were also observed before the treatment.

TABLE 1

Mean body weight (kg)of immature female rhesus monkeys(n=4) before and after the FSH and hCG treatment

Animal No	Body weight(kg)	
	Before treatment	After treatment
942	2.1	2.3
943	2.3	2.2
944	2.6	2.3
945	2.2	2.1
Mean±SEM	2.3 ± 0.11	2.2 ± 0.05

P>0.05

TABLE.2. Effect of FSH and hCG treatment on ovarian size.

Animal No	Before treatment						After treatment					
	Length(cm)		Width(cm)		Thickness(cm)		Length(cm)		Width(cm)		Thickness(cm)	
	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt
942	0.85	0.72	0.45	0.45	0.31	0.42	1.2	1.2	1	0.8	1	0.8
943	1.1	1.1	0.52	0.61	0.52	0.41	1.4	1.9	0.8	1.2	0.9	1
944	0.75	0.82	0.45	0.42	0.34	0.35	1.6	2	1.3	1.2	1	1.8
945	0.72	0.62	0.53	0.43	0.43	0.35	1.8 **	– *	1 **	– **	1.2 ***	– *
Mean±SEM	0.85 ± 0.08	0.81 ± 0.1	0.48 ± 0.02	0.47 ± 0.04	0.4 ± 0.04	0.38 ± 0.01	1.5 ± 0.12	1.7 ± 0.21	1.02 ± 0.1	1.06 ± 0.11	1.02 ± 0.06	1.2 ± 0.26

* = p<0.05
** = p<0.01
*** = p<0.001

TABLE 3

Effect of gonadotropins on follicular development as observed by transillumination during laprotomy.

Animal no	Before treatment No of follicles		After treatment No of follicles	
	Catagory A 1-2 mm	Catagory B 3-8 mm	Catagory A 1-2 mm	Catagory B 3-8 mm
942	28	0	11	12
943	23	0	8	2
944	11	0	–	–
945	18	0	2	7

TABLE 4

Corpous luteum formation after treatment with gonadotropins as observed by transillumination during laprotomy

Animal no	No of Corpora lutea	
	Catagory A < 6 mm	Catagory B > 6 mm
942	6	1
943	3	6
944	5	3
945	3	1

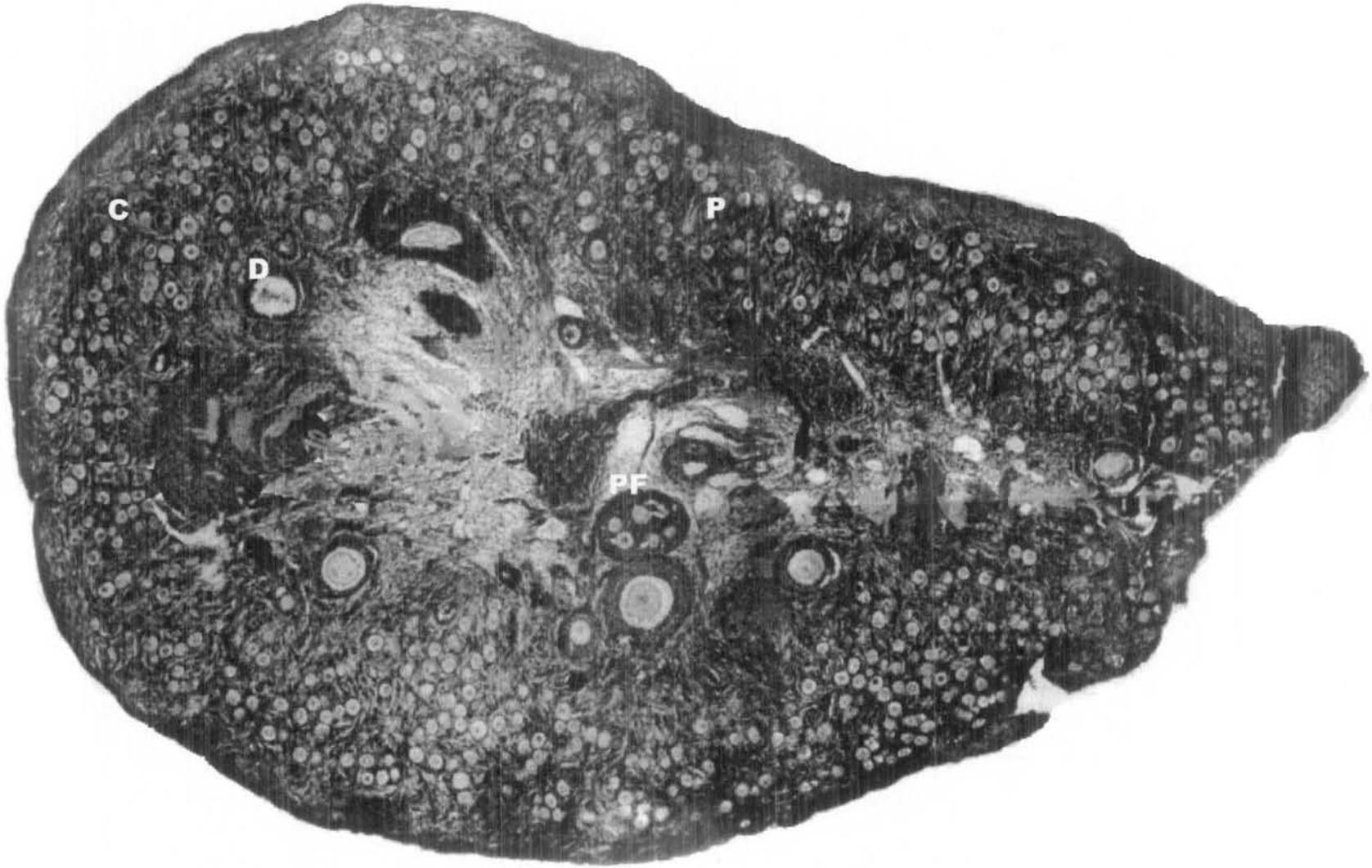


Fig.2 Photomicrograph of the representative section from the left ovary of a monkey (# 945) showing the distribution, size and status of primodial follicles (P) in the cortical (C) region, developing follicle (D) and poly ovular follicle (PF) in the medullary region before the gonadotropin treatment x 350

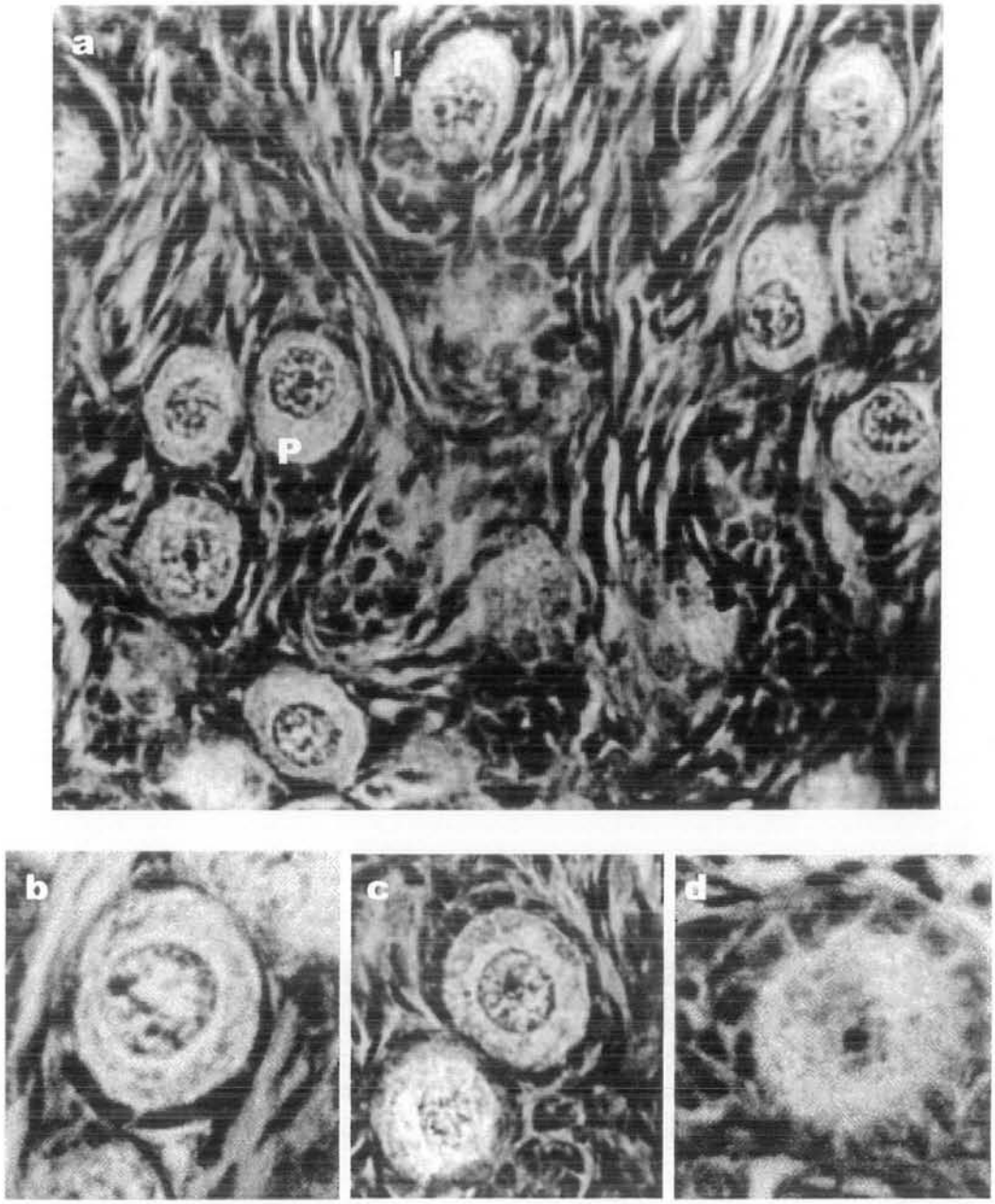


Fig.3.Portion of ovarian cortex of monkey ovary before gonadotropin treatment a) showing primodial follicle (P) and intermediary follicle (I) x 580 b) Primodial follicle in which the oocyte is surrounded by flattened granulosa cells. c) Intermediary follicle in which the oocyte is surrounded by a mixture of flattened and cuboidal granulosa cells d) Primary follicle in which the oocyte is surrounded by a single layer of cuboidal granulosa cells x1200

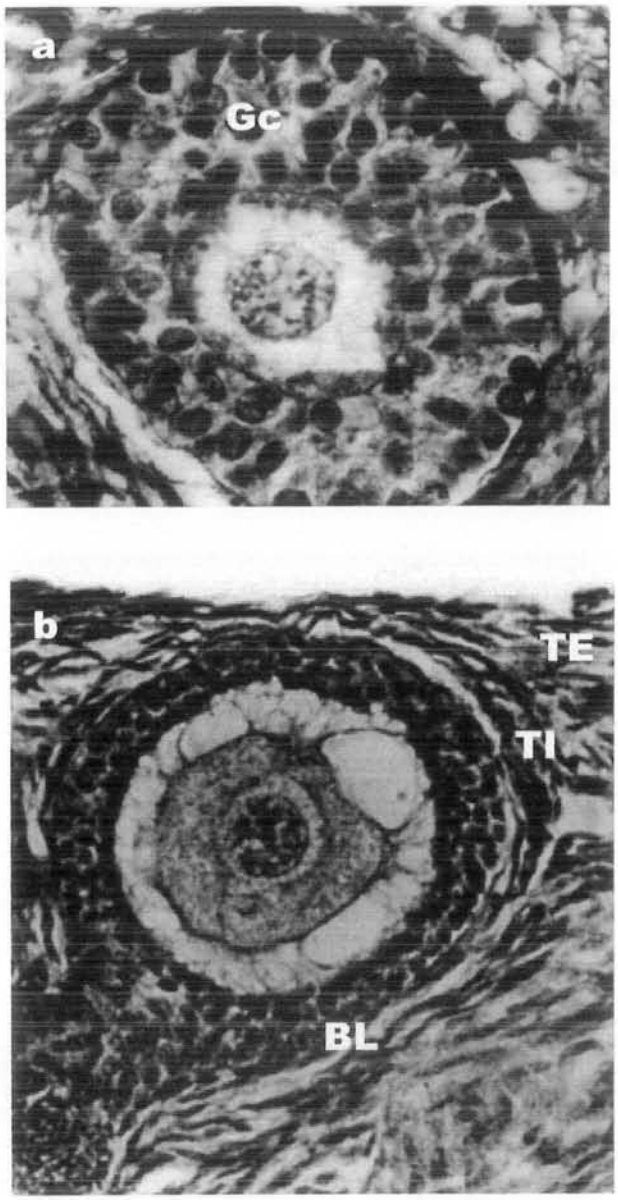


Fig.4. Photomicrograph from the middle portion of the monkey ovary before treatment a) Preantral follicle with 3-4 layers of granulosa cells (Gc) x1800 b) Preantral follicle with 2-3 layers of granulosa cells. Theca interna (TI), Theca externa (TE), basal lamina (BL) x750 c) Early antral follicle :Note antrum(a) x1800

Ovaries of treated monkeys showed a marked response to gonadotropin treatment by way of enlargement in size, follicular development (Table 2 and 3). The ovarian size before and after the gonadotropin treatment was significantly different (Table 2). In these monkeys, the ovaries were enlarged primarily due to the presence of large follicles of various sizes. Ovaries of animals examined on day 11 possessed large developing follicles ranging from 3-8mm in diameter and ranged from 2-12 in number (Table 3) as assessed by transillumination. Histological examination of these ovaries showed developing and atretic antral follicles in addition to other types of small follicles. In large fluid filled follicles, the granulosa cells layer was greatly compressed and was only a few cells thick (Fig 5-6). An antral follicle with oocyte were also seen on the 11 day of FSH treatment as shown in Fig 5.

Laparotomy performed on day 15 of FSH treatment and 48 h following a single injection of hCG, showed ovulation points on the surface of the large follicles filled with a dark red fluid. In general, the size and number of follicles were similar to the ovaries examined on day 11 of FSH treatment. However, few follicles had further increased in size (9mm).

Abundant luteal tissue was observed in ovaries of animals on day 22 of treatment. Gross examination of ovaries on day 22 revealed presence of corpora lutea (category A and category B). In category A mostly the corpora lutea were <6 mm in diameter and ranged between 3-6 in number (Table 4). In category B the corpora lutea were >6 mm in diameter and ranged between 1-6 in number. In addition some of the ovaries contained large rounded dark structures, which apparently represented ruptured follicle with persistent heamatomas. The dark bodies present in the ovaries of some animals on day 22 appeared to be derived from large follicles almost completely filled with coagulated hemorrhagic fluid forming a heamatoma like structure with some loose dispersed connective tissue cells (Fig 8c). This mass was surrounded by a thin peripheral layer of partially lutenized cells derived from the granulosa elements. The presence of this structure made it difficult to observe smaller follicles with the help of transillumination. Histological examination of the luteal tissue in these animals consisted of two different types of cells, namely, large and small luteal cells (Fig 8b). Large luteal cells were in close association with blood vessels, appeared spherical in shape and possessed an

average diameter of 22.75±0.96 μm which contained secretary granules and vacuoles. Nuclei of the large luteal cells were spherical and were central in position with intact nuclear membrane. Small luteal cells were spindle shaped and possessed an average length 28.25±1.33μm. They contained lightly stained cytoplasm and darkly stained nuclei which were eccentric in position. The copora lutea were delimited fibrous capsule. The central region of most of the corpora lutea contained varying amounts of hemorrhagic fluid and connective tissue cells. In these immature monkeys before and after treatment, polyovular follicles were also observed in which oocytes were enclosed in the follicular envelop (Fig.7).

The ovary of monkey number 944 at day 39 had considerably decreased in size and contained regressed corpora lutea. Transillumination revealed few follicles larger than 1-2mm. Microscopic examinations of ovarian sections showed degenerating corpora lutea with varying stages of luteal cell regression and their replacement by fibrous tissue (Fig 9).

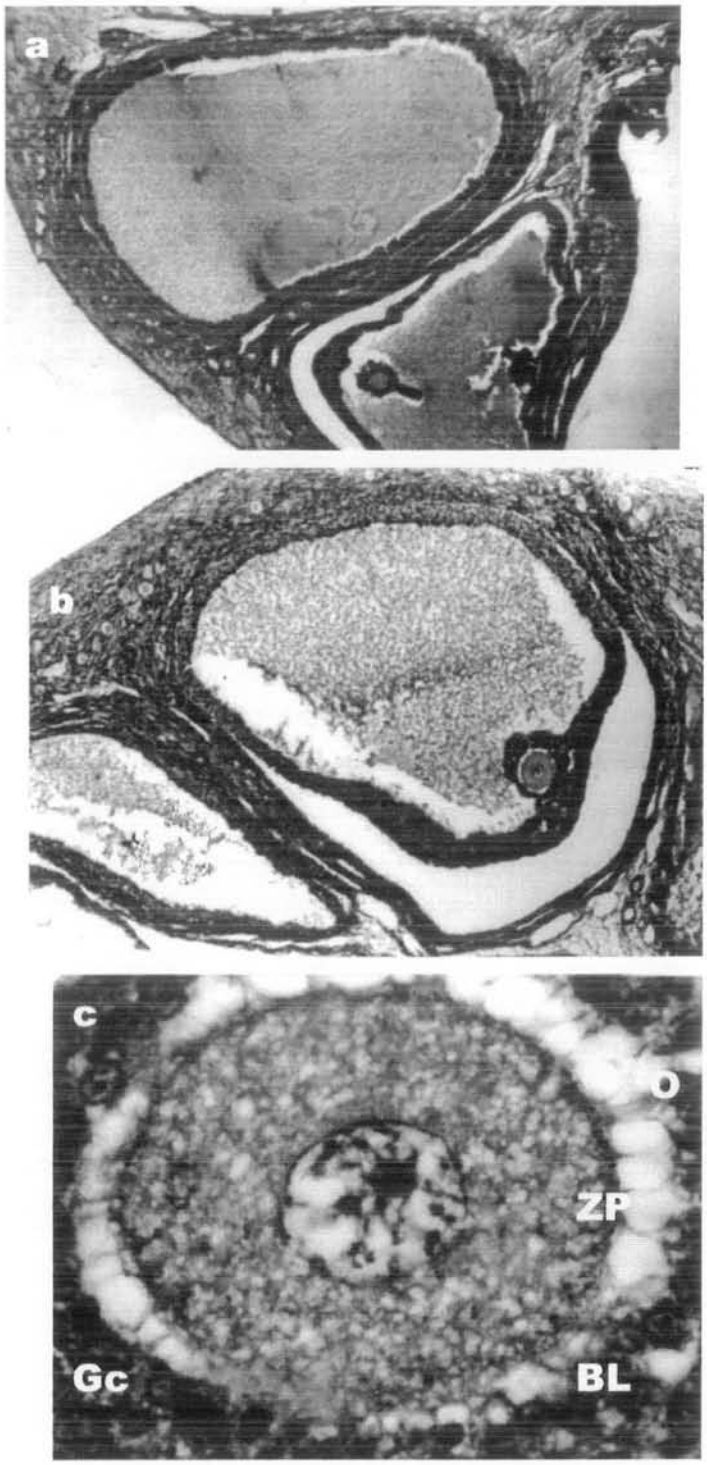


Fig. 5. Photomicrograph of the peripheral portion of the monkey ovary after 11 day of FSH treatment a) showing large antral follicles b) an antral follicle with oocyte x 60 c) Oocyte at higher magnification with zona pellucida (ZP), ooplasmic extention (O), basal lamina (BL) and granulosa cells (Gc) x1300

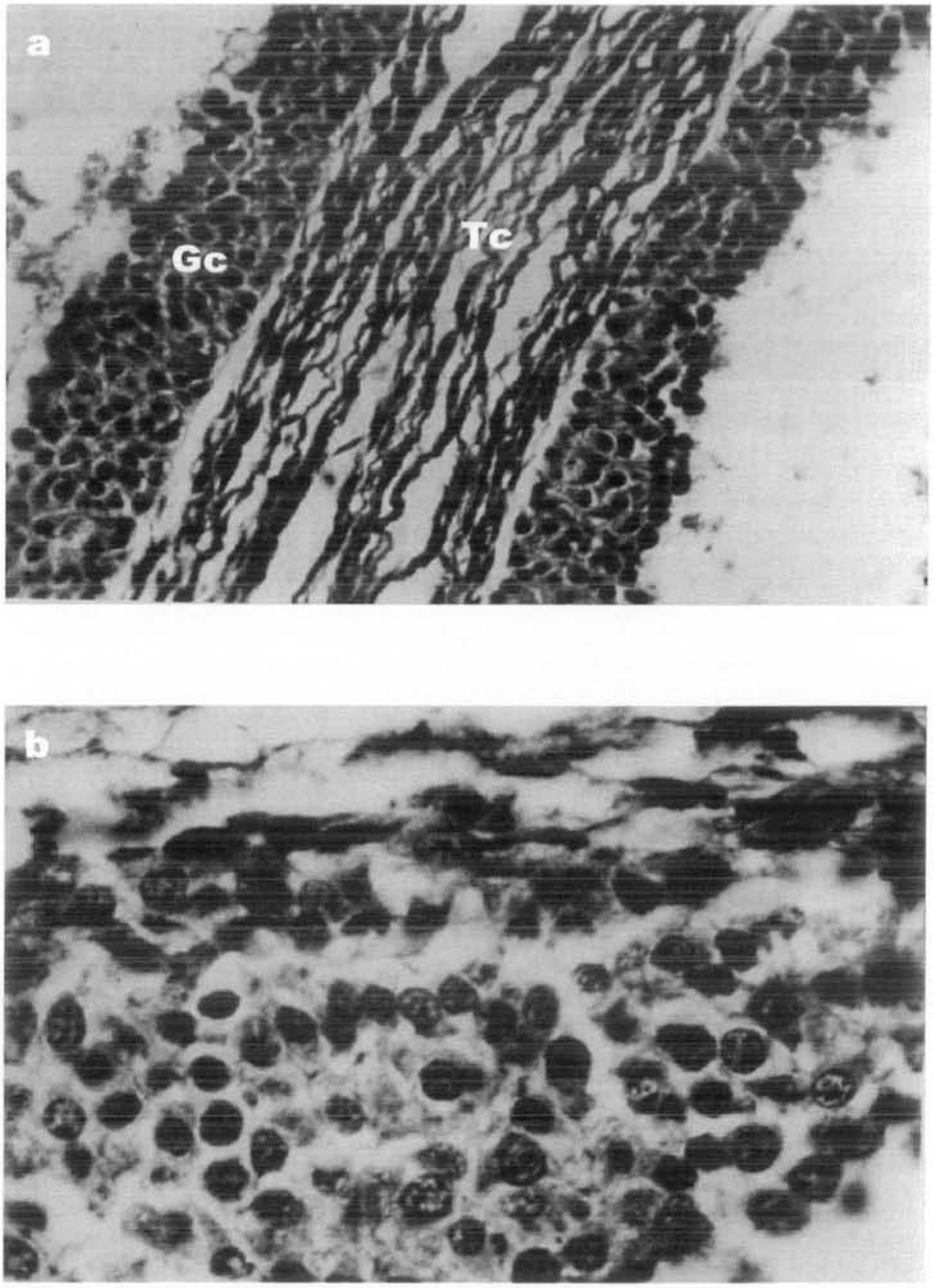


Fig.6.Photomicrograph of a portion of a) antral follicular walls of two adjacent follicles, showing granulosa (Gc) and thecal cells (Tc) on day 11 of FSH treatment x 170 b) same at higher magnification with granulosa cells and thecal cells x 680

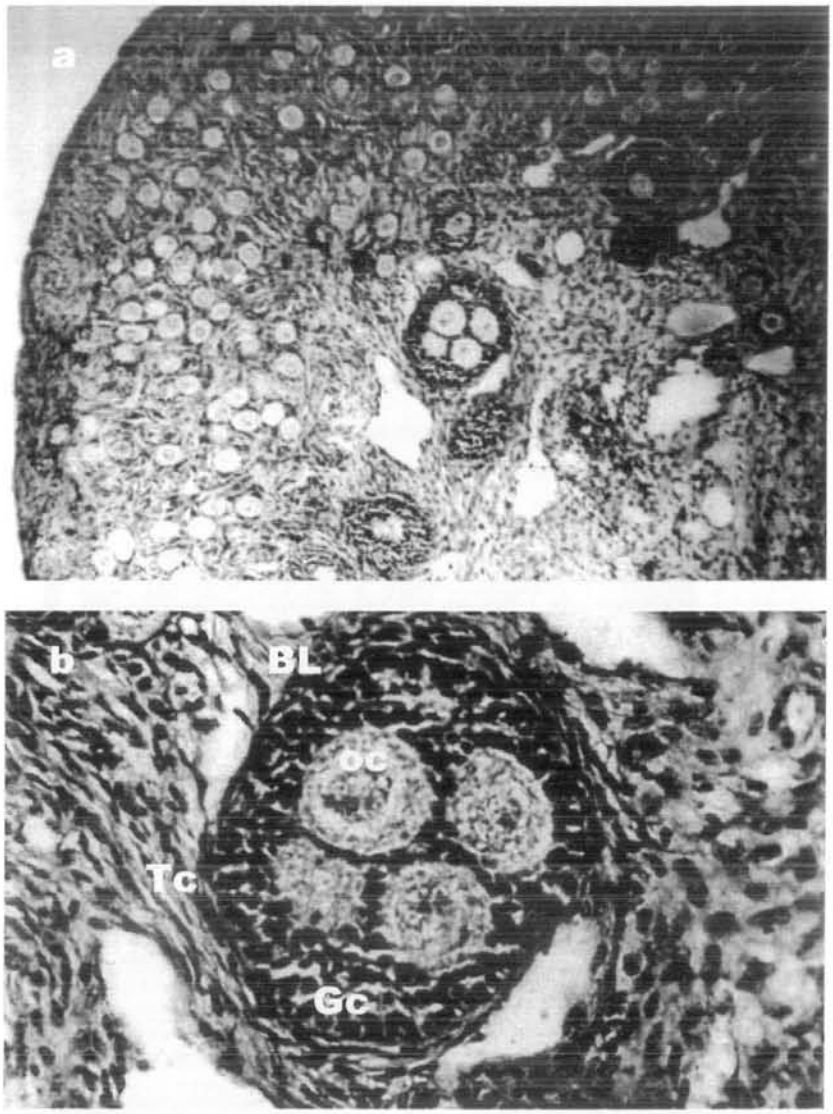


Fig.7. Photomicrograph of the cortex of monkey ovary after 22 day of gonadotropins treatment showing a) multiovular follicle towards medullary region possess four oocytes (oc) surrounded by granulosa (Gc) and thecal cells (Tc) x 160 b) same at higher magnification x 650

DISCUSSION

The process of selection and growth of the dominant follicle (Goodman et al., 1983) is the mechanism of recruitment that supplies the pool of follicles from which the selection is made. In both the adult cycling monkey and human, this includes the stimulation of certain primordial follicles and their development into antral follicles. After attaining about 1mm in diameter in the monkey (Koering et al., 1969) and 4-5 mm in diameter in human (Baired et al., 1987; Pache et al., 1990). These follicles degenerate if not adequately supported by FSH (Koering et al., 1969) The similar growth pattern is seen in ovaries from immature rhesus monkeys (Van Wagenen et al., 1973), where the hypothalamic-pituitary axis is still juvenile (Wildt et al., 1980). In the present study selection of the juvenile monkey for the model was based on the responsiveness of the immature macaque ovary to exogenous hormonal stimulation (Van Wagenen et al., 1973; Kenigsberg et al., 1984) and the lack of GnRH supply (Wildt et al., 1980).

After 13 day of FSH and hCG exposure, the enlargement in ovarian size, follicular development and process of luetinization were noticed. Ovaries of monkeys showed a marked response to gonadotropin treatment by way of enlargement in size ($P<0.05$). The size of the ovary in human and monkey increase in rectilinear fashion from infancy to adulthood. Moreover, growth and atresia of ovarian follicles occurs throughout infantile and juvenile development (Van Wagenen et al., 1973; Ross et al., 1974; Peters et al., 1976). The enlargement of ovary prior to puberty is the result of age related increase in the number and size of antral follicles and in the quantity of medullary stroma. The preovulatory follicles were observed after 11 days of the treatment. The finding that, in juvenile monkey, ovarian vein concentration of estradiol are three to fourfold greater than peripheral levels (William et al., 1982) suggest that the follicles within the prepubertal ovary are steroidogenically active. The circulating concentration of estradiol decline after ovariectomy in prepubertal monkey (Winter et al., 1977). Histological examination of ovaries before treatment showed abundant primordial (32.75±0.69μm) and primary follicle (43±0.67μm) in the outer cortical region and developing follicles were seen in the middle and inner peripheral region of the cortex. The preantral and antral follicle were 180 μm and 210μm, respectively before gonadotropin treatment.Morphological studies

have shown that definitive theca layers appear only when follicles contain three layers of Gcs(follicle diameter: 100-125 μm) in monkey (Koering unpublished data) and three to six layers of Gcs (follicle diameter 103-163 μm) in women (Gougeon, unpublished data) Follicle from Macaca mulatta pass from pre antral to antral stage between 200-250 μm (Koering et al., 1983).

After 11 day of FSH treatment examination of ovaries revealed developing (3-8 mm in diameter) and atretic antral follicles. Clark in 1979 demonstrated that during the early follicular phase, the largest healthy follicles are 2 mm and these are usually two or three follicles about the same size. However, by day 7 to 9, one follicle was considerably larger than the other and this was further accentuated by day 11-13 (preovulatory stage) when the largest follicle was 6mm. Based on in vivo observation, the largest follicle grows from 1.7-9.4 mm during the 11 day before the LH surge and the dominant follicle destined to ovulate (Clark et al., 1978). In the present finding the large follicle observed was 9 mm in diameter after 15 day of treatment. A significant increase in the mean percentage of follicles 100-200 μm is evident in the peri ovulatory period, evidently influenced by the increase in the steroids and gonadotropin (Koering et al., 1983).

Formation of corpous luteum (CL) is initiated by a series of morphological and biochemical changes in the cells of the theca interna and membrana granulosa of the preovulatory follicles. In the present study ovaries at this age reveled presence of corpora lutea and abundant luteal tissues were observed in on day 22 of treatment. Few were >6 mm and some were <6 mm in diameter. Ovaries contained large rounded structure, which represent ruptured follicles with persistent heamatoma. The mass was surrounded by a thin peripheral layer of partially luteinized cells derived from the granulose elements. It is presumed in such cases that the granulosa cells failed to invade the anterior of the follicle following its rupture, resulting in the persistence of the heamatomous structure occupying the antral cavity. The major component of the corpus luteum in rhesus is the granulosa lutein cell. The ovulated follicle have a large fluid filled antrum contain fibrin and a few leukocytes, the granulasa cells are elongated and appear to be streaming into the antrum (Catchpole and Van Wagenen, 1975). The theca lutein cells are elongated and are eripheral and associated with blood vessels, which have not yet invaded the forming

corpous. Hemorrhage in the cavity is frequent in rhesus. By cycle day 21 the CL, estimated to be 11-12 days old, attain its maximal size, and may occupy a whole diameter of the ovary. At this time, also, the cells are maximally differentiated as lutein cells. In the present study, two different types of luteal cells were differentiated small and large luteal cells with a diameter of 22.75±0.96 μm and 28.25+1.33 μm respectively. The large luteal cells are the largest, endocrine cells in the body and range from approximately 20 μm in diameter in rodent to 40 μm in human (Enders et al., 1973). Small luteal cells have diameter of 22 μm or less and appear spindle shaped (Mossman et al., 1973; Sinha et al., 1971). They contained lightly stained and darkly stained nuclei, which were eccentric in position. The corpora lutea were delimited by fibrous capsules. The cells of the CL to be derived from at least to different types of follicular steroids secreting cells (theca and granulosa cells), it is not surprising that the CL consist of at least two distinct type of steroidogenic luteal cells in several species (Warbritton et al., 1934; Mossman et al.,1973). The large luteal cells were in close association with blood vessels, appeared spherical, the nuclei were spherical and were central in position with intact nuclear membrane (Donaldson et al., 1965; Mossman et al., 1973). They contained lightly stained cytoplasm and darkly stained nuclei, which were eccentric in position.

In the immature female rhesus monkey before and after day 22 of the gonadotropin treatment the polyovular follicles were observed. Polyovular follicles, however, are common. Brambell (1956) lists their occurrence in man and monkey. They are also seen in lower primates (Harrison et al., 1949). They are often listed as abnormalities, might indeed be a normal occurrence at some time during folliculogenesis. They occur most frequently in the embryonic and immature age, at the time when follicle organization is beginning. The development of polyovular follicle is not clear, but most likely they can arise in different ways, they might be formed during the process of follicle organization. Oocyte lying within rete-tubules can easily become elongated in the rapidly proliferating rete-granulosa cells thus forming polyovular follicle. Bacsich (1946) believes their presence in the ovaries of newborn children to be due to a withdrawal of gonadotropic influence, while Harrison (1949) suggests that their presence in the goat is related to excessive estrogenic stimulation.

The ovary of monkey #944 examined on day 39 after menstruation contained regressed corpora lutea. The corpora lutea showed varying stages of luteal cells regression. The corpora lutea tend to disappear fairly slowly. The granulosa lutein cells have a shrunken cytoplasm and stain deeply. Pale staining theca lutein cells accompanying blood vessels become prominent. A number of morphological changes appear to be common among species during luteolysis. The increase in number of lipid droplet has been observed in rabbit (Koering et al., 1978) and pig (Paavola et al., 1979) and in human (Van Lennys et al., 1965).

In conclusion, present finding are noteworthy in demonstrating the enhancing role of FSH on ovarian morphology. The data suggest that FSH priming is the primary stimulus for early follicle growth in primate. Moreover, it appears that exogenous FSH administration stimulate aspects of early antral follicle development. Furthermore, treatment of rhesus monkey with human chronic gonadotropin has been shown to evoke the formation of macroscopically visible antral follicles indicating that the preantral follicles observed in the present study were fully capable of advancing preovulatory stage when provided with adequate gonadotropin stimulus.

CHAPTER 3

NMA INDUCED PROLACTIN RELEASE IN ESTROGEN, PROGESTERONE AND ESTROGEN PLUS PROGESTERONE TREATED OVARIECTOMIZED IMMATURE FEMALE RHESUS MONKEYS

Prolactin is used by different species to control a wide variety of functions, many of which are species specific. From comparative studies, osmoregulation, growth, and development may be the most fundamental actions of the hormone. In adult humans, PRL's role is incompletely delineated but seems to be involved primarily with reproductive functions.

The role that PRL plays in ovarian function is complex, differing among species and within each species during various stages of reproductive function. The frequent development of amenorrhea in women with hyper-prolactinemia demonstrates the critical role of PRL in human ovarian function. In contrast, hypoprolactinemia does not appear to interfere with cyclicity. There is a preovulatory PRL surge in rodents and ruminants but not in primates. This appears to be due to the rising titer of estrogen at this time and appears to require the posterior pituitary (Muari et al., 1990). PRL receptors are present in the luteinizied rat ovary. PRL has an important role in maintaining the corpus luteum in the rat, but not in humans, during early pregnancy. This includes the stimulation of luteal cell growth and progesterone secretion by the corpus luteum. PRL does this by maintaining appropriate numbers of LH receptors on the developing corpus luteum, thereby enhancing its steroidogenic response to LH (Richards et al., 1976). PRL down-regulates annexin production in the corpus luteum, and annexins (or lipocortins) inhibit phospholipase A2, an important enzyme in prostaglandin synthesis, and prostaglandin in turn decreases progesterone biosynthesis in the ovary (Albarracin et al., 1991).

Serum PRL levels are generally higher in women than men. This reflects the effects of estrogen.The day time peak of PRL secretion is more pronounced during the luteal phase (Tennekoon et al., 1985).In women, an optimal window of PRL levels appears to be necessary for normal corpus luteum function. For example, lactation impairs follicular development in all mammals. Paradoxically, high PRL levels suppress progesterone synthesis by the ovary and decrease induction of aromatase, resulting in decreased estrogen production (Dorington et al., 1981). Such a bell shaped response curve is characteristic of many of PRL's actions and is reminiscent of the antagonistic properties of high levels of GH in preventing receptor dimerization and action. These effects could

result in direct inhibition of ovarian function, but hyperprolactinemia also acts centrally on the hypothalamus to decrease the frequency and amplitude of LH pulses, possibly at the level of the GnRH pulse generator (Cohen-Backer et al., 1986).

PRL release from the anterior pituitary gland is inhibited by dopamine secreted by the hypothalamus in the hypophysial portal system (MacLeod, 1976). However, the role of dopamine secretion in alterations of PRL release induced by stimuli such as mating and suckling remains unclear. The simplest explanation for increase in PRL release would be that stimuli such as suckling reduce tonic inhibition exerted by the hypothalamus, freeing the pituitary gland to express its inherent capacity to secrete PRL spontaneously at a very high rate. Treatment of lactating rats with sufficient amounts of a-methyl-p-tyrosine, an inhibitor of dopamine synthesis, to completely suppress dopamine secretion leads to increase in PRL release quantitatively similar to those observed after suckling. Thus, disinhibition is a potential explanation for the neurogenic stimulation of PRL release if PRL releasing stimuli could be shown to dramatically alter dopamine release (Neill, 1988). However, evidence has been derived which demonstrates that dopamine in portal blood during a simulated suckling in lactating rats, is reduced only transiently (Plotsky and Neill, 1982) and cannot fully account for the massive rise in PRL. This raises the possibility that a stimulator or PRL-releasing factor (PRF) rather than sole inhibition of dopamine (PRL-inhibiting factor) is the primary drive for the acute rise in PRL. The identity of the PRF is yet unknown (Laudon et al., 1990).

A number of putative PRF's of hypothalamic origin have been described although in many cases, a physiological requirement of a PRF is hard to establish. The potential candidates as PRF's include amongst others, thyrotropin releasing hormone (TRH), vasoactive intestinal peptide (VIP) and oxytocin. TRH and VIP have been shown to exert a stimulatory effect on PRL release by direct action on the pituitary cells (Blake, 1974; Gourdji et al, 1979). The list of naturally occurring compounds that will release PRL has now grown to include both peptidic and non-peptidic secretion like GnRH, serotonin and others (Neill, 1988). A recent study demonstrates that dopamine D1 receptor analogues act centrally to stimulate PRL secretion in sheep (Curlewis et al., 1993). These studies provide convincing evidence that D1 receptor agonists act via secretion of PRF's or PIF's. Furthermore, the steroidal milieu has been shown to influence the basal serum

PRL levels as well as the PRL response to various releasing stimuli. Thus gonadal steroids, androgens and estrogens, have been demonstrated to affect PRL secretion by the pituitary gland, in rodents (Sinha et al., 1979), primates (Neill et al., 1988) and man (Barbarino et al., 1982).

Previous studies have demonstrated that N-methyl-D, L-aspartic acid (NMA), a potent analogue of the excitatory neurotransmitter aspartate (Watkins and Evans, 1981), acutely stimulates the release of anterior pituitary hormones in rodents (Price et al., 1978), sheep (Estienne et al., 1989), cattle (Shahab et al., 1993) and primates (Wilson and Knobil, 1982; Gay and Plant, 1987). In the context of gonadotropin release, compelling evidence is already available which shows that the effect of NMA is mediated via the central nervous system mechanisms and specifically utilises the NMDA receptor (Schainker and Cicero, 1980; Plant et al., 1989) which may be blocked in a potent and specific fashion by D, L-2-amino-5-phosphonopentanoic acid (AP5), a chemically defined antagonist of NMDA receptor (Arslan et al., 1988).

Evidence has been presented indicating that qualitative changes in response of pituitary hormones to NMA stimulation may occur as a result of altered physiological states. NMA has been shown to inhibit the release of LH in ovariectomized monkeys (Reyes et al., 1990) sheep (Estienne et al., 1990), cattle (Shahab et al., 1993) and in lactating rats (Pohl et al., 1989). Similarly, NMA failed to elicit a robust discharge of PRL in chronically orchidectomized adult monkeys (Arslan et al., 1991).

In the preoptic area ^{3}H- glutamate binding to the non-NMDA binding site is increased by two days of estradiol treatment, and still further increased if the estradiol is followed by progesterone. NMDA receptors binding site density was increased selectively in CA1 region of hippocampus (Weiland et al., 1992). Interestingly, ^{3}H-muscimol binding also increase selectively in cerebral aqueduct (CA1) area of hippocampus after estrogens treatment (McCarthy et al., 1992). This paradigm also increases CA1neuronal dendritic spine density (Gould et al., 1990; Wooley et al., 1993), an effect blocked by co-administration of the NMDA receptors antagonist, MK801 (Wooley et al., 1994). Smith and colleagues (1989) found estrogen and progesterone to have antagonistic influences on the excitatory amino acid. Progesterone administration suppressed glutamate

excitation of neuroexcitatory response to glutamate where as estrogen treatment significantly increased the purkinje cell excitatory response to glutamate (Smith et al., 1989). The effect of progesterone is not dependent on prior treatment with estrogen (Smith et al., 1989). If E_2 is replaced following ovariectomy, synapses increase in number in specific E_2 regulated brain areas. This hormone is a significant factor in synaptic remodelling (Nishizuka and Arai.,1981; McEwen., 1997). E_2 in physiological amounts is essential for the maintenance of specific neurons populations (Miller et al., 1998). Brann and Mahesh (1994) reported that estradiol plus progesterone treatment increases α-amino- 3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors glutamate receptor (GluR1) subunit immunoreactive levels in the preoptic area and arcute nucleus of immature rats. In the estrogen-primed animals, progesterone significantly enhances the effect of NMDA on stimulating LH release in both the rat and monkey (Carbon et al., 1992; Reyes et al., 1991). Injection of estradiol to ovariectomized ewes produced the biphasic LH response; an initial suppression followed by a surge like LH increase together with an elevated basal secretion of PRL (Jiang et al., 1997). Antagonism of NMDA receptors in untreated ovariectomized ewes stimulated release of PRL suggesting that the endogenous NMDA ligands were stimulatory to the release of a PRL inhibiting neurohormone like dopamine (Jiang et al., 1997). The excitatory effect of NMDA on GnRH neurons was decreased in old rats. Therefore, decrease in the excitatory inputs to GnRH neurons could be directly involved in the reduction of hypothalamic pituitary ovarian axis activity observed during aging (Arias et al., 1993).

The present study was designed to investigate the effect of different steroids background on basal circulating levels of plasma PRL in the ovariectomized immature female rhesus monkey. An attempt has also been made to assess PRL responsiveness to acute stimulation by NMA in steroid deprived and steroid replaced animals of this age group and to determine whether these steroids can affect PRL responsiveness to NMDA receptors stimulation.

ANIMALS

Four immature (2.0-2.6 kg BW) female rhesus monkeys (*Macaca mulatta*) 18-24 months of age were used in these experiments (Table 1). All these monkeys were ovariectomized 6 months prior to this study. The animals were housed in individual cages and were provided standard monkey food supplemented with fresh fruits and vegetables. Water was available ad libitum.

CATHETERIZATION:

Animals were catheterized and were bled by using the same method as described previously in Chapter 1.

COLLECTION OF BLOOD SAMPLES

Sequential blood samples (~2 ml) were obtained 60 min before and 60 min after the administration of NMA (15 mg/kg BW) at 15 min intervals in heparinized syringes. Following each sampling an equal volume of heparinized(5 IU) normal saline was injected in the tubing. During the course of bleeding additional ketamine was administered as required. All the bleedings were carried out between 1200 and 1400 h to minimise diurnal variations. Blood samples were immediately centrifuged at 3000 rpm for 10 min. Plasma was separated and stored at –15 °C until analysed.

SEX SKIN

Changes in the sex skin colour were evaluated and graded + to + + + + + by using a series of colour standards in these treated animals.

EXPERIMENTAL PROTOCOL

EXP.1

EFFECT OF SINGLE IV INJECTION OF NMA ON PRL RELEASE IN OVARIECTOMIZED IMMATURE FEMALE RHESUS MONKEYS:

Four ovariectomized female rhesus monkeys were injected intravenously with NMA (15 mg/kg BW) via the cannula. Blood

samples were collected 60 min before and 60 min after the administration of NMA at 15 min intervals.

EXP.2

NMA INDUCED PRL RELEASE IN ESTROGEN TREATED OVARIECTOMIZED IMMATURE FEMALE RHESUS MONKEYS:

Four ovariectomized female rhesus monkeys were injected (im) with 500 μg Estradiol Valerate (Progynon Depot) on day 1 and on 8th day (500μg/animal/week). PRL responsiveness to NMA was studied at 0 and 15 day following estradiol treatment. Sequential blood samples were obtained 60 min before and 60 min after the administration of NMA (15 mg/kg BW) at 15 min intervals.

EXP.3

NMA INDUCED PRL RELEASE IN PROGESTERONE TREATED OVARIECTOMIZED IMMATURE FEMALE RHESUS MONKEYS:

Four ovariectomized female rhesus monkeys were injected (im) with 50 μg progesterone (Proluton Depot) on day 1 and 8th day (50μg/animal/week). PRL responsiveness to NMA was studied at 15 day following progesterone treatment. Sequential blood samples were obtained 60 min before and 60 min after the administration of NMA (15 mg/kg BW) at 15 min intervals.

EXP.4

EFFECT OF SINGLE IV INJECTION OF NMA ON PRL RELEASE IN OVARIECTOMIZED IMMATURE FEMALE RHESUS MONKEYS TREATED WITH ESTROGEN+PROGESTERONE:

Ovariectomized female rhesus monkeys (n=4) were injected (im) with 500 μg Estradiol Valerate (Progynon Depot) and 50 μg progesterone (Proluton Depot) on day 1 and on 8th day. PRL responsiveness to

NMA was studied after 2 wks following estrogen and progesterone treatment. Sequential blood samples were obtained 60 min before and 60 min after the administration of NMA (15 mg/kg BW) at 15 min intervals.

DRUGS AND HORMONES

The following drugs were used in this study:

1. Ketamine hydrochloride (ketavat; park Davis, Berlin, FRG).
2. N-methyl -D, L -aspartic acid (NMA, Sigma Chemical Co. St Louis, MO, USA).
3. Proluton Depot. (Schering AG. Federal Republic of Germany).
4. Progynon Depot. (Schering AG. Federal Republic of Germany).

HORMONE DETERMINATIONS

Plasma levels of Prolactin (PRL) were determined in duplicate using specific EIA assay system as described previously in chapter 1.

STATISTICAL ANALYSIS

Mean baseline plasma PRL concentration among the treatment groups was calculated by averaging all the concentrations obtained before NMA treatment for comparison. On the other hand, PRL responsiveness to NMA stimulation was determined by comparing basal hormone levels calculated by averaging the concentrations immediately before the injection at o min and PRL obtained by averaging the concentrations 15-min after the NMA injection. Student's test was used to determine differences between the means of basal and stimulated levels. Data were analysed using analysis of variance and Duncan's multiple range test.

RESULTS

BODY WEIGHT

The body weight of immature female rhesus monkeys (*Macaca mulatta*) recorded over a period of treatment is presented in Table 1. The mean body weights at the beginning and at the end of the experiment were, 2.17 ±0.14 kg and 2.15±0.02 kg, respectively. The changes in body weight of animals during this course of experimental period did not vary significantly (P>0.05).

Changes in sex skin colour

The pattern of sex skin changes following initiation of estradiol valarate treatment was almost similar in all the animals (Fig.1.). An increase in the intensity of sex skin colour was observed on day 4 following initiation of treatment. The colour of the sex skin in all treated monkeys ranged between (+) and (+++++). The approximate duration of the sex skin response ranges between days 4–15. Increase in the intensity of the sex skin colour was invariably accompanied by swelling of the vulva and the perineal skin. Subsequently, there was a gradual waning of the sex skin colour and by day 20 the sex skin colour changed to its normal

In the Progesterone treated group as shown in Fig.2.an increase in the intensity of sex skin colour was observed on day 3 following initiation of treatment The sex skin response ranges between days 3–15 and then there was a gradual waning of the sex skin colour and by day 20 the sex skin colour changed to its normal condition. In these animals increase in the sex skin colour was accompanied by swelling of the vulva and the perineal skin.

In the Estradiol and Progesterone treated animals (Fig.3) similar findings were noticed as described in progesterone treated group.

EXP.1

EFFECT OF SINGLE IV INJECTION OF NMA ON PRL RELEASE IN OVARIECTOMIZED IMMATURE FEMALE RHESUS MONKEYS:

The individual and mean plasma PRL profiles before and after a single intravenous injection of NMA in agonadal prepubertal female monkeys are presented in Table 2 and Fig. 4.There was no significant increase in plasma

TABLE 1

Individual and mean body weight (kg)of immature female rhesus monkeys(n=5) before and after the treatment.

Animal No	Body weight(kg)	
	Before treatment	After treatment
942	2.0	2.1
943	2.0	2.1
944	2.6	2.3
945	2.1	2.1
Mean±SEM	2.17 ± 0.14	2.15 ± 0.02

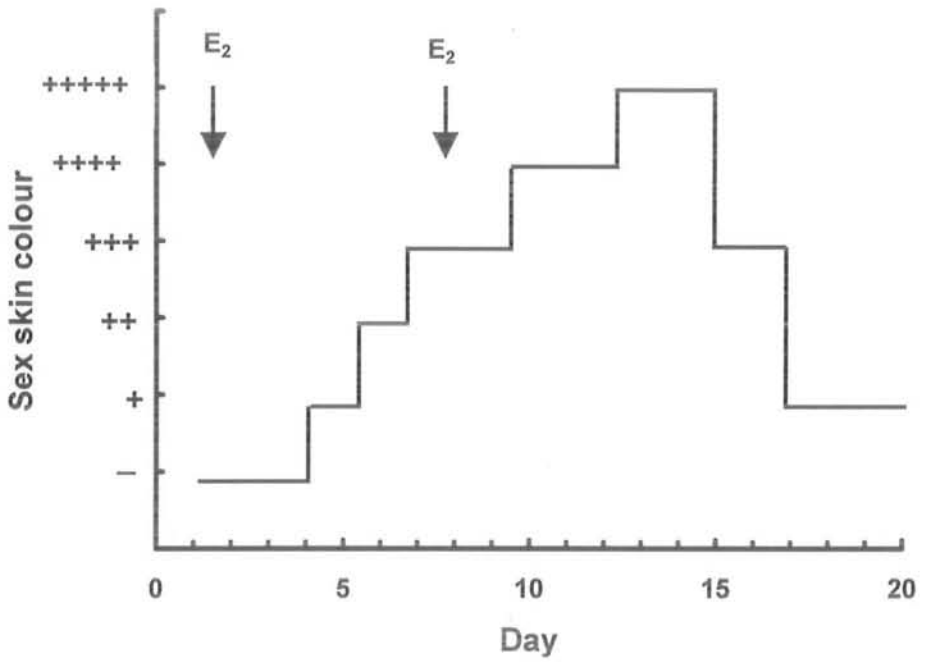


Fig.1. Change of sex skin colour in ovariectomized immature rhesus monkeys during estrogen treatment.

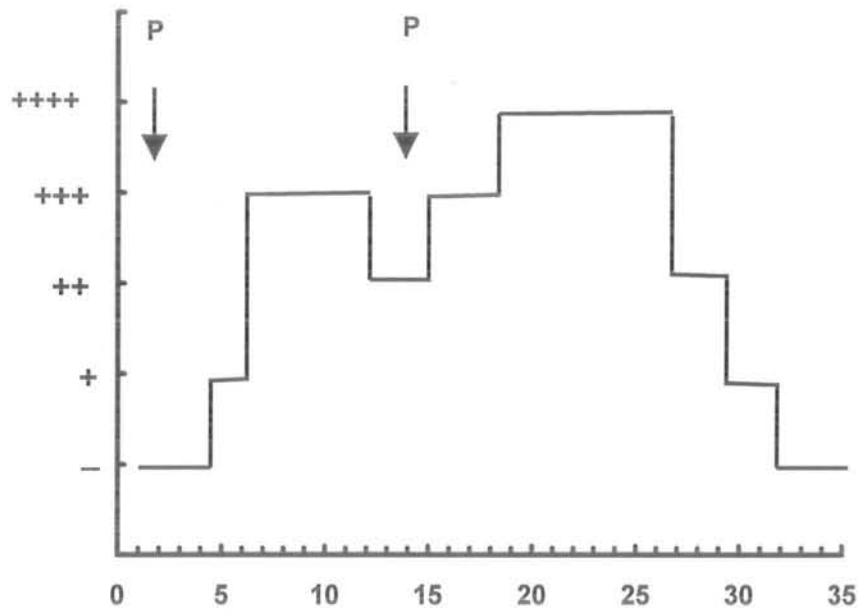


Fig.2. Change of sex skin colour in ovariectomized immature rhesus monkeys during Progesterone treatment.

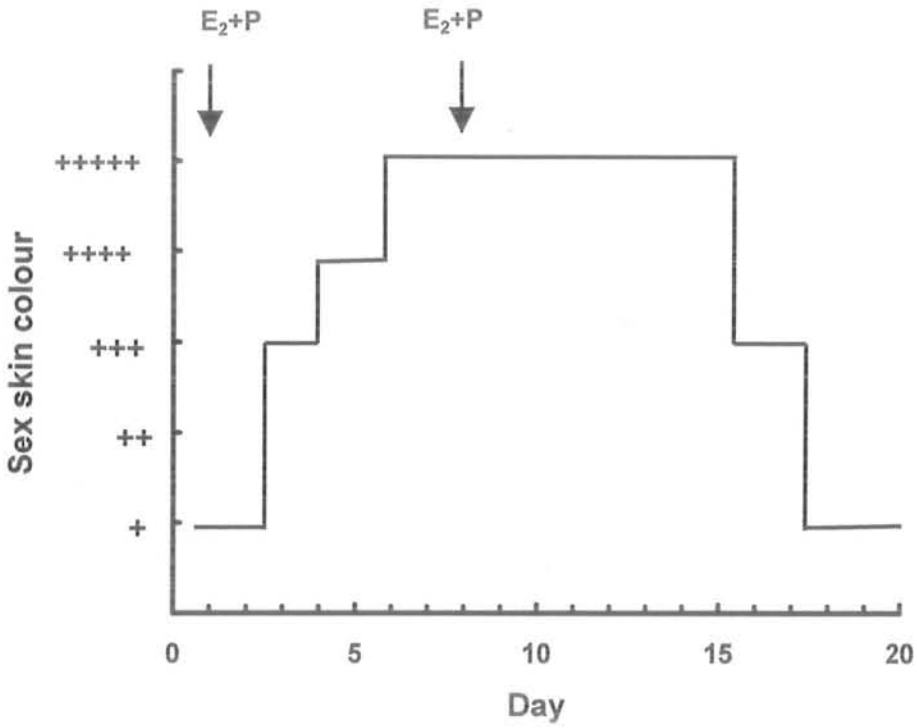


Fig.3. Change of sex skin colour in ovariectomized immature rhesus monkeys during Estrogen + Progesterone treatment.

PRL levels at 15 min following NMA administration. The mean plasma PRL concentrations before and after single iv injection were 151.01±14.29 mIU/L and 187.18±34.33 mIU/L, respectively. The increase in PRL concentration was not significant (P>0.33).

EXP.2

NMA INDUCED PRL RELEASE IN ESTROGEN TREATED OVARIECTOMIZED IMMATURE FEMALE RHESUS MONKEYS:

a Effect of Estrogen on basal levels:

Plasma PRL concentration in ovariectomized prepubertal monkeys on 0 day were 155.54±6.28 mIU/L (Table 2) and all mean basal values for 15 days treatment two weeks following the initiation of estrogen treatment were 403.43±50.40 mIU/L (Table 3) and Fig.5. The observed increase in PRL concentration in latter group was significant (P<0.05) compared to the former group.

b NMA induced PRL release in estrogen primed animals:

The individual and mean values of plasma PRL concentration, in the four monkeys challenged with intravenous injection of NMA at 0 and 2-wks of estrogen treatment, are shown in Table 2 and 3 and Fig.4. At 0 wk of treatment, plasma PRL levels following NMA administration did not show a significant increase (P>0.33). The mean plasma PRL concentrations immediately before and just after NMA injection were 151.01±14.29 mIU/L and 187.18±34.33 mIU/L, respectively. The release of PRL in response to NMA was significant (P<0.03) at the end of the 2 wks of estrogen treatment and the initial PRL concentrations increased from 300.96±88.66 mIU/L to 1678.94 ±66.22 mIU/L within 15 min of NMA injection(Table.3). The elevated levels then declined gradually from 30 min to 60 min but remained higher than the baseline levels.

EXP.3

NMA INDUCED PRL RELEASE IN PROGESTERONE TREATED OVARIECTOMIZED IMMATURE FEMALE RHESUS MONKEYS:

a Effect of Progesterone on basal levels:

Basal levels of PRL in ovariectomized female prepubertal rhesus monkeys are shown in Table 2 and Fig. 5. before the initiation of Progesterone treatment . Two weeks following the initiation of P administration, the all mean basal values of plasma PRL concentration increased from 155.54±6.28 mIU/L to 475.85±15.74 mIU/L (Table 2 and 4). The observed increase was significant ($P<0.05$).

b NMA induced PRL release in Progesterone primed animals:

The individual and mean values of plasma PRL concentration, in the four monkeys challenged with intravenous injection of NMA at 0 and 2 wks of Progesterone treatment, are shown in Table 2 and 4 and Fig.4. The mean plasma PRL concentrations immediately before and just after a NMA injection were 408.00±20.82 mIU/L and 1116.73±236.28 mIU/L, respectively at the end of the 2 wks of treatment and the release of PRL in response to NMA was significant ($P<0.005$). The elevated levels then declined gradually and remained higher than the baseline levels.

EXP.4

NMA INDUCED PRL RELEASE IN ESTROGEN PLUS PROGESTERONE TREATED OVARIECTOMIZED IMMATURE FEMALE RHESUS MONKEYS

a Effect of Estrogen and Progesterone on basal levels:

Basal levels of PRL in ovariectomized female prepubertal rhesus monkeys are shown in Table 2 and Fig. 5. before the initiation of Estrogen and Progesterone treatment . Two weeks following the initiation of E_2 plus P administration, all mean basal values of plasma PRL concentration increased

from 155.54±6.28 mIU/L to 3774.72± mIU/L (Table 2 and 5). The observed increase was highly significant (P<0.05).

b NMA induced PRL release in Estrogen plus Progesterone primed animals:

The individual and mean values of plasma PRL concentration, in the four monkeys challenged with intravenous injection of NMA after 2 wks of Estrogen and Progesterone treatment, are shown in Table5 and Fig.4. The mean plasma PRL concentrations immediately before and just after a NMA injection were 3171.02±472.70 mIU/L and 4395.28±242.70 mIU/L, respectively at the end of the 2 wks of treatment and the release of PRL in response to NMA was significant (P<0.02). The elevated levels then declined gradually and remained the same.

Mean basal plasma PRL levels showed significant increase (Fig 5, F=70.08, P<0.001) in estrogen plus progesterone treated group over the mean basal PRL levels of estrogen or progesterone treated animals. Furthermore, mean plasma PRL concentrations in treated groups were significantly different from mean PRL levels observed in non-treated animals (P<0.05).

TABLE 2

Plasma PRL(mIU/L) concentrations in immature ovariectomized female rhesus monkeys (n=4) before and after a single iv injection of NMA(15 mg/kg) prior to initiation of estrogen treatment.

Time(min)	PRL(mIU/L) Animal No					
	942	943	944	945	Mean	SEM
-60	116.8	113.9	176.9	163.3	142.71 ±	16.08
-45	162.8	157.9	164.8	189.4	168.70 ±	7.05
-30	147.2	173.8	196.5	151.6	167.25 ±	11.34
-15	116.8	188.8	108.5	178.0	148.01 ±	20.62
0	176.0	111.4	167.0	149.7	151.01 ±	14.29
15	258.0	230.2	150.4	110.2	187.18 ±	34.33
30	222.4	199.6	208.3	201.0	207.81 ±	5.22
45	200.3	221.3	166.9	186.5	193.74 ±	11.45
60	165.2	108.8	176.4	152.4	150.66 ±	14.80

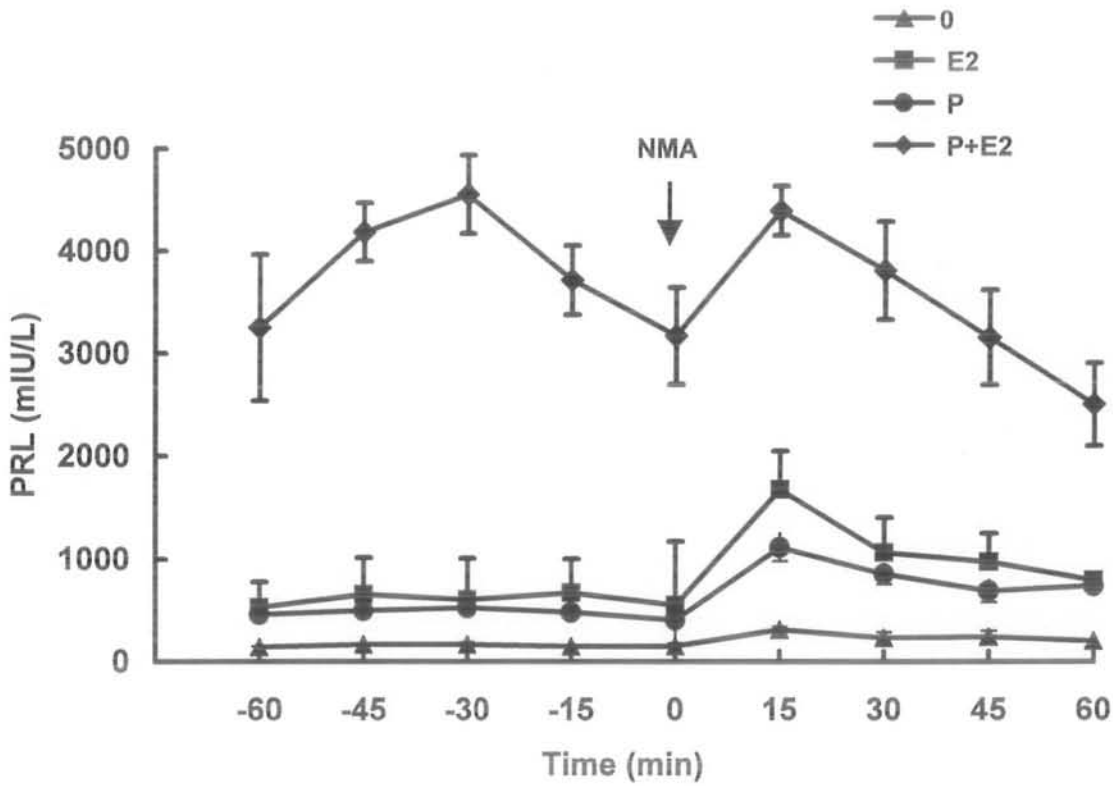


Fig.4 Mean (SEM) plasma PRL(mIU/L) concentrations in immature ovariectomized female rhesus monkeys (n=4) before and after single iv injection of NMA at 0 day, 15 days of estrogen, progesterone and estroge+progesterone treatment.

TABLE 3

Plasma PRL(mIU/L) concentrations in immature ovariectomized female rhesus monkeys (n=4) before and after a single iv injection of NMA(15 mg/kg) after 15 days of estrogen treatment.

Time(min)	PRL(mIU/L) Animal No					
	942	943	944	945	Mean	SEM
-60	997.2	412.3	236.6	475.0	530.25 ±	163.61
-45	403.4	377.6	404.5	435.4	405.21 ±	11.83
-30	689.2	189.6	270.0	281.4	357.55 ±	112.43
-15	881.3	259.4	273.2	278.8	423.19 ±	152.77
0	534.3	133.5	337.5	198.6	300.96 ±	88.66
15	2537.3	2938.7	419.2	820.6	*1678.94 ±	622.33
30	1708.4	1704.5	295.9	556.8	1066.39 ±	373.37
45	1646.6	1464.6	249.8	537.0	974.50 ±	342.62
60	1319.5	1222.8	236.6	397.2	794.02 ±	278.13

*Values significantly different from those at 0 min

P<0.03

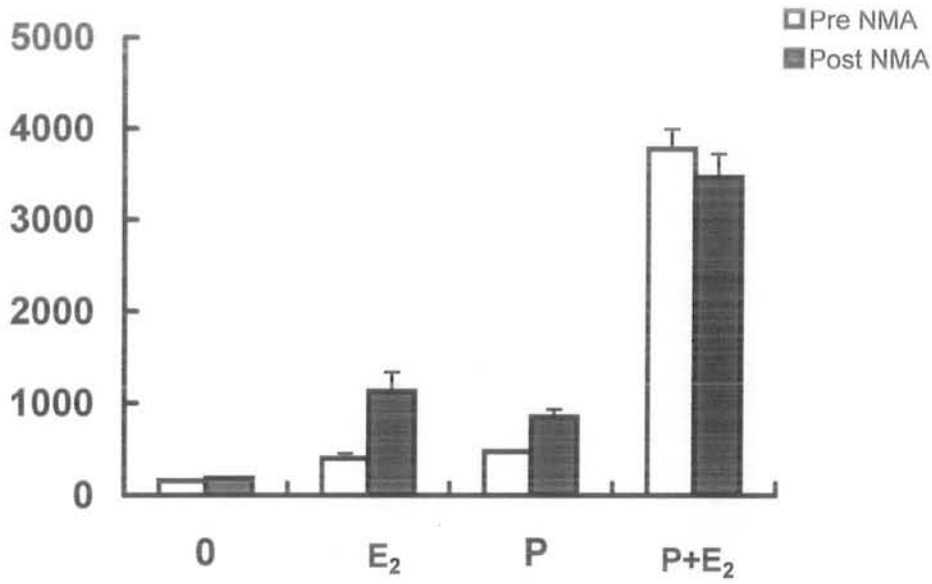


Fig.5.Mean basal and stimulated plasma PRL (mIU/L) concentrations in immature ovariectomized female rhesus monkeys (n=4) at 0 day, 15 days of estrogen, progesterone and estroge+progesterone treatment.

TABLE 4

Plasma PRL(mIU/L) concentrations in immature ovariectomized female rhesus monkeys (n=4) before and after a single iv injectic of NMA(15 mg/kg) after 15 days of progesterone treatment.

Time(min)	PRL(mIU/L) Animal No					
	942	943	944	945	Mean	SEM
-60	440.4	561.2	397.5	450.2	462.27 ±	34.88
-45	497.8	511.0	457.0	534.3	499.97 ±	16.14
-30	667.1	495.1	545.2	398.1	526.34 ±	55.99
-15	468.0	518.1	466.6	478.3	482.68 ±	12.07
0	351.5	420.4	451.1	409.4	408.00 ±	20.82
15	799.5	1787.7	770.8	1109.1	*1116.73 ±	236.38
30	745.1	1224.6	591.2	876.2	859.25 ±	134.98
45	735.2	860.3	397.5	768.2	690.27 ±	101.11
60	959.9	780.8	438.1	790.1	742.19 ±	109.40

*Values significantly different from those at 0 min

P<0.005

TABLE 5

Plasma PRL(mIU/L) concentrations in immature ovariectomized female rhesus monkeys (n=4) before and after a single iv injection of NMA(15 mg/kg) after15 days of estrogen and progesterone treatment.

Time(min)	PRL(mIU/L) Animal No					
	942	943	944	945	Mean	SEM
-60	4086.07	4660.6	2799.89	1455.06	3250.41 ±	713.75
-45	3900.62	4138.72	4996.73	3698.72	4183.70 ±	285.54
-30	4032.84	5482.12	3824.84	4873.06	4553.22 ±	383.68
-15	3311.9	4632.7	3126.4	3789.9	3715.24 ±	336.24
0	2590.0	4563.84	2572.32	2957.92	3171.02 ±	472.70
15	4372.15	5012.0	3824.84	4372.15	*4395.28 ±	242.70
30	4038.71	5019.67	2764.0	3416.1	3809.62 ±	480.01
45	3641.11	4157.0	2066.8	2767.61	3158.13 ±	463.19
60	2655.2	3491.81	1549.42	2325.37	2505.45 ±	402.26

*Values significantly different from those at 0 min

P<0.02

DISCUSSION

In the present study steroids were administered for a period of two weeks to gonadectomized prepubertal female rhesus monkeys. This resulted in significant increase in basal plasma PRL concentrations. This is in agreement with previous studies in which steroids have been shown to effectively increase PRL secretion in normal prepubertal (Herbert, 1978), stalk sectioned adult (Marshall et al., 1983) male rhesus monkeys and in ovariectomizd ewes (Jiang et al., 1997), and rats (Bouvier et al., 1991). There is sufficient evidence to indicate that the stimulatory action of steroids in enhancing PRL release is primarily enacted directly on lactotropes rather than mediated through hypothalamic activity (Nicoletti, 1984).

Estrogen administration not only increases PRL pituitary release both in women (Yen et al., 1974; Ehara et al., 1976) and men (Barbarino et al., 1982) but, also, enhances PRL response to TRH (Carlson et al., 1973) and dopamine antagonists (Buckman and Peak, 1973). Goodman (1988) demonstrated that estrogen increases the number of lactotrophes and their PRL content. A rise in plasma levels of PRL following treatment with estrogen has been observed in intact (Fernandez-Ruizet et al., 1986) and ovariectomized (Bouvier et al., 1991) rats. Furthermore, a stimulatory action of estradiol on PRL synthesis by serum free culture maintained monkey pituitary cells has been indicated (Bethea and Yuzuriha, 1986). In the present findings, the basal plasma levels of PRL concentrations in ovariectomized immature monkeys were increased following estrogen treatment. These observations are in agreement with previous reports in ovariectomized female rats in which pituitary PRL content has been shown to increase with increasing doses of estradiol (Shulaman et al., 1987). Murai and Jonathan (1990) have demonstrated the presence of PRF in the posterior pituitary of female rats, is the primary site that mediates the acute effects of estradiol on PRL release. An increase in the size and number of PRL cells or lactotropes has been documented in rats (Pasteels, 1963) and man (Pasteels et al., 1972) following administration of esrogen, progesterone (Pasteels.1963), a combination of estrogen and progesterone. The present findings clearly demonstrate the effect of steroids on plasma PRL secretion.

In the present investigation a single iv injection of NMA to ovariectomized female rhesus monkeys after two weeks of steroids treatment resulted in a rapid and significant increase in PRL circulating levels whereas a similar dose regimen of NMA failed to induce a significant rise in PRL concentrations in agonadal prepubertal monkeys. These findings are consistent with the previous observations reported from this laboratory in which NMA was shown to induce a prompt discharge of PRL in intact adult male monkeys but an identical dose had no effect on PRL secretion in orchidectomized monkeys of the same age group (Arslan et al., 1991). It has been shown that PRL response to NMA in intact prepubertal monkeys is similar to that observed in the agonadal adult males (Mahmood, 1993).

Other studies have also demonstrated that the responsiveness of pituitary hormones to NMA may change as a consequence to altered physiological states. Thus, this excitatory amino acid has been shown to be ineffective in evoking LH release in ovariectomized monkeys (Reyes et al., 1990). A change in pituitary hormone response to NMA has also been demonstrated in sheep (Estienne et al., 1990) and in lactating rats (Pohl et al., 1989).

In this study estrogen administration to female immature monkeys reinstated the PRL response to NMA injection that was similar from that observed in progesterone and estrogen plus progesterone treatment. Similarly, in chronically gonadectomized adult male monkeys, steroid treatment has been shown to re-establish the PRL releasing effect of NMA (Arslan et al., 1991).

Estrogens have been shown to influence the basal serum PRL levels as well as PRL response to various releasing stimuli. Thus oestrogen administration not only increased PRL pituitary release both in women (Yen et al., 1974; Ehara et al., 1976) and men (Barbarino et al., 1982) but also enhanced PRL response to TRH (Carlson et al., 1973) and dopamine antagonists (Buckman and Peak, 1973). It is interesting to note that in our experiments treatment of steroid deficient prepubertal female monkeys with estrogen, progesterone and estrogen plus progesterone for two weeks, initiated the PRL responsiveness to NMA stimulation and significant change was observed in basal plasma PRL concentrations following treatment with this steroid. However, the magnitude of the rise of PRL levels following NMA injection was markedly greater in estrogen or

progesterone replaced monkeys than that observed in animals treated with combination of both these steroids when basal PRL levels were significantly different from those observed in estrogen or progesterone treated animals. A stimulatory effect of estradiol on NMA-induced PRL release has also been reported in pigs (Barb et al., 1992) and Weiland (1992) in ovariectomized rats found that estradiol together with progesterone caused a significant increase in glutamate binding in the preoptic area due to an increase in non NMDA receptors activation.

There is increasing evidence that estradiol is an important determinant of the effect of excitatory amino acids on LH secretion in the female. In contrast to the stimulatory effect of NMDA on LH release in intact female rhesus monkeys, reported by Wilson and Knobil (1982), however, Reyes et al (1990) found that NMA inhibited LH release in ovariectomized female monkeys. NMA has also been shown to stimulate LH release in ovariectomized ewes in the presence but not absence of exogenous estradiol (Estienne et al., 1990). Similarly, Lauderer et al., (1990), and Brann and Mahesh (1992) have reported that NMDA administration inhibits LH secretion in ovariectomized rats not treated with estrogen, whereas it stimulates LH release in ovariectomized rats given oestrogen replacement therapy. This biphasic effect of NMA on PRL and LH secretion, depending on oestrogen background is not unique to this category of putative neurotransmitters but has also been reported for other neuroendocrine regulatory factors such as norepinephrine and neuropeptide Y (Kalra et al., 1988). The previously mentioned report by Smith (1989) that estrogens markedly increase neuronal responses to excitatory amino acids may provide a biochemical explanation for the regulatory effect of estradiol over excitatory amino acids action on PRL and LH secretion. Alternatively, since estradiol is known to sensitise the anterior pituitary to TRH and GnRH, the bisphasic excitatory amino acid effect may simply reflect different states of pituitary sensitivity in the presence and absence of estradiol. The mechanism underlying the NMDA dependent driven PRL release is not clear although a suprapituitary site of NMA action in the context of gonadotropin is well established (Olney and Price, 1980; Gay and Plant, 1987). It has been reported that this neuroexcitatory amino acid stimulates both the PRF and PIF and the actual PRL secretion depends on the relative magnitude of the existing NMDA tone to these PRL control systems (Arslan et al., 1992). Alternatively, the possibility that

NMA may directly stimulate pituitary lactotropes cannot be ruled out. Antagonists of the NMDA receptors have recently been reported to reduce the rate of PRL release from primary cultures of rat anterior pituitary cells (Login, 1990).

Taken as a whole, these studies suggest that hypothalamic NMA induced PRL release may be regulated by either E_2 or P but combination of these steroids failed to cause any further increase in PRL levels. This inhibition in further release of PRL may be attributed to the high level of PRL itself.

CHAPTER 4

NEUROENDOCRINE REGULATION OF PROLACTIN SECRETION IN ADULT FEMALE RHESUS MONKEY DURING DIFFERENT PHASES OF MENSTRUAL CYCLE: ROLE OF NEUROEXCITATORY AMINO ACID (NMA)

During the normal menstrual cycle, characteristic changes occur in the serum concentrations of Lutenizing hormone (LH) and Follicle stimulating hormone (FSH). LH levels rise slightly during the follicular phase peak at the time of mid cycle surge and then decrease during the luteal phase of the cycle. The serum FSH concentration begins to rise during the late luteal phase, increases during the early follicular phase of the next cycle and decreases just before the mid cycle FSH surge. The mid cycle FSH surge is smaller than that of LH. FSH levels then decrease during the luteal phase and increase again before the next menses (Ross et al., 1970).

The frequency of LH peaks also varies with different phases of the menstrual cycle (Filicori et al., 1986). The number of LH secretary bursts per 24 h is maximal in the late follicular phase, minimal in the mid luteal phase and intermediate in the early follicular phase (Sollenberger et al., 1990). The frequency of LH pulses remains circhoral on the day of LH surge, but the amplitude is increased (Filicori et al., 1983).

The plasma levels of ovarian steroids also undergo fluctuations during normal menstrual cycle. During the follicular phase a gradual increase in plasma estradiol concentrations occurs, whereas progesterone is barely detectable. As concentration of estradiol rises, the circulating concentration of FSH falls. Prior to the initiation of preovulatory gonadotropin surge, a sudden increase in circulating levels of estradiol occurs which lasts for a period of about 48 h. The increase in the plasma estradiol concentration is paralleled by a slight rise in progesterone concentrations. Following ovulation, the remainder of the cycle is dominated by production of progesterone, which begins to wane shortly following the midpoint of the luteal phase (Goodman and Hodgen, 1983). The corpus luteum also produces major quantities of estrogens and the time course of second estradiol peak roughly parallels that of progesterone (Knobil et al., 1988).

Prolactin (PRL) is present in ovarian follicular fluid. Although the follicular fluid levels of FSH and LH parallel serum levels, PRL levels are higher, suggesting either an active transport mechanism or local synthesis. In the early follicular phase PRL is maximal and decreased during the late follicular phase. The levels appear to be inversely related to

follicular fluid volumes and correlate with maturational changes in the preovulatory human follicle (Seibel et al., 1989). PRL has long been known as a luteotropic hormone especially in rodents. It is involved in initiating luteinization of granulosa cells, in maintaining their level of progesterone synthesis in luteal cells, and in inhibiting the activity of progesterone categorizing enzymes 20-hydroxysteroid dehydrogenase particularly in rodents (Rothchild et al., 1981). PRL has been demonstrated to enhance progesterone production in cultured granulosa cells of rats (Crisp et al., 1977) and porcine (Veldhuis et al., 1980) pre-ovulatory follicles. The appearance of specific receptors in granulosa cells late in follicular development and their induction by FSH in culture indicate the likelihood that PRL may exert a physiological action on granulosa cells at the stage of terminal differentiation when they are transformed into luteal cells. PRL injections (Advis et al., 1981) or hyperprolactinemia induced by in vivo administration of dopaminergic receptors blocker (Gay et al., 1970; Siegal et al., 1976) have been found to induce precocious puberty, as well as to increase ovarian responsiveness to LH in immature rats. In contrast to the stimulatory action of PRL or progesterone secretion, progesterone production by granulosa cells from small immature porcine follicle was markedly inhibited by physiological concentration of PRL (Bex et al., 1975) and can be reversed by estradiol exposure (De Paolo et al., 1979). Another inhibitory effect of PRL on estradiol secretion was reported for cultured rat granulosa cells obtained from follicles at both pre-antral and pre-ovulatory stages (Fujii et al., 1983; Sauder et al., 1984). Decreased estradiol secretion in vitro appears to be due, at least in part, to an inhibiting action of PRL on FSH induction of aromatase activity (Welschen et al., 1980; Chappel et al., 1979). PRL has been reported to suppress basal and gonadotropin-stimulated E_2 secretions by human ovaries perfused in vitro (Lee et al., 1983).

Prolactin like all anterior pituitary hormones is secreted episodically, with a distinctive 24-hour pattern. There are about 14 pulses of PRL secretion in 24 hours in normal human approximately one each 95 minutes (Van cauter et al., 1981). Super-imposed upon this pattern is a bimodal 24-hour pattern of secretion, with a major nocturnal peak beginning after sleep onset and peaking in mid sleep. Minimal levels were observed during noon and maximum in the evening (Sassin et al., 1973;Van cauter et al., 1981). The high levels

during the night are due to increases in the amplitude of each pulse, unaccompanied by an increase in pulse frequency (Valdhuis et al., 1988). PRL secretion remains pulsatile in patients with prolactinomas, whereas circadian variation is abolished. PRL is released in pulses of 8 to 10 minutes intervals in rat anterior pituitaries transplanted under the pituitary capsule of hypophysectomized rats. Because hypothalamic connections have been severed, this short periodicity appears to be intrinsic to the lactotroph (Shin et al., 1981). These short pulses are not controlled by the hypothalamus in human and rat studies but arise within the gland (Samuels et al., 1991).

There is a preovulatory PRL surge in rodents and ruminants but not in primates. This appears to be due to the rising titer of estrogen at this time and appears to require the posterior pituitary (Muari et al., 1990). PRL receptors are present in the luteinizied rat ovary. PRL has an important role in maintaining the corpus luteum in the rat, but not in humans, during early pregnancy. This includes the stimulation of luteal cells growth and progesterone secretion by the corpus luteum. PRL does this by maintaining appropriate numbers of LH receptors on the developing corpus luteum, thereby enhancing its steroidogenic response to LH (Richard et al., 1976). PRL down-regulates annexin production in the corpus luteum, and annexins (or lipocortins) inhibit phospholipase A2, an important enzyme in prostaglandin synthesis, and prostaglandin in turn decreases progesterone biosynthesis in the ovary (Albarracin et al., 1991).

In women, PRL levels appear to be necessary for normal corpus luteum function. For example, lactation impairs follicular development in all mammals. High PRL levels suppress progesterone synthesis by the ovary and decrease induction of aromatase, cause decreased in estrogen production (Dorington et al., 1981). Such a bell shaped response curve is characteristic of many of PRL's actions and is reminiscent of the antagonistic properties of high levels of growth hormone (GH) in preventing receptor dimerization and action. These effects could result in direct inhibition of ovarian function, but hyperprolactinemia also acts centrally on the hypothalamus to decrease the frequency and amplitude of LH pulses, possibly at the level of the GnRH pulse generator (Cohen et al., 1986).

Most investigators have not found consistent cyclic changes in individual prolactin profiles during the normal menstrual cycle in women. Although prolactin concentrations vary from day to day, the mean follicular and luteal phase concentrations appear similar in most studies, and the major peak of LH and FSH observed at midcycle is not associated with corresponding changes in prolactin, Vekeman et al (1977), however, mean luteal-phase prolactin concentrations were higher than mean follicular-phase concentrations.

High serum prolactin (PRL) levels are frequently associated with amenorrhea and anovulation (Bohnet et al., 1977). However, sites and mechanisms of action of elevated serum PRL levels in impairing the hypothalamic-pituitary-ovarian function are still controversial. Reduced or absent LH secreting episodes (Bohnet et al., 1977) and lack of LH elevation both after estrogens (Glass et al., 1975) and clomiphene (Bohnet et al., 1977) administration observed in hyperprolactinemic women suggest a site of action of hypothalamic level. On the other hand a site of action at ovarian level is suggested by the demonstration in humans (Mc Natty et al., 1979) of specific receptors for PRL on the granulosa and corpus luteum cells and by the finding of reduced in vitro progestrone secretion in incubation medium by human follicles in the presence of high PRL concentration.

In recent years, L-glutamate and the two major excitatory amino acid (EAA) postsynaptic receptors, namely, N-methyl-D-aspartic acid (NMDA) (Lopez et al., 1992) and non-NMDA receptor subtypes (Donoso et al., 1992) have been implicated in the control of basal, episodic (Donoso et al., l990) and cycle release of LH (Lopez et al., 1990). Administration of NMDA readily elicited LH secretion in intact rats (Bonovera et al., 1993) and blockade of EAA receptors in castrated rats decreased episodic LH release (Arslan et al., 1988). Activation of glutamate receptors both the NMDA and non-NMDA types stimulated GnRH release from the hypothalamus (Donoso et al., 1990). Similarly blockade of NMDA and non-NMDA receptors suppressed basal as well as pulsatile gonadotropin secretion (Mahesh and Brann, 1994).

Pharmacological evidence also suggests that EAA may participate in the cyclic release of GnRH and LH. Specific EAA receptor antagonists suppress the preovulatory LH surge and that induced by ovarian steroids in ovariectomized rats (Brann and Mahesh, 1991).

Current evidence demonstrates that NMDA antagonists block both spontaneous and steroid-induced LH surges (Urbanski and Ojeda, 1990; Meijs-Roelof et al., 1991). More recently release of glutamate itself has been observed to peak at the time of LH surge (Ping et al., 1994), suggest that NMDA neurotransmission may be an integral component of the neurotransmission line that mediates steroid induced surges of gonadotropin in the female rat (Brann and Mahesh, 1991) and may play an important role on pro-estrous in regulating the preovulatory gonadotropin and prolactin surge. Excitatory amino acid neurotransmitters also appear to be potent modulator of PRL secretion in rodents and primates (Wilson and Knobil, 1982; Gay and Plant 1987; Olney and Price, 1980; Pohl et al., 1989). NMDA elicits PRL secretion in adult rats (Olney and Price, 1980; Arslan et al., 1988) and monkeys (Wilson and Knobil, 1982; Arslan et al., 1991). Administration of NMDA antagonist, MK-801 significantly attenuates the pro-estrous gonadotropin and PRL surge in immature and the adult cycling female rat.

The ovarian steroids, estrogens and progesterone modulate effects of GnRH on pituitary LH release. These steroids have repeatedly been shown to suppress GnRH induced LH release from pituitary gonadotropes both in vivo (Kalra et al., 1982) and in vitro (Kamel et al., 1987). However, the patterns of circulating ovarian steroids during reproductive cycle are associated with basal episodic LH discharge indicating a stimulatory effect of estradiol on gonadotropin secretion. Additionally, there is a growing evidence to show that rising titers of estradiol accelerate the frequency of GnRH and LH discharge during the follicular phase of the menstrual cycle (Sollenberger et al., 1990). The stimulatory effect of estrogens on LH secretion is not exerted directly on GnRH neurons, since GnRH neurons are deficient in estrogen receptors (Watson et al., 1992). Estrogens stimulate gonadotropin secretion by regulating the effectiveness of the episodic GnRH and neuropeptide-Y (NPY) signals on pituitary LH output. Thus estrogen, either alone or in conjunction with progesterone enhances the interactive actions of GnRH and NPY (O'Conner et al., 1993).

In the adult female rhesus monkey limited data are available regarding the role of excitatory amino acid in controlling the PRL secretion during different phases of the menstrual cycle. Functional role of PRL in follicular and luteal development in primates is not established and data on secretary dynamics of PRL during the menstrual cycle are scant. During the menstrual cycle marked changes occur in steroid milieu, which provides an interesting paradigm to study excitatory amino acid and steroid interaction in controlling pituitary hormone secretion. . In the present investigation, an attempt has been made to study the endocrine events occurring during the menstrual cycle of the adult rhesus monkeys in order to get an insight into the physiological changes of the reproductive system. The major objective of the present investigation in the rhesus monkey (*Macaca mulatta*) was under taken to study some aspects of regulation of prolactin under different physiological states using neuroexcitatory amino acid, NMA, as a neuropharmacological probe in this infrahuman primate.

MATERIALS AND METHODS

ANIMALS

Four female rhesus monkeys (*Macaca mulatta*), weighing 7-9 kg and with normal menstrual cycles, were used in this study. The animals were maintained under standard colony conditions, housed in individual cages, and were offered standard monkey food supplemented with fresh fruits and vegetables. Water was available ad-libitum.

CATHETERIZATION

To permit withdrawal of sequential blood samples and intravenous administration of the drug, the animals were anesthetized with ketamine Hcl (5 mg/kg,BW) and while under sedation, they were fitted with indwelling teflon cannula (Vasocan Branule, 0.8 mm/22G O.D, B-Braun Melsungen AG, Belgium) in the sephanous vein. The free end of the cannula was attached to a syringe. The dose of ketamine used was only enough to immobilize the animals slightly but not to induce unconsciousness.

BLEEDINGS

Sequential blood samples (~2 ml) were obtained at 10 min intervals in hepranized syringes. Following each samples collection, an equal amount of hepranized (5 IU/ml) normal saline (0.9 % NaCl) was injected in the tubing . For obtaining single blood samples, animals while restrained in squeeze-back cages were injected with ketamine hydrocholoride (5 mg/kg BW; im) and blood was withdrawn from the sephenous vein.

Blood sampling and the infusion of the drug were also carried out under ketamine tranquilization (2.5 mg/kg BW at 30 min interval). The bleeding was carried out between 10:00-11:00 h. Single blood sampling was carried out between 10:30-11:30 h.

Blood samples were immediately centrifuged at 3000 rpm for 10 min. Plasma was separated and stored at -15 °C, until analyzed.

EFFECT OF SINGLE IV INJECTION OF NMA ON PLASMA PRL SECRETION IN ADULT FEMALE RHESUS MONKEYS DURING DIFFERENT PHASES OF THE MENSTRUAL CYCLE

Four adult female rhesus monkeys with normal menstrual cycles were used for this study. Each monkey was observed daily, checked for vaginal bleeding and studied thrice, i.e during the follicular (9th day), luteal (21 day) and menstrual phase (at the day of vaginal bleeding) of its menstrual cycle.

The menstrual cycle was divided into follicular and luteal phases. The former phase was defined as the period from the first day of menstruation up to the day of LH surges while the later phase was defined as the period after surge till the next menstruation. First day of the menstrual bleeding was denoted as the day one of the menstrual cycle and was used as a reference point. During examination daily for menstruation, any uterine bleeding recorded in an animal for 2 or more consecutive days was regarded as menstruation. Changes in sex skin colour were evaluated and graded + to +++ by using a series of colour standards. On menstruation, the monkeys were bled during different phases of the cycle. The effect of single i.v. injection of NMA (15 mg/kg BW, intramuscularly) on prolactin secretion was studied in ketamine sedated (5mg/ml BW at 30 min intervals) animals. The dose of NMA as established by earlier workers to elicit a maximal discharge of PRL in monkeys (Wilson and Knobil, 1982). The control animals were injected with vehicle (normal saline) in an identical regimen. Blood samples (~2 ml) were collected 30 min before and 60 min after the NMA or saline injection at 10 min interval. All samples from the same animal were analyzed in the same assay to exclude any inter-assay variation.

PLASMA PROLACTIN (PRL), ESTRADIOL(E_2) AND PROGESTERONE(P) PROFILE DURING THE MENSTRUAL CYCLE IN THE ADULT FEMALE RHESUS MONKEYS

To determine the plasma PRL, E_2 and P concentration in adult female rhesus monkeys throughout the menstrual cycle, single blood sample was withdrawn at

alternate days, until the on set of next menstruation, via the saphenous puncture 5 min after the administration of 2.5mg/kg ketamine Hcl, as described earlier.

PHARMACOLOGICAL AGENTS

The following drugs were used in this study.

1. Ketamine hydrochloride (ketavat; park Davis , Berlin, FRG).
2. N-methyal -D,L -aspartic acid (NMA;sigma chemical Co. St Louis, Mo, USA).

Hormone Assays

Plasma prolactin concentration was determined by using enzymeimmunoassay system developed for special programme of research in human reproduction by the World Health Organization. The assay is of an immunometric design and the assay procedure was similar to the one described in chapter 1.

Plasma E_2 and P concentrations were determined by using commercially available enzyme immunoassay (EIA) kits (Serono Diagnostic SA, CH-1267 coinsins, Switzerland).

Steroid Assay

In quantitative determination of steroid hormones by EIA, a high affinity polyclonal antibody was used which incorporated magnetic solid phase separation. The assay procedure involved two steps:

Immunological step

Hormone (Antigen) present in the sample, standard or control competed with a fixed amount of hormone derivative (conjugated to an enzyme Ag*) for binding to a limited amount of fluorescein labeled polyclonal antibody (Ab). This resulted in the formation of Ag-Ab+Ag*-Ab and free Ag*+Ag. After incubation anti-fluorescein coupled to a

magnetic solid phase was added in excess, which rapidly and specifically bound to the hormone derivative-antibody complex (Ag*-Ab) and was sedimented. This was followed by washing.

Enzymatic step

After decanting and washing the sediment, the enzyme substrate solution was then added to the tubes which bound to the Ag*-Ab complex. The colour produced by the enzyme reaction was measured photometrically. Intensity of the colour was inversely proportional to the concentration of hormone present in the sample.

EIA PROCEDURE

Plasma samples (500 ul) in disposable round bottomed (12 x 75 mm) polystyrene test tubes were incubated with 0.2 ml of enzyme conjugate at 37°C in a clean water bath. The incubation time varied with the type of the hormone to be measured i.e. 20 min for E_2 without derivative and additional 20 min with 0.2 ml Serozyme hormone derivative (fluorescein labeled steroid in Tris buffer with sheep and bovine serum proteins) and 15 min for progesterone with 0.2 ml derivative. Following this incubation, 0.2 ml of thoroughly mixed separation reagent (sheep anti-fluorescein bound covalently to magnetizable particles in Tris buffer with bovine serum proteins and sodium azide) was added to each tube and incubated for 5 min at 37°C in a water bath. These incubations were followed by washing. The tube rack was fixed on a magnetic separator and particles were allowed to settle for 2 min. The supernatant was decanted and 0.5 ml of diluted wash buffer (a surfactant and a preservative in tris buffer) was added to each tube. A thorough mixing was done to assure a good assay performance. Rack of the tubes was again fixed on separator and particles were allowed to sediment. This washing was repeated in case of E_2. Following removal of tube from the magnetic base, 0.3 ml of Serozyme substrate solution (phenolphthalein monophosphate and an enzyme cofactor) was dispensed into each tube including the blanks. The tubes were gently shaken and incubated for 15 min except for the E_2 assay where the tubes were incubated for a period

of 30 min. After this last incubation, 1.0 ml of serozyme stop solution (sodium hydroxide and a chelating agent in a buffer solution, PH>10) was dispensed into each tube including the blank. The rack containing the tubes was fixed to the magnetic separator and particles were allowed to settle for at least 10 min. Tubes were then read at a wavelength of 550 nm against the reagent blank on Serozyme I spectrophotometer (Serono). The hormone concentration in each sample was determined using a calibration curve. Inter and intra-assay coefficients of variation were 16.1% and 3.2% for E_2 and 17.4%, 4.1% for P respectively.

STATISTICAL ANALYSIS

Basal hormone levels were calculated by averaging the concentrations before NMA treatment, while stimulated PRL levels were calculated by averaging the peak concentrations during different phases of the menstrual cycle. Student t-test analysis of variance and Duncan's multiple range tests were used to determine difference between the means of basal and stimulated PRL levels.

RESULTS

BODY WEIGHT

The body weight of adult female rhesus monkeys (*Macaca mulatta*) recorded throughout the experiment is presented in Table 1. The mean body weight at the beginning and at the end of the experiment were, 7.98 ±0.44 kg and 8.12±0.50 kg, respectively. The changes in body weight of animals during this course of experimental period were not significantly different (P>0.05).

Changes in sex skin colour

In the adult female rhesus monkeys, sex skin was pale in colour on the day of menstruation and was scored as (–). The pattern of sex skin changes following initiation of the cycle was almost similar in all the animals (Fig.1.). The colour of the sex skin in these monkeys ranged between (+) and (+++). A discernible increase in intensity of sex skin colour was observed on day 5 of the cycle. The approximate duration of the sex skin response ranges between days 5–25. Increase in the intensity of the sex skin colour was invariably accompanied by swelling of the vulva and the perineal skin. Subsequently there was a gradual waning of the sex skin colour and by day 26-30, the sex skin colour had regressed to (-). The decline in sex skin colour intensity was followed by withdrawal bleeding. In these monkeys menstruation occurred on day 29. The uterine bleeding lasted 3-4 consecutive days.

EFFECT OF SINGLE IV INJECTION OF NMA ON PLASMA PRL SECRETION IN ADULT FEMALE MONKEYS DURING DIFFERENT PHASES OF THE MENSTRUAL CYCLE

As depicted in Fig.2. mean plasma PRL concentration in the vehicle treated group was not significantly different from that observed prior to saline injection. (P>0.05). However, the mean basal plasma PRL concentration was significantly different in the luteal phase from that observed in the follicular and menstrual phase of the reproductive cycle (p<0.05).

Follicular Phase

The individual and mean plasma PRL profile before and after NMA

TABLE 1

Mean body weight (kg)of adult female rhesus monkeys(n=4) before and after the menstural cycle

Animal No	Body weight(kg)	
	Before	After
9409	8.2	8.4
9412	7.5	7.2
9413	9.1	9.4
9414	7.1	7.5
Mean±SEM	7.98 ± 0.44	8.12 ± 0.50

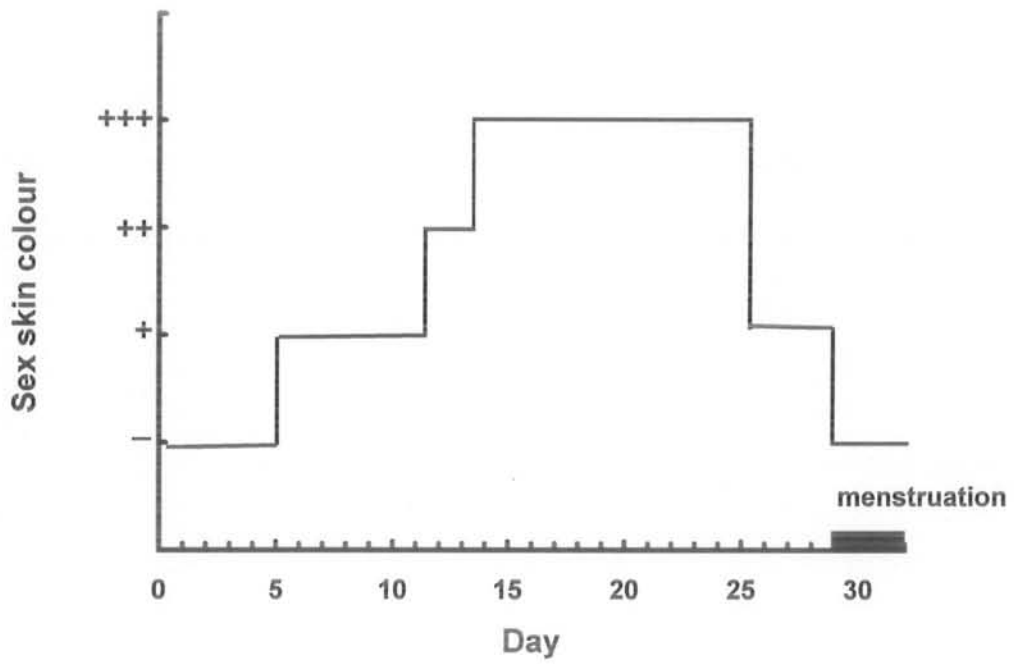


Fig.1. Changes in sex skin colour and onset and duration of uterine bleeding in adult female rhesus monkey during the menstrual cycle

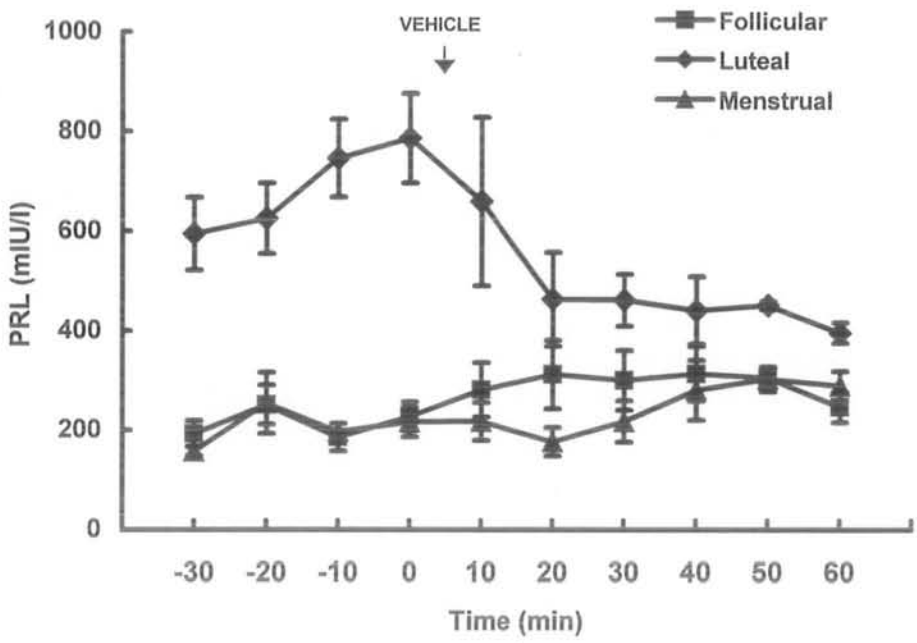


Fig.2.Mean plasma prolactin concentration in adult female rhesus monkeys (n=4) before and after the administration of vehicle alone during different phases of the menstrual cycle.

administration in four adult female monkeys, during the follicular phase (day 9) of the ovarian cycle, are given in Table 2, and Fig.3 and 4. In these monkeys a discernable increase in mean plasma PRL concentration over the basal levels at 0 min was observed following NMA injection (Fig.3.). Mean plasma PRL concentration increased significantly ($P<0.002$) from 214.06±41.06 mIU/L at 0 min to 426.04±9.53 mIU/L at 10 min.

Luteal Phase

The individual and mean plasma PRL concentration in NMA treated monkeys are shown in Table 3 and in Fig.3 and 4 during the luteal phase (day 21) of the ovarian cycle. The change in plasma PRL concentration after a single iv injection of NMA was remarkable ($p<0.01$). The mean plasma PRL concentration at 0 min and at 10 min of NMA administration were 355.87±79.63 mIU/L and 1079.59±189.49 mIU/L, respectively.

Menstrual Phase

During the menstrual phase (day 29), mean plasma PRL levels at 0 min were 227.19±32.07 mIU/L (Table 4, Fig 3 and 4). Following NMA administration at 10 min the levels were 539.05±50.83 mIU/L, being significantly higher than the levels observed at 0 min ($P<0.002$).

Maximum increase in plasma PRL concentration in response to NMA was observed on the 21 day of the ovarian cycle. During this period an increase of 67.03% over the basal levels was induced by a single iv injection of NMA. Whereas on day 9 and on the day of menstruation (29^{th} day) an increase of 49.75% and 57.85% over the basal levels was observed after the administration of the drug ($F=4.5$, $P<0.02$).

Mean basal plasma PRL concentration (Fig 5) was also significantly different in the luteal phase of the cycle from those observed in follicular and menstrual phase of the cycle ($F=11.22$, $P<0.001$).

TABLE 2

Mean and individual plasma prolactin concentrations in adult female rhesus monkeys (n=4) before and after the administration of NMA during the follicular phase of the menstrual cycle

Time(min)	PRL(mIU/L) Animal No					
	9409	9412	9413	9414	Mean	SEM
-30	205.69	241.62	245.78	192.41	221.38 ±	13.20
-20	243.37	231.21	236.14	186.53	224.31 ±	12.84
-10	268.88	267.11	240.26	173.55	237.45 ±	22.28
0	216.78	324.51	185.98	128.98	214.06 ±	41.06
10	400.53	445.45	433.73	424.45	*426.04 ±	9.53
20	399.01	364.42	199.41	177.13	284.99 ±	56.47
30	156.41	171.34	187.32	221.57	184.16 ±	13.98
40	95.71	281.32	158.11	263.35	199.62 ±	44.02
50	161.83	224.52	243.02	395.31	256.17 ±	49.53
60	240.55	232.32	251.31	335.74	264.98 ±	23.90

*Values significantly different from those at 0 min

P<0.002

TABLE 3

Mean and individual plasma prolactin concentrations in adult female rhesus monkeys (n=4) before and after the administration of NMA during the luteal phase of the menstrual cycle

Time(min)	PRL(mIU/L) Animal No					
	9409	9412	9413	9414	Mean	SEM
-30	329.81	480.65	344.98	209.93	341.34 ±	55.39
-20	316.35	423.26	304.62	251.75	324.00 ±	35.95
-10	343.14	464.55	229.28	294.74	332.93 ±	49.69
0	402.07	509.43	379.31	132.67	355.87 ±	79.63
10	619.34	1264.89	1488.09	946.04	*1079.59 ±	189.49
20	313.65	723.91	589.09	508.09	533.69 ±	85.79
30	358.06	710.21	324.62	336.92	432.45 ±	92.84
40	395.93	656.01	623.52	444.12	529.90 ±	64.53
50	194.36	619.09	385.84	366.54	391.46 ±	87.23
60	304.91	321.07	282.11	237.83	286.48 ±	18.08

*Values significantly different from those at 0 min

P<0.001

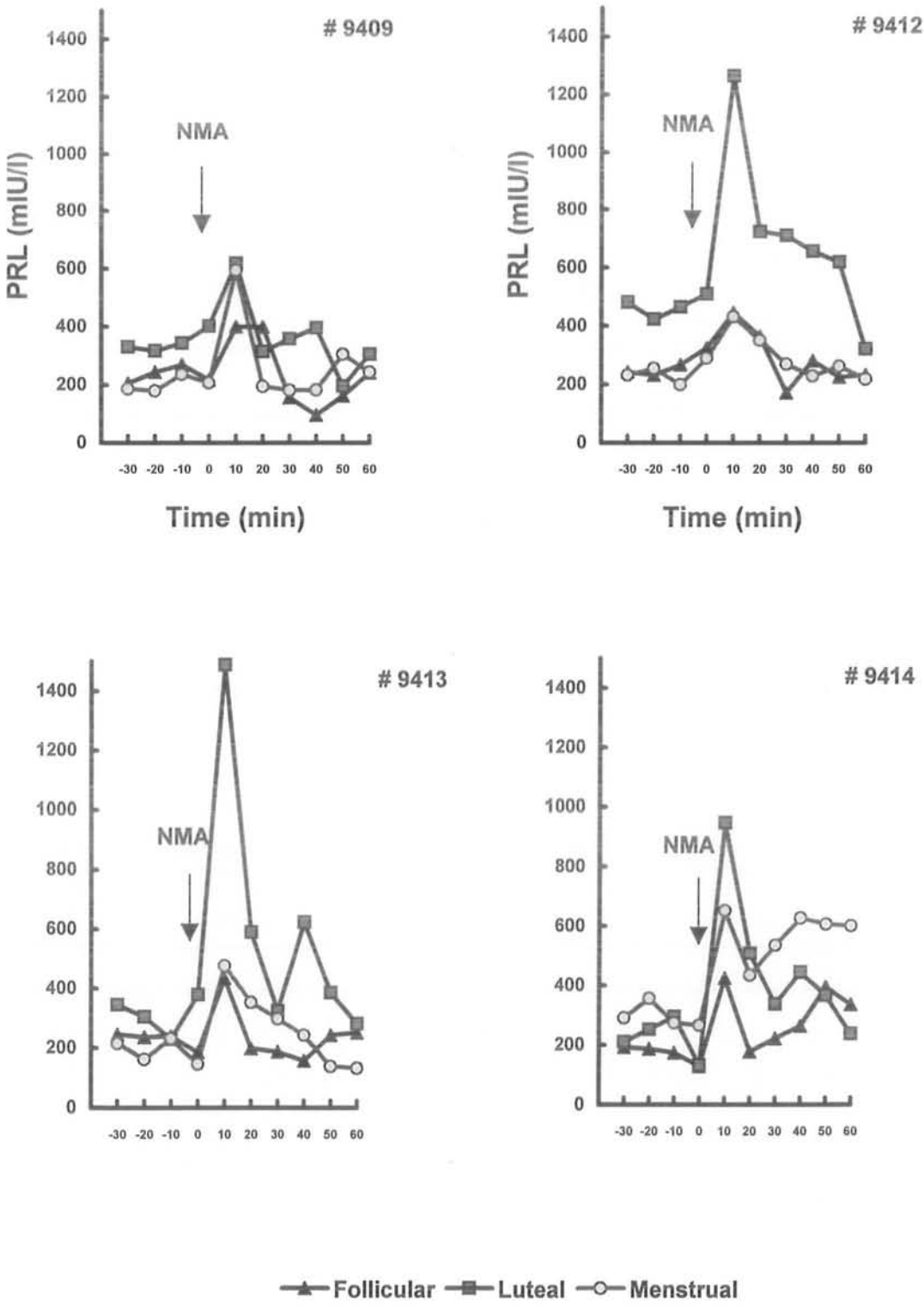


Fig.3. Individual plasma prolactin concentrations in adult female rhesus monkeys (n=4) during different phases of the menstrual cycle after iv administration of NMA.

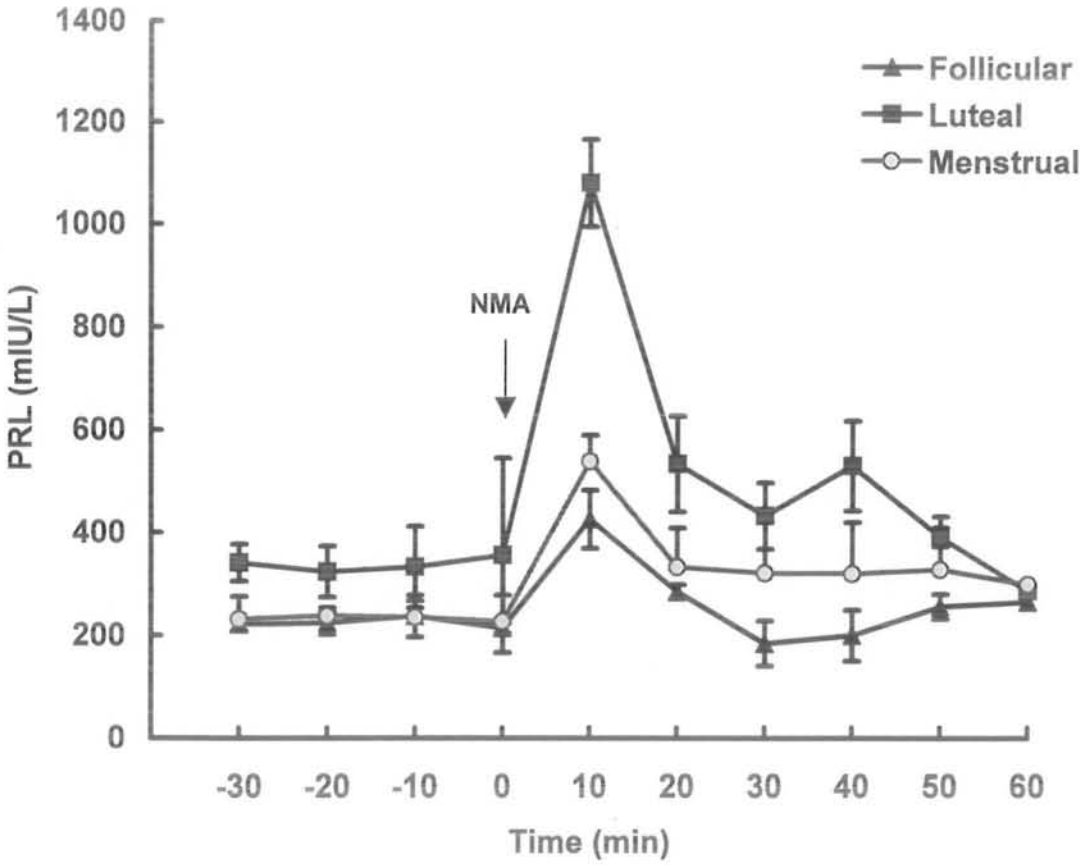


Fig.4.Mean(SEM) plasma prolactin concentrations in adult female rhesus monkeys (n=4) before and after the administration of NMA during different phases of the menstrual cycle.

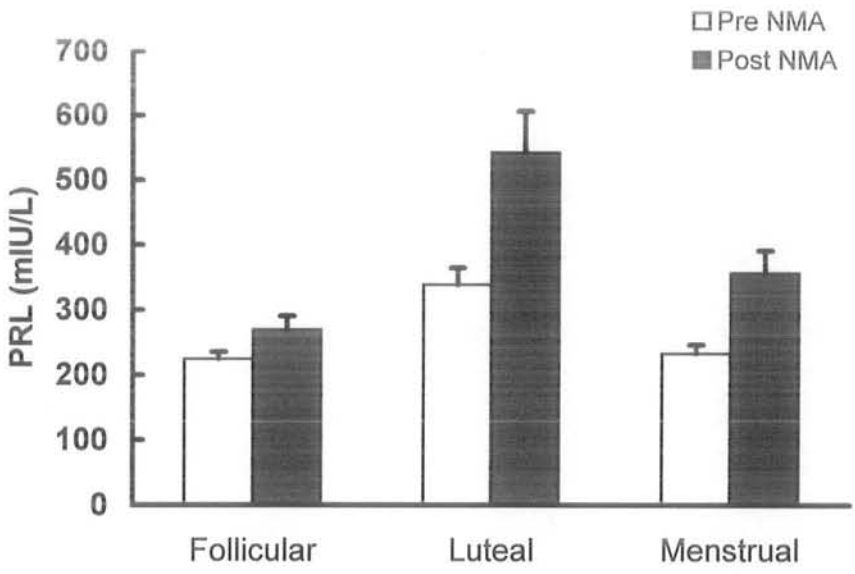


Fig.5. Mean basal and stimulated plasma PRL (mIU/L) concentrations in adult female rhesus monkeys during follicular ,luteal and menstrual phase of the cycle.

PLASMA PROLACTIN (PRL) SECRETION IN MONKEYS DURING THE OVARIAN CYCLE

The mean basal plasma PRL profile in the monkeys during the menstrual cycle is shown in Table 5 and Fig.6. The mean plasma PRL levels were low at the beginning of the cycle and remained low up to 7th day of the cycle and were not significantly different (P>0.05). The levels ranged from 146.75±14.81 mIUl/L to 167.30±9.16 mIU/L during those days. The levels began to rise on 9th day to reach 425.26±59.49 mIU/L on 23rd day. The highest levels were observed on day 23 of the cycle The plasma PRL levels showed a decline on day 25 to 29 which ranged from 337.37±55.79 mIU/L to 171.75±13.79 mIU/L, respectively.

PLASMA ESTRADIOL (E_2) SECRETION IN MONKEYS DURING THE OVARIAN CYCLE

The individual and mean basal plasma E_2 profile in the monkeys during the menstrual cycle is shown in Table 6 and Fig.7. The mean plasma E_2 levels were low during 1-7 day of the cycle. The levels ranged between 51.16±2.69 pg/ml-85.75 ±15.18 pg/ml. Then there was a significant increase in plasma E_2 concentration on day 9 and 11. The levels were 104.65±5.48 pg/ml and 137.50 ±6.96 pg/ml, respectively. The peak values of E_2 were found on day 13 and 15 during the cycle which were 305.72±2.77 pg/ml and 416.0±18.25 pg/ml, respectively, and significantly (P<0.05) different from other days of the cycle. Then there was a gradual decrease in the circulating E_2 level and the levels dropped up to 43.35±6.77 pg/ml on day 29.

PLASMA PROGESTERONE (P) SECRETION IN MONKEYS DURING THE OVARIAN CYCLE

The mean plasma P levels remained low (2.45±0.27 ng/ml to 3.88±0.17 ng/ml) till day 15 of the cycle and were not significantly different (p>0.05). The levels began to rise after day 15 to reach 9.03±0.49 ng/ml on day 23, being significantly (P<0.05) different from the other days of the cycle as shown in Table 7 and Fig 7. No further increase was noticed after day 23 and P levels declined gradually.

TABLE 4

Mean and individual plasma prolactin concentrations in adult female rhesus monkeys (n=4) before and after the administration of NMA during the menstrual phase of the menstrual cycle

Time(min)	PRL(mIU/L) Animal No					
	9409	9412	9413	9414	Mean	SEM
-30	186.27	231.86	215.64	291.24	231.25 ±	22.11
-20	179.56	254.18	162.06	357.03	238.21 ±	44.36
-10	236.34	198.75	232.02	273.82	235.23 ±	15.36
0	207.07	289.72	146.29	265.67	227.19 ±	32.07
10	595.17	432.85	476.54	651.62	*539.04 ±	50.83
20	194.65	351.43	353.55	434.25	333.47 ±	50.13
30	180.93	269.88	298.94	535.12	321.22 ±	75.59
40	182.31	228.92	243.02	626.85	320.28 ±	103.01
50	304.91	261.64	138.46	606.49	327.88 ±	99.34
60	243.37	218.29	133.25	601.12	299.01 ±	103.42

*Values significantly different from those at 0 min

$P<0.01$

TABLE 5

Plasma PRL concentration (mIU/L) in adult female rhesus monkeys(n=4) during the menstrual cycle.

Day	Plasma PRL concentration(mIU/L) Animal No 9409	9412	9413	9414	Mean	SEM
1	154.29	189.23	150.23	175.43	167.30 ±	9.16
3	180.45	125.76	162.47	118.32	146.75 ±	14.81
5	132.21	178.67	185.21	125.45	155.39 ±	15.45
7	124.23	182.56	180.23	136.21	155.81 ±	14.98
9	206.23	241.24	246.56	192.15	221.55 ±	13.27
11	325.24	269.23	170.21	194.32	239.75 ±	35.45
13	156.56	285.54	296.54	124.31	215.74 ±	44.03
15	214.87	257.21	262.29	188.25	230.66 ±	17.69
17	273.59	268.24	277.77	295.56	278.79 ±	5.92
19	211.11	395.87	467.24	257.45	332.92 ±	59.54
21	402.23	423.45	345.21	294.58	366.37 ±	29.08
23	308.29	485.25	562.32	345.21	*425.26 ±	59.49
25	206.56	358.45	475.23	309.23	337.37 ±	55.79
27	355.51	305.12	210.23	231.45	275.58 ±	33.52
29	187.39	199.45	162.95	137.23	171.755 ±	13.79

*Values are significantly different at $P<0.05$

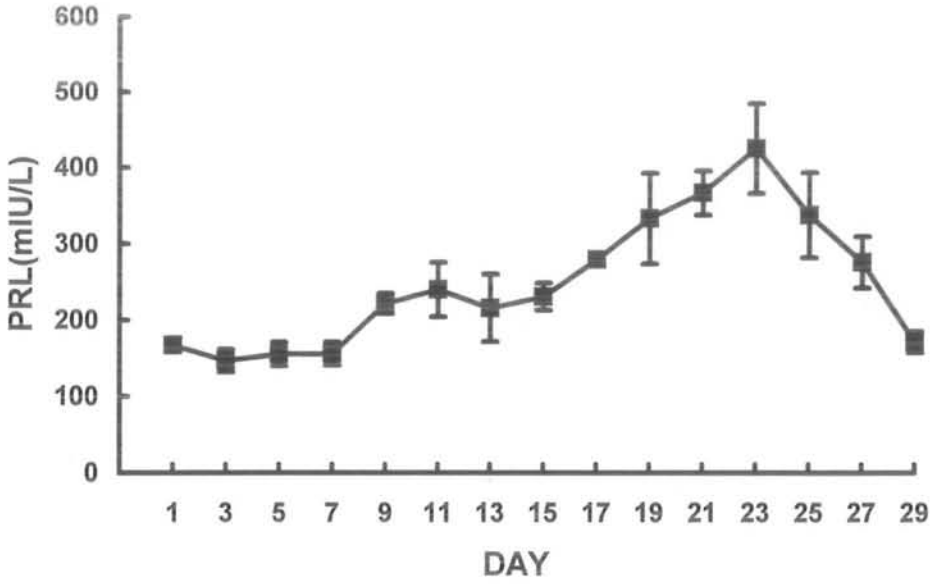


Fig.6.Mean plasma PRL (mIU/L) concentrations in adult female rhesus monkeys (n=4) during the menstrual cycle

TABLE 6

Plasma E_2 concentration (ng/ml) in adult female rhesus monkeys(n=4) during the menstrual cycle.

Day	Plasma E_2 concentration(pg/ml) Animal No 9409	9412	9413	9414	Mean	SEM
1	58.01	45.31	52.31	49.02	51.16 ±	2.69
3	45.21	49.12	61.01	58.21	53.39 ±	3.72
5	69.11	60.35	83.21	76.04	72.18 ±	4.88
7	88.21	42.43	101.32	111.05	85.75 ±	15.18
9	99.32	102.11	96.43	120.73	104.65 ±	5.48
11	131.13	150.43	121.23	148.32	137.78 ±	7.01
13	303.32	309.11	299.12	311.32	305.72 ±	2.77
15	396.13	469.43	389.32	410.11	*416.00 ±	18.25
17	380.24	405.32	360.45	299.22	361.31 ±	22.64
19	160.32	211.11	185.21	223.02	194.92 ±	13.97
21	97.02	111.43	96.22	108.11	103.20 ±	3.86
23	87.01	90.01	82.24	79.12	84.60 ±	2.43
25	72.13	72.03	90.33	85.31	79.95 ±	4.66
27	51.32	40.12	59.03	63.11	53.40 ±	5.06
29	44.04	28.32	40.01	61.01	43.35 ±	6.77

*Values are significantly different at $P<0.05$

TABLE 7

Plasma P concentration (ng/ml) in adult female rhesus monkeys(n=4) during the menstrual cycle.

Day	Plasma P concentration(ng/ml) Animal No 9409	9412	9413	9414	Mean	SEM
1	1.9	2.8	3.0	2.1	2.45	± 0.27
3	2.2	2.4	2.8	3.0	2.60	± 0.18
5	3.1	3.0	2.7	2.9	2.93	± 0.09
7	2.6	3.7	3.1	2.8	3.05	± 0.24
9	3.3	2.8	3.5	3.2	3.20	± 0.15
11	3.8	3.3	4.0	3.0	3.53	± 0.23
13	4.0	3.4	4.2	3.9	3.88	± 0.17
15	3.1	2.9	4.0	3.6	3.40	± 0.25
17	7.1	5.1	6.3	5.9	6.10	± 0.42
19	8.7	7.3	8.0	6.9	7.73	± 0.40
21	9.0	9.4	8.9	6.8	8.53	± 0.59
23	10.2	9.1	9.0	7.8	*9.03	± 0.49
25	8.1	8.0	7.6	6.8	7.63	± 0.30
27	5.1	4.6	5.4	4.2	4.83	± 0.27
29	2.0	2.8	3.1	2.2	2.63	± 0.28

*Values are significantly different at P<0.05

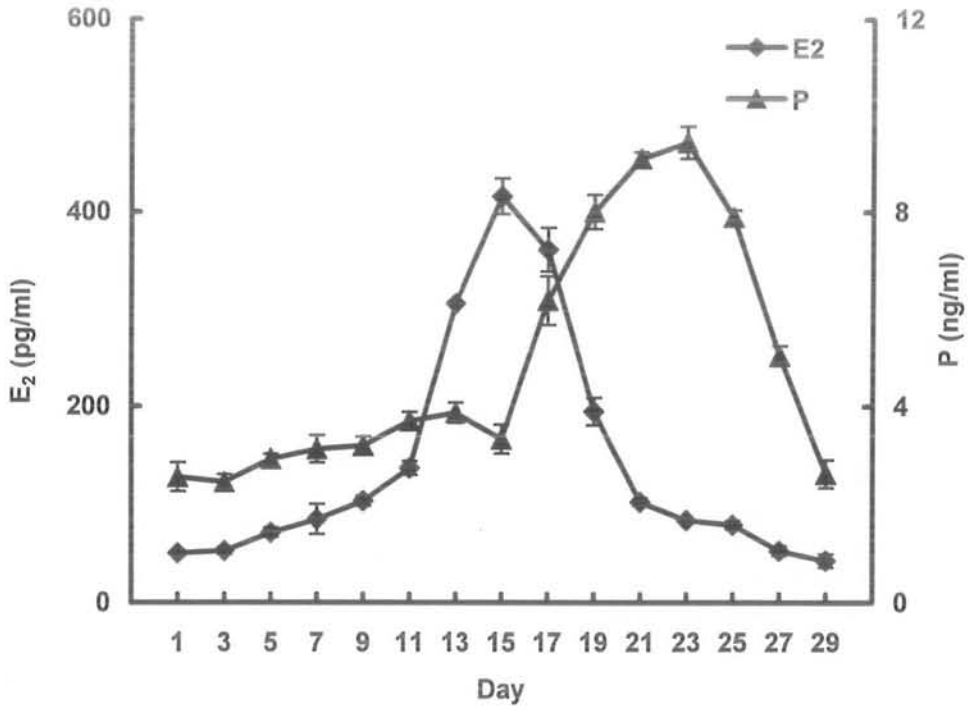


Fig.7.Mean plasma P (ng/ml) and E_2 (pg/ml) concentrations in adult female rhesus monkeys (n=4) during the menstrual cycle

DISCUSSION

In the present investigation, the iv administration of the neuroexcitatory amino acid agonist, NMA, to intact adult female rhesus monkeys resulted in a significant stimulation of prolactin (PRL) secretion during different phases of the normal reproductive cycle. In these animals the greatest response was observed during the luteal phase of the menstrual cycle. These observations are consistent with the result obtained in some of the previous studies in the female rhesus monkey (Wilson et al., 1982; Phol et al., 1989; Stojilkovics et al., 1987) in which administration of NMA could elicit a several fold increase of plasma Prolactin levels through the release of hypothalamic releasing factor, VIP and TRH (Ajika et al., 1972). Present results confirm previous reports in the literature showing that in the cycling monkey, a response of NMDA during the luteal phase was higher than that during the late follicular phase of the cycle (Stojilkovics et al., 1987).

Gonadal secretions have been shown to affect peripheral prolactin concentration and development of lactotropes (Pasteels et al., 1972; Herbert et al., 1974). NMDA stimulation of LH release has been reported to be dependent on the steroid back ground (Reyes et al., 1990; Brann et al., 1992; 1993; Carbone at al., 1992). It is possible, therefore that the highly significant rise in plasma prolactin concentration during the lutel phase of the normal ovarian cycle could partly be due to an increase in steroidal milieu.

As PRL response to NMA was significantly blunted by ovariectomy in rats (Luderer et al., 1993). This finding suggests that NMA induced release of PRF or PRL is enhanced in the presence of ovarian feedback. As stimulatory role for ovarian steroids on PRL secretion has been previously described (Ajika et al., 1972). Present finding shows a maximum increase in plasma PRL concentration in response to NMA on 21 day of the ovarian cycle. During this period an increase of 67% over the basal levels was induced by a single iv injection of NMA whereas on day 9 and on the day of menstruation an increase of 49% and 57% over the basal levels was observed after the administration of the drug. The mean basal plasma PRL profile in these monkeys during the menstrual cycle also shows the higher level of PRL on day 23 of the cycle when P concentration was highest and circulating E_2 concentration was lowest. These findings also confirm the stimulatory role of progesterone on PRL secretion during the normal ovarian cycle in an

infrahuman primate. Progesterone significantly enhances the effect of NMDA on stimulating LH release in both the rat and the monkey (Reyes et al., 1991; Carbone et al 1992). LH response to NMA was highest during the luteal phase of the monkey where serum progesterone levels were the highest. The mechanism whereby progesterone enhances the LH releasing effect of NMDA is unclear but it could be related to the ability of progesterone to increase GnRH synthesis, increase pituitary sensitively (Brann et al., 1991), increase Preoptic area release of endogenous excitatory amino acid (Ping et al., 1994).

Brann and Mahesh in 1991 showed that administration of the NMDA antagonist, MK801, blocks the proestrous prolactin surge in the female rat. Likewise, Brann et al (1993) showed that treatment with the non-NMDA antagonist dinitroquinoxaline(DNQX) significantly attenuated the preovulatory prolactin surge in the PMSG-primed immature rat. Suckling-induced prolactin release in the lactating rat has also been reported to be blocked by the administration of cyno-3,3-dihydro-7-nitro quinoxaline(CNQX), a non-NMDA antagonists (Parker et al., 1993). These studies demonstrated that excitatory amino acid neurotransmission is important in the control of prolactin secretion in a variety of physiological situation.

Progesterone appears to exhibit an action on the hypothalamic GnRH pulse generator. During the menstrual cycle, dramatic changes were observed in the frequency of pulsatile LH secretion (Filicori et al., 1983; Reame at al., 1984; Norman et al., 1984) and, therefore, by interference of the hypothalamic GnRH pulse generator. The first convincing evidence that ovarian P secretion is responsible for the deceleration of the hypothalamic GnRH Pulse generator during the luteal phase of the ovarian cycle was provided by Goodman et al, (1983) in the ewe and then in man by Soules et al (1984), who found that administration of P to normal women during the follicular phase of the menstrual cycle retards LH Pulse frequency. A neurochemical phase for the P deceleration of the hypothalamic GnRH in Primates is provided by the identification of receptor for this steroid in cytosolic extracts of macaque hypothalmi (Krey et al., 1983). Administration of nalaxone, an opiate receptor antogonist, during the luteal phase of menstrual results in dramatic acceleration of LH pulse frequency (Robert et al., 1981; Van Vugt et al., 1984).

Physiological concentrations of circulating estrogen, in contrast to those of testosterone and progesterone, are probably incapable of decelerating the hypothalamic GnRH pulse generator in primates (Plant et al., 1978). E_2 is the major ovarian component of the negative feedback loop that regulates gonadotropin secretion during the follicular phase of the menstrual cycle (Knobil E., 1974). In the monkey that microinjection of E_2 into various neural sites in the hypothalamus (Ferin et al., 1974) and miniinfusion of this steriod into the cerebro spinal fluid (CSF) of the third cerebral ventricle (Chappel et al., 1981) were followed by a suppression of LH secretion have been used as evidence for a hypothalamic site of E_2 action of major importance in the negative feedback control of gonadotropin secretion. E_2 is also able to facilitate LH and FSH release, and this so-called positive feedback action of the steroid plays a major role in eliciting the preovulatory gonadotropin surge in primates (Knobil E., 1974;Young et al., 1976).

Taken together, the present data indicate that prolactin response to NMA in an adult female rhesus monkey during the luteal phase of the menstrual cycle is markedly different from that observed in follicular and menstrual phase and that the steroids may overtly influence the NMDA dependent drive to prolactin release.

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