POSSIBLE ROLE OF NEUROPEPTIDE Y (NPY) ON HORMONES DURING DIFFERENT PHASES OF MENSTRUAL CYCLE IN ADULT RHESUS MONKEY

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BY

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DECLARATION

I hereby declare that the material contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Nahid Kausar

In the name of Allah, the Beneficent, the Merciful.

I dedicate the successful completion

of this thesis and all my achievements in the field

of Reproductive Physiology and

Neuroendocrinology to my honorable

Supervisor,

Professor Dr. Samina Jalali

Contents

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Nahid Kausar

List of Abbreviations

<	Less than
>	More than
µg	Microgram
µl	Microliter
^{0}C	Centigrade
A	Adrenaline
AgRP	Agouti-related peptide
AII	Angiotensin II
ARC	Arcuate nucleus
BW	Body weight
Ca^{++}	Calcium ion
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CPON	C-terminal of neuropeptide Y
DA	Dopamine
DMN	Dorsomedial nucleus
DPN	2,3-bis(4-Hydroxyphenyl)-propionitrile
E2	Estradiol
EA	Electro-acupuncture
ELISA	Enzyme linked immuno-sorbent assay
ER	Estrogen receptor
ERα	Estradiol receptor alpha
ERβ	Estradiol receptor beta
Fig.	Figure
FSH	Follicle stimulating hormone
G	Guanine
GABA	Gamma-aminobutyric acid
GH	Growth hormone
GHRH	Growth Hormone releasing hormone

GnRH	Gonadotropin-releasing hormone
GnRH-Rs	Gonadotropin-releasing hormone receptor
GPR	G-protein-coupled receptor
GPR54	G protein coupled receptor 54
GTI-7	GT1-7 Gonadotropin neuronal cell line
h	hour
hCG	Human chorionic gonadotropin
HCL	Hydrochloric acid
HPG-axis	Hypothalamic pituitary gonadal axis
HPO	Hypothalamic–pituitary Ovarian
i.c.v.	intracerebroventricular
i.m.	Intramuscular
i.v.t.	Intraventricular
IGF-I	Insulin- like growth factor-I
IU	International unit
i.v.	Intravenous
K^+	Potassium
Kg	Kilogram
L	Leucine
LH	Luteinizing hormone
M	Methionine
MAPK	Mitogen activated protein kinase
MBH	Mediobasal hypothalamus
ME	Median eminence
mg	Milligram
min	Minute
ml	Milliliter
mRNA	Messenger ribonucleic acid
n	Number
NA	Noradrenaline
NE	Norepinephrine

ng	Nano gram
nm	Nanometer
NPY Y1R	Neuropeptide Y1 receptor
NPY Y2R	Neuropeptide Y2 receptor
NPY	Neuropeptide Y
OVX	Ovariectomized
P	Progesterone
pg	Pico gram
PIF	Prolactin inhibitory factor
PKA	Phosphokinase activated
PL	Placental lactogen
PLC- β	Phospholipase C- β
POA	Preoptic area
PP	Pancreatic peptide
PPT	4,4’,4’’-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol
PRL	Prolactin
PVN	Paraventricular nucleus
PVR	Pseudorabies virus
PYY	Peptide YY
rmp	Revolution per minute
SE	Standard error
SEM	Standard error mean
SS	Somatostatin
T	Thymine
TH	Tyrosine hyroxylase
THDA	Tuberohypophyseal dopaminergic neurons
TIDA	Tuberoinfundibular dopaminergic neurons
TMB	3,3′,5,5′-tetramethylbenzidine
TRH	Thyroid releasing hormone

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end of experiment.

26b Mean plasma prolactin (PRL) concentration before and after 200µg NPY administration during different phases of menstrual cycle. NPY single bolus i.v. injection has stimulatory and inhibitory effect on plasma PRL level during different phases of menstrual cycle. Plasma prolactin concentration on day 1 (menstrual phase) 15 minutes after NPY administration increased non-significantly with subsequent non-significant temporal increase till 30 minutes followed by significant temporal decrease till the end of experiment. Whereas plasma PRL level increased non-significantly on day 7 (follicular phase) and significantly on day 15 (peri-ovulatory phase) 15 minutes after NPY administration compared to baseline level followed by significant temporal decrease till the end of experiment. However on day 21 (luteal phase) a non-significant decrease in plasma PRL level 15 minutes after NPY i.v. injection compared to baseline level followed by significant temporal decrease till the end of experiment. 136

26c Mean plasma growth hormone (GH) concentration before and after 200µg NPY administration during different phases of menstrual cycle. NPY single bolus i.v. injection has inhibitory effect on plasma GH level during all the four phases of menstrual cycle. On day 1 (menstrual phase) plasma growth hormone (GH) concentration decreased non-significantly after 15 minutes of NPY administration with subsequent non-significant temporal decrease till 45 minutes followed by significant temporal increase till the end of experiment but level remained low than the baseline plasma GH level. Whereas on day 7 (follicular phase) significant decrease in plasma GH level after 15 minutes of NPY administration followed by non-significant temporal decrease till the end of experiment. However on day 15 (peri-ovulatory phase) and day 21 (luteal phase) plasma growth hormone concentration decreased non-significantly 15 minutes after NPY administration followed by non-significant temporal decrease on day 15 and significant temporal decrease (with slight fluctuation) on day 21 till the end of experiment 137

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ABSTRACT

Abstract

Neuropeptide Y (NPY) acts at the hypothalamus to regulate the reproductive function by stimulating the release of GnRH from hypothalamus. In the present study a group of 5 female adult rhesus monkeys (*Macaca mulatta*), 5.5-9 years old, mean body weight of 10.31±0.90 kg and with menstrual cycle of 31 days was used. Changes in their body weight, behavior and sex skin color were observed throughout the cycle. Menstrual cycle of each monkey was monitored daily by recording the onset and duration of menstrual bleeding with vaginal swabs. Baseline profile of estradiol (E2), progesterone (P), prolactin (PRL) and growth hormone (GH) were measured by collecting blood sample (2 ml) on different days throughout the menstrual cycle of 31 days. Sequential blood samples (2 ml) were collected at an interval of 15 minutes for one hour before NPY administration for the hormonal baseline and for 2 hours and 15 minutes after NPY administration. In order to study the effect of NPY on plasma E_2, P, PRL and GH levels on day 1 (menstrual phase), day 7 (follicular phase), day 15 (peri-ovulatory phase) and day 21 (luteal phase) of menstrual cycle, 200 µg of NPY in single bolus intravenous injection was given. Individual and mean body weight during the menstrual cycle was not significantly different. After NPY administration monkeys were relaxed and comfortable. Sex skin coloration changed progressively from whitish pink to deep red following menstrual to periovulatory phase and then decrease in colour intensity occurred during luteal phase. Baseline profile of estradiol showed that plasma E_2 concentration was significantly high ($P<0.001$) in the periovulatory phase of menstrual cycle compared to menstrual, follicular and luteal phases. The luteal phase plasma E_2 level was significantly low compared to follicular phase ($P<0.003$) but not significantly different from the menstrual phase. Plasma estradiol level 15 minutes after NPY administration increased non-significantly in all the four phases of menstrual cycle compared to baseline at 0 minute. Then, subsequent significant temporal increase till 45 minutes on day 1, 75 minutes on day 15, 60 minutes on day 7 and day 21 followed by subsequent significant temporal decrease. At the end of experiment plasma estradiol attained the basal level in all the four phases. Baseline profile of plasma progesterone showed significantly low

levels during menstrual, follicular and periovulatory phases compared to the luteal phase. No significant difference was observed in the plasma P concentration between menstrual, follicular, and ovulatory phases. In all the four phases of menstrual cycle plasma progesterone level 15 minutes after NPY administration increased non-significantly followed by significant temporal increase till 60 minutes on day 1, 105 minutes on day 7, 135 minutes (i.e. till the end of experiment) on day 15 and 30 minutes on day 21. After then non-significant temporal decrease on day 7 and significant on day 1 ($P<0.0002$) and day 21 ($P<0.0007$) was observed. The baseline profile of plasma PRL showed that plasma PRL levels were significantly high during menstrual ($P<0.013$) and periovulatory phases ($P<0.023$) compared to luteal phase. Plasma prolactin level of follicular phase was non-significantly lower than menstrual and peri-ovulatory phases. The plasma prolactin levels of follicular and luteal phases were not different. In plasma prolactin concentration after 15 minutes of NPY bolus injection a non-significant rise was observed on day 1 followed by non-significant temporal increase till 30 minutes and then significant temporal decrease till the end of experiment. On day 7 non-significant and on day 15 significant increase in plasma prolactin level was observed 15 minutes after NPY administration followed by significant temporal decrease on day 7 ($P<0.0005$) and day 15 ($P<0.009$). On day 21 a non-significant decrease in plasma prolactin level after 15 minutes of NPY administration followed by significant temporal decreased till the end of experiment. Regression analysis of variance showed highly significant temporal decrease ($P<0.0003$). The base line plasma in all the four phases of menstrual cycle GH levels in all the four phases of menstrual cycle were non-significantly different ($P>0.05$). NPY administration inhibited the plasma GH level in all the four phases of menstrual cycle. On day 1 (menstrual phase) of menstrual cycle plasma growth hormone level 15 minutes after NPY administration decreased non-significantly with subsequent non-significant temporal decrease till 45 minutes followed by significant temporal increase till the end of experiment. A highly significant decrease in plasma GH level was observed on day 7 (follicular phase) and non-significantly on day 15 (periovulatory phase) and day 21 (luteal phase) of menstrual cycle 15 minutes after NPY administration followed by non-

significant temporal decrease on day 7 and day 15, but significant temporal decrease on day 21 ($P<0.004$) till the end of experiment. These results show that NPY has stimulatory and inhibitory effects on the ovarian and pituitary hormones by acting as a modulator, neurotransmitter and neurohormone. NPY has applications in pharmacological fields and can be used for further research.

INTRODUCTION

Introduction

The continuation of succeeding generations of a species depends upon its ability to reproduce. According to Plant (2008), in primates like in other mammal's reproduction is controlled by complex interaction of hypothalamic pituitary gonadal (HPG) axis involving signals and feedback loop. It comprises of hypothalamus, pituitary and gonads. In this axis, the release of pituitary gonadotropins (luteinizing hormone and follicle-stimulating hormone) is controlled by pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. The gonadotropins control the regulation of gonadal maturation and functions (Plant, 2008). The key regulator of the vertebrate reproductive axis is GnRH (Ebling, 2005). The GnRH release from the hypothalamus is modulated by inhibitory and stimulatory factors. The stimulatory factors comprise of neuropeptide, norepinephrine and glutamate, whereas inhibitory factors are corticotrophin releasing hormone, endogenous opioids and gamma-aminobutyric acid (GABA) (Horton et al., 1989). Gonadal steroids have negative effect during luteal and follicular phases and positive during pre-ovulatory period (for ovulation trigger) reported by Zeleznik and Pohl (2006). GPR54 (Kisspeptin/ G protein coupled receptor 54) system in the past years is crucial for the regulation of GnRH secretion. Evidences suggested that HPG- axis (hypothalamic-pituitary-gonadal) can communicate with neurons involved in control of energy metabolism through contact with GnRH neurons. Neuropeptide Y (NPY) is an orexigenic hormone, expression of NPY receptors on GnRH neurons suggested direct link between reproduction and metabolism. NPY variable effect on GnRH cells depends upon reproductive and metabolic status of the animal.

Neuropeptide Y (NPY)

Over 30 year ago, Neuropeptide Y (NPY) was isolated in 1982 by Tatemoto et al. (1982). NPY is a neuropeptide, neurotransmitter and neuromodulator belonging to the pancreatic polypeptide family consisting of 36 amino acids. According to Allen et al. (1987) all these peptides of pancreatic polypeptide family have a common hairpin-like tertiary

structure, consisting of an N-terminal poly-proline helix and a long alpha-helix connected by a beta turn. Neuropeptide Y (NPY) has 5 tyrosine residues in its primary structure, therefore, it is named neuropeptide Y or neuropeptide tyrosine, as "Y" is an abbreviation of tyrosine (Fig.1a).

Tyr^{1}-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu^{10}-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Arg-**Tyr**^{20}-**Tyr**-Ser-Ala-Leu-Arg-His-**Tyr**-IIe-Asn-Leu^{30}-IIe-Thr-Arg-Gln-Arg-**Tyr**^{36}-NH_2

Fig. 1a: Primary structure of NPY (human, rat) (Allen et al., 1987).

Other peptides like pancreatic polypeptide (PY) and peptide YY (PYY) categorized as the NPY family share with NPY high degree of homology (Larhammar et al., 1993). The human NPY amino acid sequence from the brain was found to be matching with that of rat, guinea-pig and rabbit peptides, but dissimilar to those of bovine and porcine. The single structural alteration between the known mammalian NPY molecules is at position 17, where the methionine (**M**) residue in the human, rat, guinea-pig and rabbit peptides is substituted by leucine (**L**) (Fig. 1b).

Human

1 5 10 15 **17** 20 25 30 35

Y P S K P D N P G E D A P A E D **M** A R Y Y S A L R H Y I N L I T R Q R Y

Rat

Y P S K P D N P G E D A P A E D **M** A R Y Y S A L R H Y I N L I T R Q R Y

Guinea

Y P S K P D N P G E D A P A E D **M** A R Y Y S A L R H Y I N L I T R Q R Y

Rabbit

Y P S K P D N P G E D A P A E D **M** A R Y Y S A L R H Y I N L I T R Q R Y

Porcine

Y P S K P D N P G E D A P A E D **L** A R Y Y S A L R H Y I N L I T R Q R Y

Bovine

Y P S K P D N P G E D A P A E D **L** A R Y Y S A L R H Y I N L I T R Q R Y

Fig. 1b: Primary structure of Human, Rat, Guinea Pig, Rabbit, Porcine and Bovine (McHenry R, 2008).

Location of NPY

NPY is an exceedingly well-preserved peptide during the progression of evolution (Larhammar, 1996a), and owing to this nature it is advocated that it plays a crucial role in the regulation of prime physiological functions. In the brain, the most abundant and widely distributed neuropeptide is NPY (Everitt et al., 1984; Allen et al., 1987; Zukowska et al., 2003), and is present in central and peripheral neurons. Its presence in the hippocampus, hypothalamus, cortex, and hindbrain in the brain is also documented (Chronwall et al., 1985). In abundance synthesis of NPY occurs in arcuate nucleus (ARC) as stated by Beck (2005). Agouti-related peptide (AgRP) is another orexigenic peptide, also co-synthesized in these neurons (Hahn et al., 1998). The axons of these neurons are directed towards dorsomedial nucleus (DMN), in the median preoptic and to the paraventricular nucleus (PVN) (Bai et al., 1985; Kerkerian and Pelletier, 1986). The projections from NPY synthesizing neurons existing in the brainstem are also received by PVN (Sahu et al., 1988b). NPY often coexists with noradrenaline (NA) and adrenaline (A) in some projecting catecholaminergic neurons, and with serotonin in the brainstem, in human and rabbit (but not in rat) medullary neurons (Everitt and Hökfelt, 1989). NPY is present mainly in inhibitory interneurons in the forebrain, where it coexists with GABA and often with somatostatin (Everitt and Hökfelt, 1989). NPY also occurs in the periphery in chromaffin cells of the adrenal medulla (coexisting with A and NA), in sympathetic neurons (coexisting with NA) and in a few parasympathetic neurons. NPY is also found in blood cells, spleen, bone marrow, megakaryocytes and thrombocytes (Ericsson et al., 1987).

Synthesis of NPY

NPY is synthesized within the endoplasmic reticulum as a large precursor protein, after its synthesis, NPY moves to the golgi apparatus, which is then followed by its translocation into the trans-golgi network. Here the peptide is stored till further activation. The large dense-core vesicles store majority of NPY (Michel, 2004). When NPY is needed post-translational modifications in precursor protein made before it is

exocytosed into the extracellular space. Further cleavage occurring by proteolysis upon its entry into the extracellular space and changed into different sizes peptides.

Functions of NPY

Neuropeptide Y high percentage of conservation reflected that NPY must plays an important physiological role in the body. NPY was found to perform similar functions in animal kingdom for controlling behavior and organism adaptation during environmental challenges as in starvation, predator attack or infection (Sokolowski, 2003). Wide distribution of NPY neurons involves it in many biological actions, comprising of cardiovascular regulation, control of seizure, cognition and stress, control of appetite, modulation and regulation of neuroendocrine systems (Walker et al., 1991; Thorsell and Heilig, 2002; Zukowska et al., 2003). NPY is also associated in many other physiological functions involving body temperature regulation; control of the release of gonadotropin releasing hormone (GnRH) from hypothalamus. The release of corticotropin releasing factor and sexual behavior is likewise controlled by NPY. Besides this NPY controls pain, anxiety, circadian rhythms, memory processing and inhibition of the release of other neurotransmitters (Danger et al., 1990; Gray and Morley, 1986). Furthermore, NPY plays a role to reduce epileptiform activity in the hippocampus and has an important role in the control of neuronal activity (Woldbye et al., 1997) by its inhibitory action on glutamate secretion (Vezzani et al., 1999). It is also documented that NPY performs its role in the regulation of PRL and GH.

Receptors of NPY

NPY and peptides of pancreatic polypeptide family can act on NPY multiple G-protein coupled receptors: Y1, Y2, Y4, Y5, and Y6 which is known to be nonfunctional in humans depending on the length (Brothers and Wahlestedt, 2010; Michel, 2004). NPY mediates all of its biological actions and physiological effects through NPY receptors from Y_1-Y_6 (Bader et al., 2001). In all the mammalian species NPY Y_6 receptor is not present (Widdowson et al., 1997b; Burkhoff et al., 1998). These receptors may be derived from three Y receptor genes which lead to the Y_1, Y_2 and Y_5 subfamilies (Larhammar and

Salaneck, 2004). All three Y_1, Y_2 and Y_5 subfamilies seem to play a role in the control of feeding (Henry et al., 2005). More than two decades ago five receptors of NPY were cloned (Michel et al., 1998). These G-protein-coupled receptors of NPY are linked to inhibitory pathways that upon stimulation, cause membrane hyperpolarization (Sun et al., 1998), inhibition of adenylatecyclase and an increase in intracellular calcium concentration. The NPY receptors are extensively located in the nervous system. The receptors differ in their location, as well as in their anatomical and functional roles. Individual distribution patterns of NPY receptors distribution was observed in the hypothalamus by (Fetissov et al., 2004).

Mechanism of action of NPY

NPY receptors are G protein coupled receptors, which generally couples with G_i or G_0 protein to inhibit the adenylatecyclase activity to hang-up cAMP buildup and modulate the potassium (K^+) and calcium ions (Ca^{++}) channels (Holliday et al., 2004). NPY Y_2 and Y_4 receptors have the ability to couple with Gq protein to increase the synthesis via the stimulation of phospholipaseC- β (PLC) reported by Misra et al. (2004) in smooth muscles of rabbits. It has been shown that in the central nervous system (CNS) on the basis of ligand binding and pharmacological studies, at least two neuropeptide Y receptors might occur in the CNS (Kalra and Crowley, 1992). Phospholipase C and inositol phosphate metabolism is coupled to Y_1 receptor metabolism and furthermore upon activation enhances intracellular Ca^{++} mobilization, whereas the influx of Ca^{++} is decreased by the Y_2 receptor resulting in further inhibition of secretory activity of the target cells (Ewald, 1988). Adenylcyclase is negatively linked to both Y_1 and Y_2 (Kalra and Crowley, 1992). Pertussis toxin inhibition of all the neuropeptide effects shows the mediated involvement of G_0/Gi (Ewald, 1988; Kalra and Crowley, 1992).

Role of NPY in the regulation of GnRH/LH system/Reproduction

Reproductive function is controlled by NPY via regulation of GnRH release at hypothalamic level (Kaynard et al., 1990; Khorram et al., 1987; McDonald et al., 1989). In the arcuate region of hypothalamus NPY neurons projected on cell bodies of GnRH

neuron in POA and in medien eminence up to GnRH pre- synaptic terminals (Li et al., 1999), whereas morphological evidences indicated that NPY receptors co-localization with GnRH neurons, potential mechanism has been suggested. These neuroanatomical arrangements between NPY and GnRH, suggested a probable mechanism by NPY influence on reproductive axis. Episodic hormone discharge is a distinctive feature of the HPG axis (Maeda et al., 2010). In vitro study Dhillon et al. (2009) found that GnRH gene expression was significantly increased on GT1-7 neurons cell line treated with NPY from mHypoE-38 cell line and inhibited by NPY Y_1-receptor antagonist BIBP-3226. GT1-7 neurons express Y_1, Y_2, and Y_4 NPY receptors, but not of Y_5. NPY-mediated increase in GnRH transcription was reduced by Mitogen activated protein kinase (MAPK) and protein kinase A (PKA) signal transduction pathway pharmacological inhibitors (Dhillon.et al., 2009). These studies by Dhillon et al. (2009) strengthen the evidence of NPY importance in the control of reproductive function.

The normal female reproductive cycle, important for completion of reproductive process, is entirely dependent upon modulatory factors such as neuropeptide Y which coordinate the secretion of reproductive hormones. GnRH pulsatile release in hypophyseal portal blood vessels accompanies NPY during the cycle (Woller and Terasawa, 1992). NPY immunoneutralization significantly decreases the LH surge in the portal circulation (Sutton et al., 1988), and in NPY-knockout mice LH surge is stunted (Xu et al., 2000). NPY supplements LH secretion in proestrus (Bauer-Dantoin et al., 1991; Bauer-Dantoin et al., 1993; Besecke and Levine, 1994; Leupen, 1997). Without proestrus hormonal milieu, the significant release of LH by GnRH in presence of NPY is not possible. NPY enhances GnRH-stimulated LH release from adenohypophyses of proestrous rats and not from metestrous (Bauer-Dantoin et al., 1993). In vivo NPY has no effect in metestrous, or ovariectomized and pentobarbital-treated rats on GnRH-stimulated LH release (Bauer-Dantoin et al., 1992). In NPY-knockout mice primed with estrogen, the GnRH action on LH release is attenuated (Xu et al., 2000). GnRH secretion in the monkey is not only regulated but also enhanced by NPY (Gore et al., 1993; Pau et al., 1995).

Data showed that during initiation of prolactin and luteinizing hormone (LH) surge in rats the rise in GnRH release is parallel with NPY neurosecretion (Woller et al., 1992; Sutton et al., 1988) GnRH ability is amplified to trigger the LH preovulatory surge by NPY surge. In addition gonadotrophs secretion of LH may be directly controlled. In animals NPY can simulate GnRH secretion in presence of both steroids (estrogen and progesterone) (Mizuno et al., 2000). These changes in GnRH release parallel with NPY suggested that NPY is important for preovulatory LH surge during estrous cycle.

In the last decade, Plant and Shahab (2002) have been following the hypothesis that NPY expressing neurons in the hypothalamus provided the major component for upstream brake on pulsatile GnRH release, this was on the basis of findings that GnRH pulsatility in agonadal post-pubertal monkey (both female and male) is first arrested by NPY central administration (Kaynard et al., 1990; Pau et al., 1995; Shahab et al., 2003). GnRH release at particular development stage in the mediobasal hypothalamus of the agonadal monkey (male) are inversely related to changes in NPY gene expression and peptide content at the 3rd major stage of postnatal development (El Majdoubi et al., 2000a). Moreover, during the development stages of the rhesus monkey (male) this pattern in the ontogeny in NPY gene expression correlated with a structural re-modeling of the hypothalamus neurons, interactions between GnRH perikarya and NPY axonal varicosities (Plant, 2001; Plant and Shahab, 2002). The appositions between GnRH perikarya and NPY varicosities in the mediobasal hypothalamus are less in pubertal animals compared to animals at the juvenile stage. Central administration of NPY at post pubertal stage inhibited the release of GnRH from the hypothalamus by NPY Y_1 receptor but the same receptor mediates the break on GnRH release at prepubertal are vague as supported by pharmacological data regarding the role NPY Y_1 receptor (Shahab et al., 2003). In another approach to test the hypothesis that increased hypothalamic NPY signaling is a major component of the brake that is exerted on pulsatile GnRH release prior to puberty, researchers have looked for loss of function mutations in the NPY Y_1 receptor in children with GnRH-dependent precocious gonad arche (Barker-Gibb et al., 2002a; Freitas et al., 2003). In a total of 35 patients, a heterozygous substitution of T (Thymine) for G (Guanine) at nucleotide 1330

of exon 3 of the gene encoding the Y_1 receptor was found in one girl with precocity. Although this mutation results in the substitution of lysine by threonine at position 374 in the carboxy terminus of the receptor, the mother of this subject was found to have the same heterozygous mutation, yet had apparently normal pubertal development (Freitas et al., 2003). Zhaohui and co-workers had shown that the repeated electro-acupuncture (EA) down-regulated the HPG axis during puberty in rat and rabbits, especially GnRH expression, serum testosterone, and number of sperms (Zhaohui et al., 2007). They subsequently hypothesized that NPY should be a crucial part in down-regulation of HPG axis during puberty, and that the repeated low frequency EA could down-regulate the HPG axis via the intervening NPY. In order to test that hypothesis, Zhaohui and co-workers detected the expression of NPY at hypothalamus level in female and male rats (Zhaohui et al., 2007) at different development stages (from juvenile to adult stage) after repeated low frequency EA. They also compared the trend of fluctuation of NPY expression with that of GnRH expression in the hypothalamus and that of body weight of rats at different development stages. In another study (Zhaohui et al., 2012) demonstrated that NPY expression in hypothalamus of rats is significantly down-regulated in early puberty after repeated low frequency EA, which did not affect the increase of body weights of rats. The finding that NPY expression level waves similarly with that of GnRH during the early puberty of rats supports the view that metabolic peptidergic systems in the hypothalamus such as NPY may not play critical roles in controlling fertility (Ward et al., 2009).

A number of studies showed that NPY stimulated the LH release through NPY Y_1 receptor and BIBP3226 a selective NPY Y_1 antagonist inhibited the GnRH induced and proestrus LH release when injected peripherally (Rudolf et al., 1997; Leupen et al., 1997). The expression of NPY Y_1R expression is increased in presence of estrogen during proestrus afternoon showed that NPY Y_1 expression is steroid dependent (In addition, Musso et al., in 2000 found that E2 treatment induces Y_1R gene expression in transfected neuroblastoma-glioma cells through the direct interaction of estrogen receptor (ER) with three hemipallindromic estrogen-responsive elements flanking the Y_1R gene.

In a recent study Roa and Herbison (2012), showed that porcine Y_1, Y_2, Y_3 NPY receptor agonists are direct inhibitors of GnRH, firing in less than 50 percent neurons, the other tested agonist showed the role of NPY Y_1 to inhibit the GnRH activity where Y_4 is stimulatory at postsynaptic level, these findings suggested that neuropeptides regulate the GnRH activity in complex way (Roa and Herbison, 2012). The mode of NPY action seems to depend on the activity of the reproductive system.

Endocrine circuits of hypothalamic–pituitary Gonadal/Ovarian axis

This hypothalamic–pituitary Gonadal/Ovarian (HPG/O) axis is the central unit for the upkeep of the endocrine poise and fertility. Ovarian jobs are under the directive of endocrine factors of the brain. GnRH is produced and secreted via the hypothalamus and excites the gonadotrophs of pituitary to synthesize and release LH and FSH (McCann et al., 1989). These hormones enter the blood circulation to act on gonadal targets for the regulation of steroidogenesis and gametogenesis. Ovarian theca cells are acted upon by LH subsequently fuelling the manufacture and release of androgens from these theca cells. Androgens act on granulosa cells within the follicle, altered consequently into estradiol, although follicular growth is stimulated by FSH on granulosa cells and FSH receptors appearance on this cell type is observed. Positive and negative feedback mechanisms secondarily control the gonadal steroids, which in turn maintain normal hormonal levels and permit standard reproductive functions (Kalra, 1986). The hypothalamus and pituitary is controlled by gonadal steroids to govern the secretion of GnRH and pituitary gonadotropes. LH surge occur preceding ovulation due to positive feedback effect of estrogens on gonadotrophs, whereas during follicular and luteal phase estradiol has a negative feedback consequence on gonadotropes release. Progesterone peak released in luteal phase along with estrogen in response to LH surge has inhibitory effect on pituitary for gonadotropin release which leads to luteal inhibition and early follicular development. Granulosa cells expressed FSH receptors and response to follicular stimulating hormone (FSH) which acts directly on the granulosa cells to stimulate to stimulate follicular growth. High concentration of estrogens by its positive

feedback mechanism stimulates the gonadotropin for pre-ovulatory LH surge, whereas estrogen by its negative feedback inhibits the gonadotropin release during early follicular phase and after ovulation in luteal phase. LH stimulates progesterone production in the luteal phase, and has negative influence on gonadotropin secretion along with estrogen for early follicular growth.

Hypothalamo-Hypophyseal Loop

Among primates, the reproductive axis is controlled by GnRH, which is predominantly synthesized in neurons of the mediobasal hypothalamus and the arcuate nucleus and secreted in a pulsatile fashion (Conn and Crowley, 1994). GnRH transported to the anterior pituitary through the portal circulation and stimulates release of gonadotropins (FSH and LH) via GnRH receptor (GnRH-R) stimulation (Conn and Crowley, 1991). FSH and LH act on the gonads and control the secretion of estrogen and progesterone (Naor, 2009). In vertebrates GnRH exists in 23 forms of which are decapeptides, and amino acids in peptide at positions 5 to 8 changed to develop different from one another (Mathias and Clench, 1998; Millar, 2005). It is a highly conserved decapeptide through evolution and expressed from one to three forms in each vertebrate (Naor, 2009; Millar, 2005; White et al., 2008). In mammals it exists in two common GnRH types include - GnRH I, which regulate the hypothalamic-pituitary axis and gonadotropin production, whereas GnRH II, which is distributed particularly in the hind brain and spinal cord, so being extra-hypothalamic. In reproduction and sexual behavior it participated through a neuromodulator role (Millar, 2005; Barnett et al., 2006). GnRH has different receptor GnRH-I and II in mammals, although GnRH-RII is not fully functional receptor, because of a genomic stop sequence (Morgan et al., 2003; Naor, 2009; Stewart et al., 2009). So GnRH II also signals through the type I receptor (Millar, 2005; White et al., 1998). New findings show that an ancestral extinct vertebrate, the lamprey, has two types III-like GnRH-Rs that are closely related to GnRH-R type-II (Joseph et al., 2012). Isolation and characterization of GnRH was made by Nobel Prize winners Roger Guillemin and Andrew Schally (1977). The GnRH performed different functions by acting through its

receptors which are G-protein-coupled receptor (GPR) with seven trans-membrane domains (White et al., 2008). GnRH receptors are found in hypothalamus, brain, ovary, endometrium, myometrium, decidua, placenta, breast/mammary glands, lymphocytes, T cells, prostate, testis, sperm, mononuclear blood cells, spleen, liver, pancreas, adrenal glands, kidney, heart, skeletal muscle, gastric parietal cells, sub-maxillary glands, spinal cord, retina, gastric parietal cells and various cancers and cancer cell lines (Naor, 2009). The pulsatile release of GnRH from the hypothalamus is obligatory for the release of LH and FSH from intact pituitary and in turn the ovarian function. The idea GnRH secretion in episodic manner into portal vein of hypophysis is important for the subsequent pulsatile release of the hypophyseal gonadotropins as was demonstrated by Knobil (1980). The central neurobiological mechanisms that drive pulsatile release of GnRH have been embodied in a concept of a so called "GnRH". In the mediobasal hypothalamus the GnRH pulse generator has been restricted in the arcuate region (Dhillon et al., 2009; Maeda et al., 2010). The rhythmic release of GnRH is the instrinsic property of GnRH secreting neurons (Krsmanović, 1992; Moenter et al., 2003). More recently, the electrophysiological correlates of GnRH pulse generator have been unfolded. Pulsatile release of LH synchronized with GnRH pulse. The elimination of endogenous GnRH synthesis from mediobasal hypothalamus bilateral lesions was first demonstrated in female rhesus monkeys reflected the physiological significance of pulsatile GnRH release (Knobil et al., 1980). The continuous GnRH infusion into mediobasal hypothalamus failed to withstand gonadotropin release. However pulsatile GnRH administration of physiological frequency regenerated the LH and FSH to pre-lesion levels. Maeda et al. (2010) reported on the basis of similar finding in man and other species that the episodic release of GnRH is important for its therapeutic application. In turn, the activities of GnRH pulse generator are directed by interplay of excitatory and inhibitory neurotransmitters and neuropeptides (Plant and Barker-Gibb, 2004; Ebling, 2005). Some of them mediate the actions of internal factors (e.g. gonadal steroids, metabolic hormones) while others facilitate the effect of environmental factors (temperature, light). The kisspeptin-GPR54 also involved in GnRH release as a major

neuroendocrine regulator on the basis of pharmacological and genetic studies documented by Gottsch et al. (2006); Plant (2006) and Tena-Sempere (2006). Kisspeptine-GPR54 not only played a role in the initiation of puberty (Han et al., 2005), but also involved in in regulation of GnRH release (Dhillon et al., 2005; Ramaswamy et al., 2007). Whereas more recently, a peptide acting as putative suppressor of gonadotropin secretion termed as gonadotropin inhibiting hormone (GnIH) has been characterized in several mammalian species including primates (Tsutsui, 2010). The proper functioning of the HPG axis is sensitive to many external and internal factors (Bronson, 1985; Cameron, 1996; Wade et al., 1996; Wade and Jones, 2004).

Hypothalamo-Hypophyseal-Ovarian Loop

The GnRH-driven production and release of gonadotropic hormones (FSH and LH) is controlled by ovarian steroid and inhibin protein. Estradiol greater production from maturing follicles during the early follicular phase through its negative feedback effect reduces the FSH/LH release by depressing pulse amplitudes. Whereas increased estradiol level to a certain threshold over a definite period during the follicular phase by its positive feedback action both at the hypothalamic (for GnRH release) and pituitary level induced the mid cycle peak of gonadotropin (FSH/LH) release and stimulate ovulation. Midcycle progesterone increase also participated in this positive feedback activity as progesterone antagonists block delay or ovulation, this effect can be reversed by administration of progesterone (Batista et al., 1992). A study by (Borman et al., 2004) on rhesus monkey proposes the direct beneficial effect of progesterone on oocyte maturation/follicle survival but progesterone is not able to provoke ovulation in the absence of LH. Number of other factors like neurokinin, or substance P, may be participated in the control of the preovulatory LH release because substance P antagonist administration acting via the neurokinin receptor, significantly reduced LH concentration (Kerdelhue et al., 2000). Estradiol and progesterone exhibit negative feedback effect after ovulation, on both gonadotropic hormones to restore the preovulatory levels through decreasing the pulse frequency instead of pulse amplitude. This reflects that pulse

amplitude and frequency are inversely related to produce LH output during luteal phase comparable to follicular phase (Veldhuis and Johnson, 1990). Peptides follistatin, activin and inhibin, mediated feedback loop regulate the release of FSH (Bilezikjian et al., 2006; Messinis, 2006). LH and FSH stimulate the inhibin release from granulosa cells which in turn inhibits FSH secretion. In FSH feedback regulation follistatin and activin play role as local regulators than as endocrine regulator. In the pituitary other factors and regulator of FSH versus LH production might also involve pituitary-adenylasecyclase-activating peptide produced in folliculostellate cells (Winters and Moore, 2007). The inhibins and activins feedback action occurs at the pituitary level. Follistatin by binding to activin selectively enhances FSH release,

Menstrual Cycle/ Ovarian Cycle

The menstrual cycle is divided into two major phases, the follicular and luteal phases. Whereas ovarian triad term comprises of follicle, oocyte, and corpus luteum for the menstrual cycle of primate was used many years ago reported by (Goodman and Hodgen, 1983). In the ovarian cycle during follicular phase follicular maturation and ovulation takes place. During luteal phase establishment of the corpus luteum occurs, followed by corpus luteum regression and menstruation. The first day of menstrual bleeding is designated as the first day of the ovarian cycle. The entire duration of the ovarian cycle is 28 to 32 days in the cynomolgus monkey and the rhesus monkey (Weinebauer et al., 2008). The follicular phase ranged from 12 to 14 days, the peri-ovulatory interval is roughly 3 days, and the luteal phase comprises of 14 to 16 days. Ovarian cycle can be determined by blood sampling throughout the menstrual cycle (for hormonal profile to determine the cycle status), duration of cycle can also be determined by daily vaginal smear or by observing sex skin colour changes. Variation in the length of the ovarian cycle is described by Shaikh et al. (1978). In macaques the endocrine control and cyclicity are basically similar (Hotchkiss and Knobil, 1994).

Follicular Phase

The follicular phase is characterized by the absences of corpus luteum, and the gonadotropin-dependent follicular maturation leading up to ovulation. The maturating follicle release estradiol and inhibin but plasma progesterone is very low at early follicular phase. The length is variable and this variability account for the overall variability of the length of menstrual cycle. The ovarian steroid, activin and follistatin modulates the gonadotropic hormones production and release drive by GnRH. Increase production of estradiol from maturing follicle has negative feedback effect on gonadotropin release in follicular phase. Whereas the high level of estradiol concentration has a positive feedback effect on gonadotrophs release, particulary LH surge at mid-cycle to induce ovulation. Estradiol in this regard has direct effect on GnRH release from hypothalamus and also at pituitary level. Increase secretion of progesterone during periovulatory phase also participate in positive feedback action and progesterone action is inhibits or delays ovulation by its antagonist and reversed after progesterone administration (Batista et al., 1992). Borman et al. (2004) showed the importance of progesterone in rhesus monkey for follicle development and oocyte maturation (Stouffer, 2003), but progesterone alone is not capable of causing preovulatory LH surge and ovulation. After ovulation estradiol level decreased and prolactin as well as testosterone concentration changes widely but not related to ovarian cycle phases. The level of other peptides like inhibin B increased during early follicular phase but inhibin A increased in luteal phase (Fraser et al., 1999).

Ovulatory Phase

Midcycle gonadotropin surge induced ovulation, which activates and starts oocyte maturation, break down of follicles to release oocyte and luteinization of follicular cells. Estradiol concentrations when overcome a threshold level for at least 36 hours in the rhesus monkey, by its positive feedback effect stimulate FSH and LH surge and induces ovulation. Primary stimulator is LH. After this surge, granulosa cells gradually become mitotically inactive. The follicular steroidogenesis shifts during the periovulatory interval from the synthesis of androgens and estrogen to synthesize progesterone. The change in

steriodogenesis is regulated by gonadotropins via enzyme expression. Progesterone plays an important role through the periovulatory period by stopping atresia of the inspired follicle, promoting follicle modification and oocyte maturation (Stouffer, 2003). Number of local factors also plays a part during release of the oocyte in ovulatory period.

Luteal Phase

After ovulation luteinization of granulosa cells occurs within a few hours. Follicular cell develops to produce progesterone structure (corpus luteum) during luteinization, LH primarily control the production of the corpus luteum. LH receptor synthesis on granulose is induced by FSH. LH is essential for its production and function. FSH alone is not able to maintain normal luteal function (Zelinski-Wooten et al., 1998). As LH binds to LH receptors, granulosa cells lutolysis occurs and progesterone production starts. During this phase no antral follicle formation which indicates that this activity is modulated by FSH (Zeleznik, 2001). After ovulation the gonadotropin hormone decreases and attaines preovulatory level by reduction in pulse frequency due to estradiol and progesterone negative feedback effect on hypothalamus and pituitary gland. This showes that LH release during follicular and luteal stages is inversely related to amplitude and frequency of pulse (Veldhuis and Johnson, 1990). Additionally FSH release is mediated by peptide like inhibin, follistatin and actin feedback loop (Bilezikjian et al., 2006; Messinis, 2006). FSH and LH stimulate the release of inhibin from granulosa cell which in-turn inhibits the FSH release. The activin and follistatin feedback regulation of FSH is not an endocrine but act as local factor. The folliculostellate cells produced pituitary-adenylasecyclase-activating peptide which might also be involved in the FSH and LH synthesis within the pituitary described by Winters and Moore (2007). Peripheral feedback effects of inhibins and activins occur at the level of the pituitary. Luteal phase also called secretory phase starts with the development of corpus luteum from ruptured follicles after ovulation under the influence of LH. The luteal phase is progesterone-dependent and progesterone is the characteristic and determinant hormone of the luteal phase. However, the corpus luteum also secretes estradiol and inhibin A. In human and non-human primate in response to cyclic changes patterns of ovarian steroid hormone,

vascularization and remodeling/maturation of uterine endometrium occurs (Girling and Rogers, 2005) which makes it suitable for the implantation of the blastocyst. The length of luteal phase is relatively invariable (14±2 days) and determined by life-span of the corpus luteum.

Menstrual Phase

If no fertilization occurs in that cycle then ovarian steroid hormone secretion collapses at the termination of luteal stage, leading to vasoconstriction, reduction of endometrium vasculature and menstruation. This process is also influenced by locally produced vasoactive and endothelial growth factors (Chwennazhi and Nayak, 2009), endothelins (Cameron et.al., 1995) and angiotensin (Salamonsen et al., 1999). Moreover endotheline paracrine factors regulate endometrial blood flow (Economos et al., 1992) and menstruation phase starts: The first day of menstrual cycle is the day one of menstrual bleeding with demise of corpus luteum of the previous cycle, plasma levels of progesterone decrease and the endometrium is shed.

The regulatory mechanisms functioning in adult males (regulation of gonadotropin-gonadal axis) are significantly modified in females. In female the circulating estrogens secreted directly from the ovaries serve in lieu of locally converted androgen. In females, hormone secretion follows an approximately 28-days cycle dictated by the growth, differentiation, and apoptosis of ovarian steroidogenic tissues.

The most important endocrine effectors in reproduction are steroid hormones (estrogens and progesterone) which are involved in sexual differentiation, regulation of mood, cognition, neuroprotection, neuronal growth, neuronal differentiation and cardiovascular physiology (Korach, 2000; Gruber et al., 2002; and Labrie, 2003). The stimulatory and inhibitory effect of estradiol on the brain may due to its action as neuroactive steroid hormone, (Zinder and Dar, 1999; Rupprecht and Holsboer, 1999a, b; Rupprecht, 2003), may reduce sadness (Su et al., 1993; Pope and Brower, 2000; Pope et al., 2000; Rupprecht, 2003; Kanayama et al, 2007; Rupprecht et al, 2009). It is also a positive allosteric modulator of receptors of $GABA_A$ (Reddy, 2003; Eser et al., 2006; Schumacher

et al., 2007) and can change the production and release of dopamine under some situations. Wade et al. (1985); Cooke and Naaz (2004), reported anorexigenic action of estrogen in controlling obesity in addition to its critical role to regulate normal reproductive behavior, signifying that estrogen plays two-fold role in feeding and reproduction. Receptors of progesterone and estrogen are concentrated in hypothalamus (Bethea et al., 1996; Shughrue et al., 1997; Kuiper et al., 1998) and the gene expression of orexigenic and anorexigenic neuropeptides including NPY has been affected by progesterone and estrogen (Treiser and Wardlaw, 1992; Sahu et al., 1995; Roy et al., 1999; Titolo et al., 2006). In female for the cyclic ovulatory cycle and homeostatic regulation the estrogen control on hypothalamic–pituitary gonadal hormone axis is necessary. A few years before estrogen effect GnRH gene expression reported by Yewade et al. (2009). They demonstrated that decrease in ERα and ERβ mRNA levels in cell line of GnRH neurons cell lines (GN11 and GT1-7) by estradiol was dependent upon dose. In GN11 cells GnRH gene expression was inhibited by both 4,4',4''-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT) and 2,3-bis(4-Hydroxyphenyl)-propionitrile (DPN) specific agonist of ERα and ERβ respectively but in GT1-7 cells GnRH gene expression is only inhibited by DPN consistent with their undetectable levels of ERα expression. These studies also demonstrated that increase in progesterone receptors (PR) is mediated by estradiol in both cell lines. Data describes that the PR gene expression controlled by the dual effect of estradiol was both inhibitory and stimulatory, and for this regulation in GnRH, functional overlap of ERα and ERβ existed. Shivers et al. (1983) found no ER immunoreactivity in GnRH neurons whereas Butler et al. (1999) and Hrabovszky et al. (2001, 2007) demonstrated in vivo estrogen functional receptors expression on GnRH neurons and Navarro et al. (2003) reported GnRH expression on neuronal cells lines.

Prolactin

The prolactin (PRL) a pituitary hormone was initially identified in late 1920s and so named because it stimulates the growth of mammary tissue and lactogenesis in many

species (Trott et al., 2008). Prolactin (PRL) is an exceptional hormone with significant effects on reproduction and sexual behavior of primate and non-primates (Ben-Jonathan et al., 2008). More than 300 functions of prolactin have been recognized in various species including fish, birds and mammals (Bole-Feysot et al., 1998; Freeman et al., 2000; Grattan and Kokay, 2008). Prolactin is a member of the lactogenic group and during the course of evolution was derived from growth hormone like ancestral gene in fish. It is mainly synthesized and secreted by the lactotrop cells of the anterior lobe of pituitary (Freeman et al., 2000) and also by extra-pituitary sites such as mammary gland, placenta, uterus and T lymphocytes (Ben-Jonathan et al., 1996). Its amino acid sequence is similar to that of growth hormone (GH) and placental lactogen (PL) and belongs to the same PRL/GH/PL protein family. It is now considered that PRL is a cytokine based on both molecular and functional studies. Prolactin is also called luteotrophic hormone because of its role in corpus luteum maintenance and secretion (Morishige et al., 1974). Luteinizing hormone induced steroidogenesis in the granulosa-luteal cells, potentiated by PRL (Richard and Williams, 1976) and it maintains the progesterone production by its inhibitory action on 20-hydroxysteroid dehydrogenase enzyme (Freeman, 1994). PRL in primates and rodents plays an important role being part of FSH and LH (luteotrophic complex) (Richardson et al., 1985). Luteinization of granulosa cell is inhibited by prolactin in humans (Adashi and Resnick, 1987). Evidences showed that prolactin and progesterone synergize to stimulate mammary gland growth beyond their individual effects. Prolactin and progesterone serum levels fluctuate in rodents during different phases of estrus cycle with both prolactin and progesterone increasing during proestrus (Ben- Jonathan et al., 2008). Progesterone peaks at estrus and decreases during diestrus 1 and 2 whereas PRL level declines slightly in estrus period and then dramatically in period of diestrus 1 and 2 (Ben-Jonathan et al., 2008).

Role of prolactin is critical in different aspects of reproduction in many species. In primates prolactin not only plays a crucial role for lactogenesis, it directly affects the menstrual cycle. The patterns of PRL release in women are similar to those explained in female rats. Vekemans et al. (1977) and Suganuma et al. (1988) reported that during

ovulation prolactin surge occurs in women corresponding with the secretory peak of LH like rodents but is weaker in human female than rats (Ben-Jonathan et al., 2008). Furthermore, (L'Hermite and Robn, 1972; Brumstead and Riddick, 1992) reported elevated levels of prolactin during late follicular and luteal phases of the normal menstrual cycle. This elevated level of prolactin might be due to high level of plasma estradiol. However, according to Ben-Jonathan et al. (2008), in humans, estrogens effect in regulation of prolactin release is controversial. Normal ovulation disrupts in high level of prolactin, potentially leading to amenorrhoea and infertility (Bohnet et al., 1976; Kauppila et al., 1982). Demura et al. (1982) and McNeilly et al. (1982) described central effect of prolactin on the hypothalamic-pituitary axis and direct effect on ovarian function. The high concentration of prolactin affects various stages of ovarian physiology including follicular maturation, steroidogenesis, ovulation, luteinization and corpus luteum function. In various species including mice, pigs, rats, and sheep the prolactin is part of the luteotropic complex (Denamur et al., 1973), and in humans during the early stages of luteal formation it may also be luteotrophic (McNatty et al., 1974). Ovulatory processes have been affected by hyperprolactinaemia (Lin et al., 1980; McNeilly, 1993; Yoshimura et al., 1994) and cannot be superseded by increasing concentrations of gonadotrophin (Hamada et al., 1980; Yoshimura et al., 1991).

PRL is unique in nature amongst hormones of pituitary because it is under dual hypothalamic regulation (Neill, 1974; Shin et al., 1987), primarily through inhibitory control. In vivo studies the spontaneous PRL release due to lesion of median eminence or pituitary stalk showed the inhibitory influence of hypothalamus over pituitary PRL release (Neill, 1980; Anderson et al., 1991). In this regard dopamine importance is supported by substantial data (Macleod, 1976; Neill, 1980; Ben–Jonathan, 1980 and 1992). Dopamine is secreted from Hypothalamus into hypophyseal portal blood inhibiting the secretion of prolactin by affecting D2 dopamine receptors which are present on the surface of lactotropes (Goldsmith et al., 1979; Holzbanct and Racke, 1985). The blockers of Dopamine receptor such as haloperidol (Langer et al., 1978) and domperidone (Carter et al., 1982) stimulated the pituitary PRL secretion.

Dopamine is a PRL-inhibiting factor. Tuberoinfundibular dopaminergic neurons (TIDA) produce dopamine. Tuberohypophyseal dopaminergic neurons (THDA) located in hypothalamus arcuate nucleus (Arbogast et al., 1989) also play a part in dopamine release. Dopamine performs its effect through its receptor D2, coupled with $G_{\alpha/i}$ to stop adenylyl cyclase activity. In addition dopamine D2 receptors act by decreasing intracellular concentration of Ca^{++} and activating K^{+}-channels. In addition prolactin release is also affected by metabolism of catecholamine (Arimura et al., 1972). However in median eminence and anterior pituitary stalk blood, greater concentration of dopamine suggested that the major physiological hypothalamic prolactin inhibitory factor (PIF) is dopamine (Plotsky et al., 1978), hyperprolactinism is resulted by pituitary stalk disruption. PRL injections (Advis et al., 1981) or hyperprolactinemia induced by in vivo administration of dopaminergic blockers have been found to induce precocious puberty.

In prolactin release ovarian steroid hormones also play an important role. During reproductive ages, in women PRL level is more than in men, this being due to circulating level of estrogen which is an ovarian hormone. Prolactin release is affected by estrogen in two ways, by direct stimulation of prolactin gene expression on lactotrophs of pituitary (Shull and Gorski, 1984; Shull and Gorski, 1989), or by inhibiting the dopamine turnover by inhibiting activity of TIDA. Estrogen may increase PRL release by decreasing D_2 receptor expression on pituitary. Androgens (Tong et al., 1989) and Progesterone (Cho et al., 1993) inhibit prolactin gene expression induced by estrogen. Estrogen increases the PRL release by stimulating the proliferation of lactotrophs of anterior pituitary and increasing PRL-mRNA expression (Scarbrough et al., 1991). Moreover, prolactin release is stimulated through a constitutive pathway by estradiol since voltage-sensitive calcium channels blocker nifedipine, required for release, does not interfere in the enhancement of prolactin mRNA induced by estradiol.

In addition to this, there are a number of neuropeptides which participate in controlling of prolactin release which includes, opioids, somatostatin, vasopressin, prolactin releasing peptide, oxytocin, thyroid releasing hormone (TRH) and neuropeptide Y. Beside this angiotensin II (AII) is in high content in the median eminence and hypothalamus

(Brownfield et al., 1982). AII receptors are widely distributed in hypothalamus and pituitary (Hauger et al., 1982) and have control over pituitary hormones. Angiotensin II has dual control on prolactin release (Steele, 1992).

Role of NPY in prolactin secretion

NPY plays an obligatory role in LH release but for PRL regulation its role is less clear. In the mediobasal part of hypothalamus, NPY expression during lactation increased (Smith, 1983; Ciofi et al., 1991). Neuropeptide Y stimulates prolactin release from cultured pituitary cells, obtained from random cycling rat reported Chabot et al. (1988) and from dispersed estrogen primed cultured pituitary cells (Hill et al., 2004). On the other hand, NPY decreases PRL release in dose dependent manner from estradiol-treated ovariectomized animal's primary cultures of pituitary cells. The NPY inhibitory effect was additive to inhibit the prolactin release stimulated by dopamine and showed similar inhibitory effect at withdrawal of dopamine (Wang et al., 1996). The anatomical arrangement of neuropeptide Y in the median eminence in peri-portal nerve terminals and in TIDA neurons indicates that prolactin release may be inhibited by modulating dopamine secretion presynaptically by the NPY and/or by affecting dopamine's action in the pituitary (Wang et al., 1996).

The NPY intra cerebroventricular administration in male rat, decreases prolactin release (Rettori et al., 1990). The NPY central inhibitory effect for prolactin release may be due to increased activity of dopaminergic neurons in TIDA mediated by neuropeptide Y after its central administration (Fuxe et al., 1989). Moreover, electron microscopic examination reveals synaptic connections at neuroanatomical level, between neuropeptide Y-positive fibers and tyrosine hyroxylase (TH) positive cells (Guy and Pelletier, 1988). These observations suggested the central action of NPY potential to regulate the release of PRL. The NPY inhibitory action may depend upon sex. Central administration of Neuropeptide Y antiserum increased plasma prolactin level only in intact male rats (Ghosh et al., 1991).

Growth Hormone

Growth hormone (GH) is another anterior pituitary hormone which plays an obligatory role in mammalian growth, development (Simpson et al., 1944), and reproduction. Sexual maturation and growth are closely linked to somatogenic and gonadotrophic axes. Mainly FSH and LH regulate functions of gonads in gametogenesis. In the process of sexual differentiation, puberty, gonadal maturation, gametogenesis, steroidogenesis and ovulation, growth hormone plays an important role (Zachmann, 1992). Growth hormone regulates the ovarian function by its direct or indirect role through locally produced or systemic insulin- like growth factor IGF-I (GH), (Bartke et al., 1996). Investigators reported that GH plays an important endocrine role related to reproduction. Thus (1) long term changes in peripheral GH levels are associated with alterations in the activity of hypothalamic catecholaminergic neurons which are believed to control the release of gonadotropins and PRL; (2) both GH deficiency and GH excess are associated with abnormalities in gonadotropin levels and feedback control of LH release; (3) in female mice, chronic elevation of plasma GH levels can alter the "basal" PRL release and suppress mating-induced surges of PRL which are necessary for maintenance of luteal function in this species; (4) in males, overexpression of GH can lead to reduced fertility, presumably via inhibitory influences on copulatory behavior; and (5) life-long GH excess dramatically shortens the life span (Bartke, 1996). Growth hormone plays an important role in reproductive function; it modifies steroidogenesis, gametogenesis, gonadal differentiation, LH/FSH release and responsiveness (Zachmann, 1992). The placental and mammary roles for GH have also been proposed by Mol et al. (1996) and Alsat et al. (1997). GH deficiency often associated with delayed/absent puberty and GH administration initiating puberty reflects that GH is usually but not always needed for the timing of sexual maturation. Stimulation of sexual maturation in GH depleted monkeys (Wilson et al., 1989) and GH-deficient children (Darendeliler et al., 1990; Stanhope et al., 1992) by exogenous GH reflected the importance of GH in sexual maturation. Puberty may be accelerated through activation of GnRH pulse generator by growth hormone (Bartke et al., 1999) and/or by potentiating action of androgen (Ilondo et al., 1982). GH

acts on ovary to affect gametogenesis and steroidogenesis, suggested on the basis of in vitro studies. During early folliculogenesis GH may play its role in early follicular development which is independent of FSH, since during early folliculogenesis GH-binding activity peaks in fish ovarian homogenates (Gomez et al., 1998) and in porcine follicles (Quesnel, 1999). In vitro and in vivo studies also suggest that GH arouses growth in small follicles and checks atresia of follicles. Ativin, an ovarian growth factor may also mediate the actions. Since both GH and gonadotrophins are mandatory to prevent atresia of larger follicles (>2 mm), in sheep GH acts in combination with gonadotrophins during late folliculogenesis and luteinization to stimulate later stages of folliculogenesis and luteinization after hypophysectomy (Eckery et al., 1997).

In luteinized human granulosa cells the stimulatory effect of GH on follicle number and size reflects by increased cell proliferation (Ovesen et al., 1994), but this is also suggestive of the suppressive effect of GH on apoptosis (Eisenhauer et al., 1995; Sirotkin and Makarevich, 1999; Danilovich et al., 2000). GH may potentiate LH action to enhance follicular survival and cell proliferation. Reduced LH receptor gene expression and LH sensitivity in rats is associated with GH deficiency and administration of GH corrects both faults (Advis et al., 1981). Ovesen et al. (1994) and Ovesen, (1998), reported that in human luteinized granulosa cells the effect of GH in folliculogenesis is FSH-dependent and IGF-I-independent. In ovulation GH plays a facilitatory but non-essential role. Although GH alone fails to cause ovulation in sheep (Davis et al., 1990), pigs (Gilbertson et al., 1991) or rabbits (Yoshimura et al., 1993), gonadotrophin-induced ovulation in perifused rabbit ovaries is significantly improved by GH co-administration (Yoshimura et al., 1994). Moreover, fertility is reduced but not abolished in GHR-knockout mice (Bartke, 1999) and egg production and fertility are not impaired in GH-resistant sex-linked dwarf chickens (Decuypere et al., 1991). GH may facilitate ovulation by increasing sensitivity to gonadotrophins and by reducing the incidence of apoptosis in preovulatory ovarian follicles. The increased number of corpora lutea and reduced numbers of atretic follicles in the ovaries of mice transgenically expressing GH supports this view (Danilovich et al., 2000). GH also plays a role in fertility. de Boer et al. (1997)

reported that GH-deficient women (in specific proportion) and GH-resistant women have normal menstrual cycles and conceive normally reported by (Menashe et al., 1991; Dor et al., 1992). However, many women with GH-deficiency have menstrual cycles and conceive normally need assisted reproductive technologies, especially to induce ovulation, and other GH-deficient women may have reproductive dysfunctions (de Boer et al., 1997). In vitro studies represent that changes in ovarian steroidogenesis mediated the ovarian actions of GH. In GHR deficient cattle partial progesterone deficiency is responsible for this (Chase et al., 1998). Increase in plasma estradiol-17 levels by exogenous GH in female killifish prevents the hypophysectomy-induced decline in gonadal weight (Singh et al., 1988). Reduction in plasma androgen concentrations in humans by GH administration reported by (Tapanainen et al., 1992), stimulatory action of GH on murine preantral follicle growth blocks by folliculostatin (which binds and inactivates activin) (Liu et al., 1998). Thus GH may be particularly important in the recruitment of follicles and initiation of oocyte growth, possibly by matching nutritional position with the number of growing oocytes.

Manifold neurotransmitter pathways, as well as a multitude of peripheral feedback signals, adjust GH episodic secretion either by acting directly on the anterior pituitary gland and/or by hypothalamic hormones like somatostatin release, ghrelin and GH-releasing hormone (GHRH) (Gahete et al., 2009). The somatotrophic axis consists of GH, insulin like growth factor 1 and 2, GH binding protein, insulin like growth factor binding protein 1 to 6 and cell surface receptor for GH and insulin like growth factors which has major effects on growth, lactation and reproduction (Lucy, 2012). The somatotropic axis can be acted upon by the reproductive axis through positive estradiol effect on GH release and GHRH expression in liver (Colak et al., 2011). Furuta et al. (2001) reported that ghrelin not only control food intake but also regulates the LH through NPY/Y1 system. However in OVX rats, ghrelin increased the growth hormone but suppressed the LH release.

GH release is also affected by a number of neuropeptides, one of which is NPY.

Role of NPY in Growth Hormone Regulation

NPY intracerebroventricular (ICV) infusion stimulated the GH secretion in the sheep (Morrison et al., 2003) and cattle (Thomas et al., 1999). According to Garcia et al. (2004) Leptin-induced GH release in fasted rat was suppressed by NPY administration (Carro et al., 1998). In vitro studies showed GH release increased from estrogen incubated perfused pituitary cell of rat only in the absence of NPY whereas NPY alone or in combination with estrogen has no effect on GH release (Hill et al., 2004).

Studies indicated that in both sexes of rats NPY inhibited the GH release (Harfstrand et al., 1987; Rettori et al., 1990; Catzeflis et al., 1993). NPY intracerebroventricular infusion either in sex steroid primed or steroid nonprime ovariectomized or in intact female and male rat, decreased GH release, and increased GH secretion with antibody to NPY in these animals (rats). NPY-Y1 and NPY-Y2 receptors may be involved to mediate the inhibitory action of NPY on the bases of expression of these receptors in the mediobasal hypothalamus (Suzuki et al., 1996). It is possible that hypothalamic somatostain secretion increased in response to NPY and inhibited the release of GH in rats. The action of NPY may be in a little part direct on the pituitary gland anterior portion (Rettori et al., 1990). Adams et al. (1987) detected that NPY reduced GH release, human somatotroph tumor secretion in vitro and in rat reduces the proliferation of somatotroph cells probably by using mechanisms depending on gonadotroph-paracrine factors (Tilemans et al., 1992). A sole clinical experiment showed that NPY stimulated the release of GH from 60% patients of prolactinomas (Watanobe and Tamura, 1996). The vitro studies indicated stimulatory role of NPY on GH release from pituitary of goldfish (Peng et al., 1990). Thus, the multitude of indications favor the NPY inhibitory effect on GH axis in rats which could be specie specific e.g., it stimulates the secretion of GH in humans whereas inhibits its release in rats (Tilemans et al., 1992; Chan et al., 1996). Due to anatomical distribution of NPY neurons of the arcuate region and expression of GH receptor and c-fos gene in these neurons Kamegai et al. (1996) suggested that NPY has stimulatory effect on somatostatin secretion and inhibit the GHRH to suppress the release of GH (Kamegai et al., 1994) and enhances in

hypophysectomized rats the expression of NPY mRNA in the hypothalamus (Chan et al., 1996). Another definitive role of NPY came forward in a study where restrictive feeding of sheep initiated GH release whereas inhibited the GH release in rats in a similar situation.

Aims and objectives

During the primate menstrual cycle, noticeable variations occur in the steroidal milieu, which offers an interesting model for observing the role of NPY and steroidal communications in governing pituitary hormone secretion. In the current inquiry efforts were made to study endocrine actions that occur during the menstrual cycle of the adult rhesus monkey to understand the physiological changes that occur in the reproductive steroids and some features of the regulation of PRL and GH from the pituitary gland.

The objective of this study was to investigate the effect of NPY on plasma estradiol, progesterone, prolactin and growth hormone especially during different phases of the menstrual cycle of adult female rhesus monkey. In addition to this baseline profile of all these hormones was also studied for comparison and cycle status. Rhesus monkey has menstrual cycle that is similar to human menstrual cycle in both length and hormonal fluctuation. Data obtained from rhesus monkey will aid as a prototypical model for judging the effects of NPY throughout the menstrual cycle in humans.

MATERIALS AND METHODS

Materials and Methods

Experimental Animal (Monkey)

Nine adult female monkeys (*Macaca mulatta*), 5.5-9 years old, weighing 7.5-12.5 kilogram (kg) were obtained from a commercial supplier. The animals were housed in individual cages, under standard colony conditions in the Department's primate facility. The animals were fed daily with fresh fruits (0900-0930 h), hard boiled eggs at 1100 h, bread at 1300-1330 h and nuts or fruits in the evening 1700 h. Water was available *ad libitum*. Appetite was monitored for a month prior to the beginning of the experiments. All animals consumed their feed in 10-20 min. The animals were given diet according to their body weights (BW). Animals were maintained under a controlled set of conditions spanning light 12 hour dark 12 hour (12h:12h), photoperiod (lights on, 0700 am–1900 pm) in winter and summer (automatic electricity adjustment circuit). Constant temperature of 20°C-25°C was maintained. The change in body weight was noted daily between 0900 am to 1000 am. Animals were also given multiple vitamins. All monkeys were acclimatized to the animal house for at least one year before these studies began. The health of the monkeys was routinely monitored by a veterinarian and the research personal.

Physiological Measures

The menstrual cycle of each monkey was monitored daily by recording the onset and duration of menstrual bleeding with vaginal swabs (2a). Sex skin color change was also observed throughout the menstrual cycle. Color changes were observed at the perennial, basal of the tail, on caudal aspect of abdomen and hind legs. Daily photographs were taken between 0900 am to 1000 am. Coloration was objectively quantified using general system of grading from whitish pink (0), pink (2), pinkish red (3), red (4) and deep red/saturated red (5) referring to score of color change.

Pharmacologic Agents

The following drugs were used in the present study:

1. Human (h) NPY (American Peptide Laboratories USA).
2. Ketamine hydrochloride (Ketavet; Park-Davis, Berlin, FRG)
3. Heprin sodium, (Lot No. 1021, Huonsco. Ltd. 907-6 Sangshin-ri Hyangnammy, Hwaseong – city Kyunggi-do Korea).
4. Normal saline (0.9%NaCl) (Saline, Otsuka Pakistan Ltd., F/4-9, H.I.T.E, Hub, Balochistan, Pakistan.)
5. Plasma samples were analyzed using ELISA kits (Aumgnix Inc.) for the determination of estradiol, progesterone, prolactin and growth hormone.

Access to venous circulation

Venous catheterization

To permit sequential withdrawal of blood samples and i.v. administration of NPY, the animals were sedated with ketamine hydrochloride (Ketler, Astarapin, Germany 10 mg/kg BW *i.m.*) and a teflon cannula (Vasocan Branule, 0.8 mm/22 G O.D, B. Braun Melsungen AG, Belgium) was inserted in the saphenous vein of leg. The distal end of the cannula was attached to a syringe via a butterfly tube (length 300 mm, volume 0.29 ml, 20 GX3/4", JMS Singapore). Experiments were not initiated until the animals had fully recovered from the sedation. During the experiments the chair-restrained animals were isolated and kept in quiet laboratory of primate facility. All experiments were approved by the Departmental Committee for Care and Use of Animals.

Chair restraining method

To reduce the effects of stress on blood sampling, animals were habituated to chair restraint several weeks prior to commencement of the experiments. The duration of restraint was gradually increased until a daily period of 3 h was attained. The animals were sedated with ketamine hydrochloride (Ketler, Astarapin, Germany 5 mg/kg BW, i.m.) for placement in and removal from the restraining chair.

Sample collection and NPY administration

Bleeding

Blood samples (2 ml) for baseline profile of estradiol, progesterone, prolactin and growth hormone were taken from saphenous vein of leg of monkeys in heparinized syringes at different days of menstrual cycle (Fig. 2b).

Sequential blood samples (2 ml) were obtained at 15 minutes interval in heparinized syringes by venipuncture of the saphenous vein through a butterfly cannula in one leg. Following withdrawal of each sample an equal volume of heparinized (5 IU/ml) normal saline (0.9% NaCl) was injected into tubing to maintain patency. Immediately after obtaining a baseline blood sample for plasma estradiol, progesterone, growth hormone and prolactin levels for an hour (-60, -45, -30, -15, 0), 200 µg NPY (dissolved in 2 ml of saline) was administered in single bolus intravenous (i.v.) injection. After NPY treatment blood samples were obtained at an interval of 15 minutes for 2 hours and 15 minutes. Immediately before each sample, 0.5 ml of blood was withdrawn from the i.v. line and discarded. Each session lasted approximately 3.15 hours. Blood sampling was conducted between 1300-1700 h, i.e., starting about one and half hour after completion of daily feeding. Over the course of an entire session, a total of 28 ml blood was drawn. Blood samples were centrifuged at 3,000 revolutions per minute (rpm) for 15 min. Plasma was separated and stored at -20 °C until analysis of hormones. Studies of the effects of NPY were conducted during a period of three months (December to February 2011-12).

Experiment protocol

Baseline profile of estradiol, progesterone, prolactin and growth hormone at different days of menstrual cycle

Blood samples were collected from five adult female monkeys (n=5) (Fig. 2b) according to procedure described above for baseline profile on day 1, 2, 4, 7, 10, 13, 14, 15, 16, 19, 21, 22, 25, 28 and 31 of menstrual cycle.

Effect of 200 µg NPY (in single bolus intravenous injection) on plasma estradiol, progesterone, growth hormone and prolactin level during different phases of menstrual cycle in adult female rhesus monkeys

Sequential blood samples were collected from five adult female monkeys (Fig 3a) according to procedure described above, at day 1 (menstrual phase), day 7 (follicular phase), day 15 (periovulatory phase) and day 21 (luteal phase) before and after administering of single dose of 200 µg NPY (human) per animal during sampling of each phase separately (Fig 3b). Experiment was carried on fed animals but no food was provided during the sampling period.

Out of nine monkeys a group monkeys (n=5) with a mean body weight of 10.31±0.90 kg and with menstrual cycle of 31 days was used in this study.

ELISA for the measurement of estradiol (E_2) in blood plasma

Assay Procedure

All the coated wells were dispensed with 25 µl of standards, specimens and controls. Estradiol-HRP, conjugate reagent (100 µl) and 50 µl of rabbit anti-estradiol (E_2) reagent were added to each well and the wells were incubated for 90 minutes at room temperature. After incubation the microwells were washed five times with deionized or distilled water in an automated washer (Platos W96, AMP Diagnostic). In each well 100 µl of TMB (3,3′,5,5′-tetramethylbenzidine) reagent was dispensed and incubated for 20 minutes at 18-25^{o}C. TMB is a chromogen that yields a blue color when oxidized, typically as a result of oxygen radicals produced by the hydrolysis of hydrogen peroxide by HRP. The reaction was stopped by adding a stop solution (5 N HCl) to each well. The color then changes to yellow with the addition of stop solution with maximum

absorbance at 450 nm. The absorbency was read at 450 nm and the results were expressed in pg/ml.

The sensitivity of the estradiol (E_2) assay was 2 pg/ml and intra-assay coefficient of variation was < 7%.

ELISA for the measurement of progesterone (P) in blood plasma

All the coated wells were dispensed with 25 µl of standards, specimens and controls. Progesterone-HRP conjugate reagent (100 µl) and 50 µl of rabbit anti-progesterone reagent were added to each well and incubated for 90 minutes at room temperature. After incubation the micro wells were washed five times with deionized or distilled water in an automated washer (Platos W96, AMP Diagnostic). In each well 100 µl of TMB reagent was dispensed and incubated for 20 minutes at room temperature. The reaction was stopped by adding stop solution to each well. The absorbency was read at 450 nm and the results were expressed in ng/ml.

The sensitivity of the progesterone (P) assay was 0.3 ng/ml and intra-assay coefficient of variation was < 6%.

ELISA for the measurement of prolactin (PRL) hormone in blood plasma

All the coated wells were dispensed with 50µl of standards, specimens and controls. Enzyme conjugate reagent (100 µl) was added to each well and it was incubated for 60 minutes at room temperature. After incubation the micro wells were washed five times with wash buffer in an automated washer (Platos W96, AMP Diagnostic). In each well 100 µl of TMB reagent was dispensed and incubated for 20 minutes at room temperature. The reaction was stopped by adding stop solution to each well. The absorbency was read at 450 nm and the results were expressed in ng/ml within 10 minutes.

The sensitivity of the prolactin (PRL) assay was 2 ng/ml and intra-assay coefficient of variation was < 6%.

ELISA for the measurement of growth hormone (GH) in blood plasma

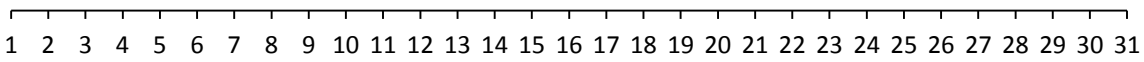
All the coated wells were dispensed with 50 µl of standards, specimens and controls. Enzyme conjugate reagent (100 µl) was added to each well and incubated for 60 minutes at room temperature. After incubation the micro wells were washed five times with wash buffer in an automated washer (Platos W96, AMP Diagnostic). In each well 100 µl of TMB reagent was dispensed and incubated for 20 minutes at room temperature.in dark. The reaction was stopped by adding stop solution to each well. The absorbency was read at 450 nm and the results were expressed in ng/ml.

The sensitivity of the growth hormone (GH) assay was 0.5 ng/ml and intra-assay coefficient of variation was < 8%.

Statistical Analysis

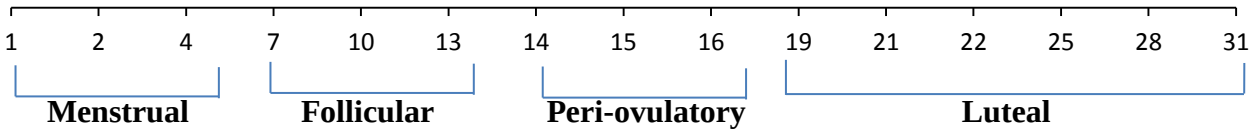
Data are presented in tables and text as mean ± SE for control and treatment phases. For the comparison of base line plasma estradiol, progesterone, prolactin and growth hormone concentration before the treatment, hormone levels were calculated by averaging all the concentrations before the treatment. On the other hand E2, P, PRL and GH responsiveness to NPY was determined by comparing basal levels of the hormones calculated by averaging the concentration immediately before NPY injection at 0 minute and the levels worked out by averaging the concentrations at 15 minutes interval after inducing the drug, Student's t-test was used to determined difference between means of basal and stimulated level of hormones. Linear Regression analysis of variance (ANOVA) was used to find the time dependent trends by evaluating the means from data of particular day of different phases of menstrual cycle in present study. P values are mentioned for the t-test applied. Where analysis of variance was carried out both values for F and P are given. Results were considered statistically significant at $P<0.05$. Statistical analyses were performed with Prism 5 (Graph Pad Software, San Diego, CA).

Protocol for baseline profile of hormones



Menstrual cycle (days)

Fig 2a: Average length of menstrual cycle of rhesus monkey.

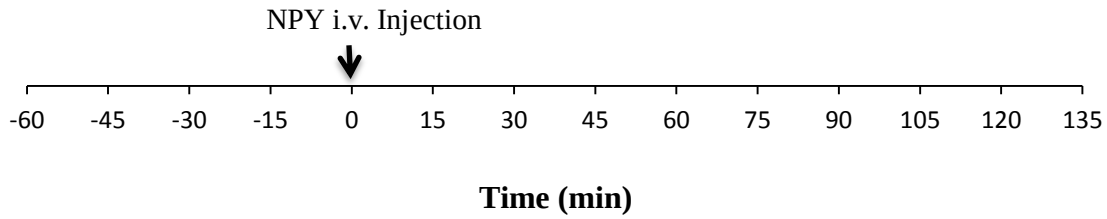


Menstrual Cycle (Phases)

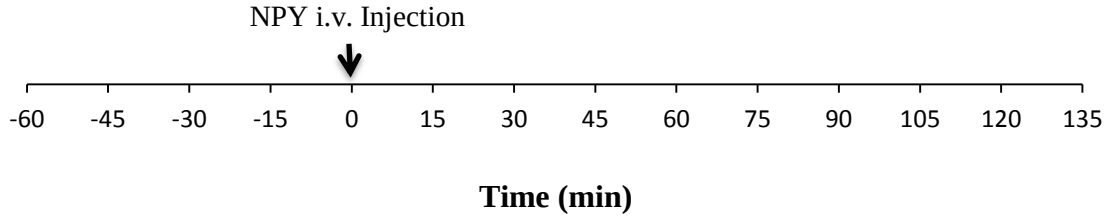
Fig 2b: Protocol showing the days of blood sample collection for baseline profile of estradiol, progesterone, prolactin and growth hormone.

Protocol for sequential blood sampling

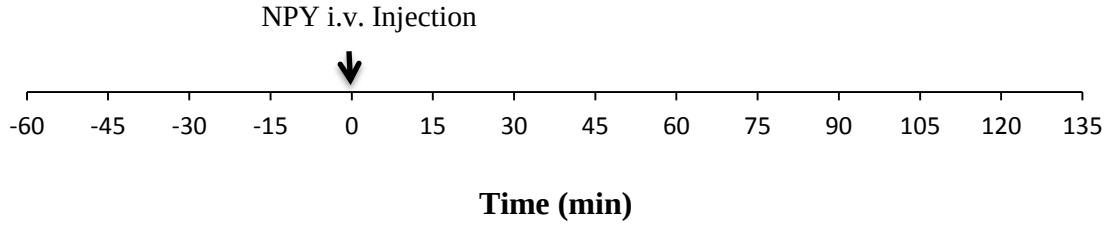
Day 1 (Menstrual phase)



Day 7 (Follicular phase)



Day 15 (Periovulatory Phase)



Day 21 (Luteal Phase)

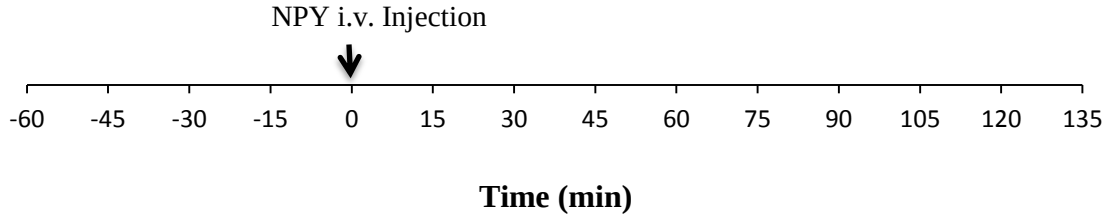


Fig 3: Protocol of sequential blood sampling before and after Neuropeptide single bolus intravenous injection during different phases of menstrual cycle.

RESULTS

Results

Present study was conducted to observe the possible role of neuropeptide Y (NPY) on hormones during different phases of menstrual cycle in adult female rhesus monkey. The changes on plasma estradiol, progesterone, prolactin (PRL) and growth hormone (GH) levels were recorded on day 1 (menstrual phase), day 7 (follicular phase), day 15 (periovulatory phase) and day 21 (luteal phase) of menstrual cycle before and after 200 µg NPY single bolus intravenous injection. Besides this change in body weight, behavior, sex skin colour and baseline profile of ovarian steroid (estradiol and progesterone) and pituitary hormones (GH and PRL) were recorded throughout the menstrual cycle of 31 days

Body Weight

Mean body weight taken during the menstrual cycle of all the five (5) adult female rhesus monkeys (*Macaca mulatta*) used in this study is represented in Table 1 and Fig. 4. The changes in the body weight of the monkeys during the menstrual cycle were not significantly different. Individual and mean body weight during menstrual (day 1-4), and Follicular (day 7-13), ovulatory (day 14-16) and luteal phase (day 19-31) of menstrual cycle were also non-significantly different (Table 2).

Behavioral Response

All the monkeys showed similar type of behavioral reaction after the administration of neuropeptide Y (NPY). NPY administration caused relaxation in the monkeys and flushing the face of two of the monkeys. All the monkeys were comfortable.

Changes in sex skin colour

Perineal sex-skin swelling and sex-skin color changes in the adult female monkey were observed and correlated with menstrual cycle phase (Fig 5a and 5b). Sex skin colour intensity increased with increase in plasma estradiol concentration. At first day of menstrual cycle the skin colour was whitish pink scored as (0), (Then color changed to pink (1) on day 2, pinkish red (2) on day 4, red (3) on day 7 and day 10. Then the color changed to deep red (4) on day 13, saturated red (5) from day 14 to 15 and again changed

to deep red on day 16, red on day 19, then pinkish red from day 21 to 22, pink from day 25 to 28 and whitish pink on last day of the cycle. The concentration of coloration was also observed over the pubis, buttock, perineum, lower back and legs. The sex skin colour was invariably accompanied by swelling of the vulva and the perineal skin.

Estradiol

Plasma estradiol (E_2) concentrations in female monkeys during the menstrual cycle

Baseline profile of estradiol during menstrual cycle

Mean basal plasma estradiol (E_2) profile in the female monkey during the menstrual cycle is shown in Table 3 and Fig. 6. Mean plasma E_2 concentration was low at the beginning of the cycle during menstrual phase (day 1-4) and then increased progressively and significantly during follicular phase (day 7-13) compared to menstrual phase ($t_{(8)}$ =5.87, P=.0003). The increase in E_2 concentration continued during periovulatory phase (day 14-16) and highest increase was observed on day 14 (periovulatory phase) of the menstrual cycle (68.62 ± 8.43) (pg/ml). The plasma E_2 concentration was significantly high during periovulatory phase compared to menstrual ($t_{(8)}$ =5.14, P=.0008), follicular ($t_{(8)}$ =2.78, P=.02) and luteal phase (day 19-31) ($t_{(8)}$ =4.41, P=.002) of menstrual cycle. The increase in plasma estradiol concentration was also significantly high in follicular phase compared to luteal phase ($t_{(8)}$ =5.19, P=.003), whereas there was no significantly difference in plasma E_2 concentration between menstrual and luteal phase (P=>0.05) (Table 3a).

Table 1: Individual and mean body weight (kg) of adult female rhesus monkey on different days of the Menstrual cycle.

	Body Weight (kg)					
Menstrual cycle	**Monkeys #**					
Days	**1**	**2**	**3**	**4**	**5**	**Mean ± SE**
1	9.56	12.50	10.00	12.00	7.50	10.31 ± 0.90
4	9.57	12.50	10.10	12.00	7.50	10.33 ± 0.90
7	9.60	12.50	10.10	12.20	7.50	10.38 ± 0.92
10	9.64	12.60	10.30	12.30	7.60	10.49 ± 0.92
13	9.65	12.88	10.30	12.50	7.80	10.63 ± 0.94
14	9.65	13.50	10.33	13.00	7.90	10.65 ± 1.00
15	9.66	13.50	10.35	13.00	8.00	10.89 ± 1.04
16	9.82	13.95	10.31	13.20	8.20	10.89 ± 1.04
19	9.80	13.50	10.30	13.00	8.00	10.92 ± 1.03
21	9.75	12.90	10.30	13.00	7.80	10.75 ± 0.99
22	9.70	12.90	10.30	13.00	7.70	10.72 ± 1.01
25	9.69	12.90	10.50	12.80	7.60	10.70 ± 1.00
28	9.68	12.90	10.30	12.50	7.60	10.60 ± 0.97
31	9.50	12.50	10.20	12.10	7.60	10.38 ± 0.89

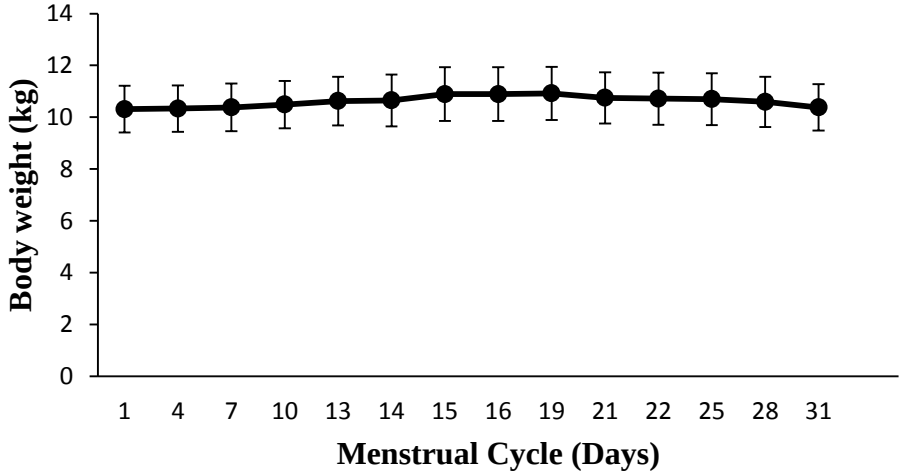


Fig. 4: Mean body weight (kg) of adult female rhesus monkey on different days of the menstrual cycle.

Table 2: Individual and mean body weight (kg) of adult female rhesus monkey during different phases of the menstrual cycle.

	Body Weight (kg)			
Monkey #	**Menstrual phase (Day 1-4)**	**Follicular phase (Day 7-13)**	**Periovulatory phase (Day 14-16)**	**Luteal phase (Day 19-31)**
1	9.58 ± 0.01	9.65 ± 0.00	9.71 ± 0.04	9.69 ± 0.04
2	12.50 ± 0.00	12.99 ± 0.22	13.65 ± 0.12	12.93 ± 0.08
3	10.07 ± 0.03	10.31 ± 0.01	10.33 ± 0.01	10.32 ± 0.05
4	12.07 ± 0.05	12.60 ± 0.17	13.07 ± 0.05	12.73 ± 0.13
5	7.50 ± 0.00	7.77 ± 0.07	8.03 ± 0.07	7.72 ± 0.02
Mean±SE	10.34 ± 0.02	10.66 ± 0.09	10.96 ± 0.06	10.68 ± 0.07

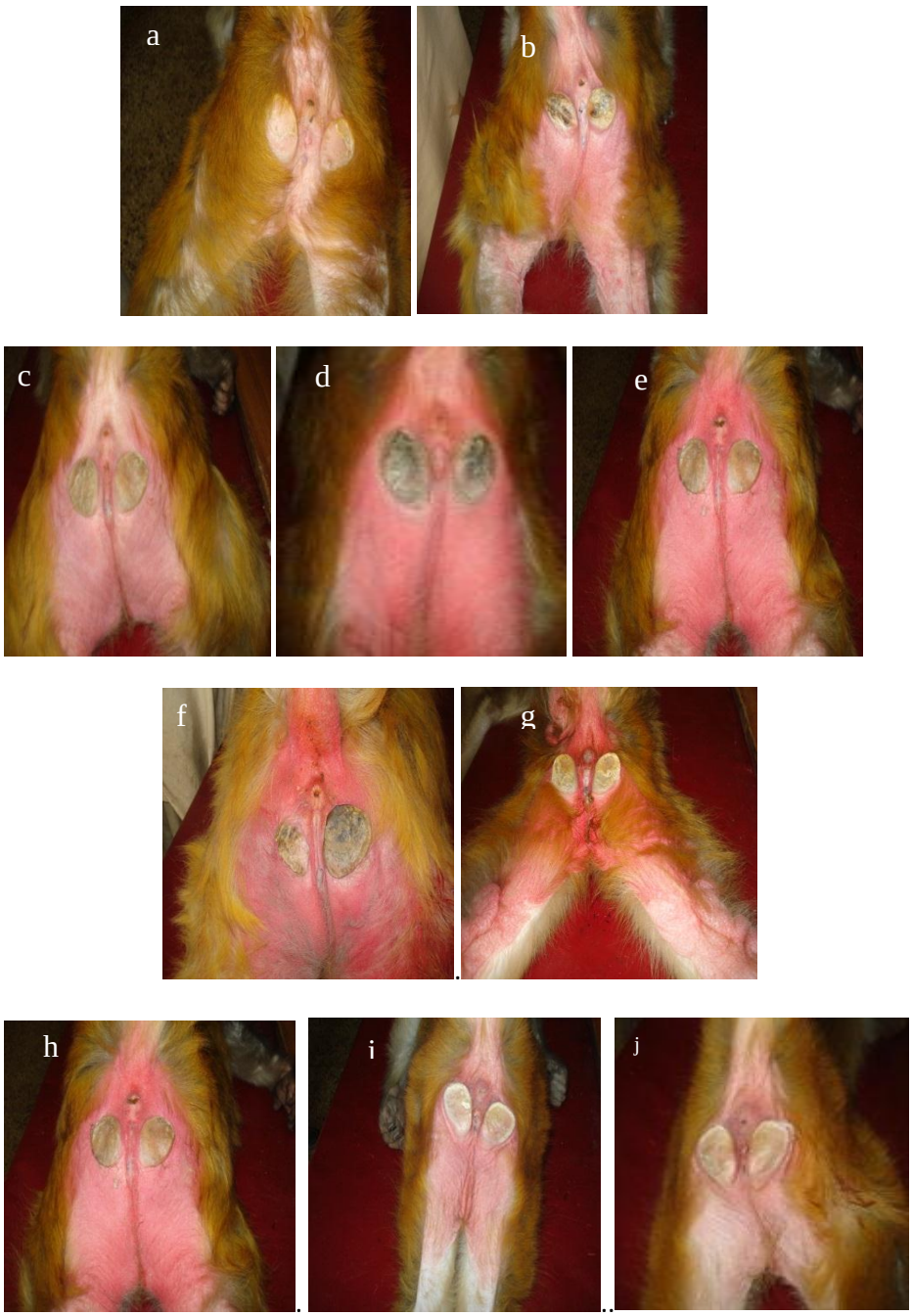


Fig. 5a: Perineal sex skin color changes (including caudal region, rump and hind limbs) in adult rhesus monkey during the menstrual cycle. a-b, (menstrual phase) colour changed from light pink to pink, c-e, (follicular phase) from pink to red, f-g, (periovulatory phase) saturated red and h-j, (luteal phase) from bright pinkish red to whitish pink.

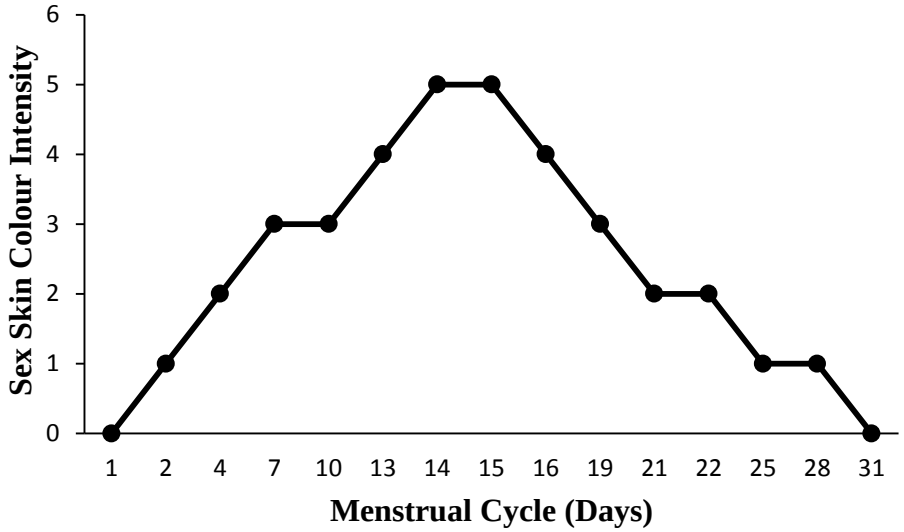


Fig. 5b: Change in Sex skin color in adult female rhesus monkey on different days of menstrual cycle.
Colour score: Whitish pink (0), pink (1), pinkish red (2), red (3), deep red (4) and saturated red (5).

Table 3: Individual and mean baseline profile of plasma estradiol concentration (pg/ml) on different days of the menstrual cycle in adult female rhesus monkey.

Plasma Estradiol Concentration (pg/ml)						
Menstrual cycle	**Monkeys #**					
Days	**1**	**2**	**3**	**4**	**5**	**Mean ± SE**
1	20.64	30.38	25.30	23.60	25.98	25.18 ± 1.59
2	22.90	35.15	20.72	20.70	22.37	24.37 ± 2.73
4	23.31	38.48	20.16	23.88	28.21	26.81 ± 3.19
7	25.97	44.99	32.01	30.52	32.00	33.10 ± 3.17
10	35.74	46.86	45.05	43.33	41.77	42.55 ± 1.90
13	46.11	48.01	56.78	59.32	62.31	54.51 ± 3.18
14	48.79	99.22	59.33	69.35	66.41	68.62 ± 8.43
15	50.91	90.50	55.70	57.77	63.72	63.72 ± 7.00
16	46.40	79.75	50.93	55.09	60.54	58.54 ± 5.79
19	39.19	54.75	42.48	42.96	51.60	46.20 ± 2.96
21	26.03	41.83	30.02	31.27	39.86	33.80 ± 3.02
22	29.21	39.83	24.15	25.75	30.24	29.84 ± 2.73
25	24.75	34.25	20.85	23.90	27.76	26.30 ± 2.27
28	22.77	32.24	24.90	22.08	26.75	25.75 ± 1.82
31	21.12	28.73	23.79	22.94	26.65	24.65 ± 1.36

Table 3a: Mean Plasma estradiol concentration (pg/ml) during different phases of menstrual cycle in adult female rhesus monkey.

Plasma Estradiol concentration (pg/ml)			
Menstrual Phase (1-4 Days)	**Folicular Phase (7-13 Days)**	**Periovulatory Phase (14-16 Days)**	**Luteal Phase (19-31 Days)**
25.45 ± 2.39	43.38 ± 1.90	63.63 ± 7.02	31.09 ± 2.23

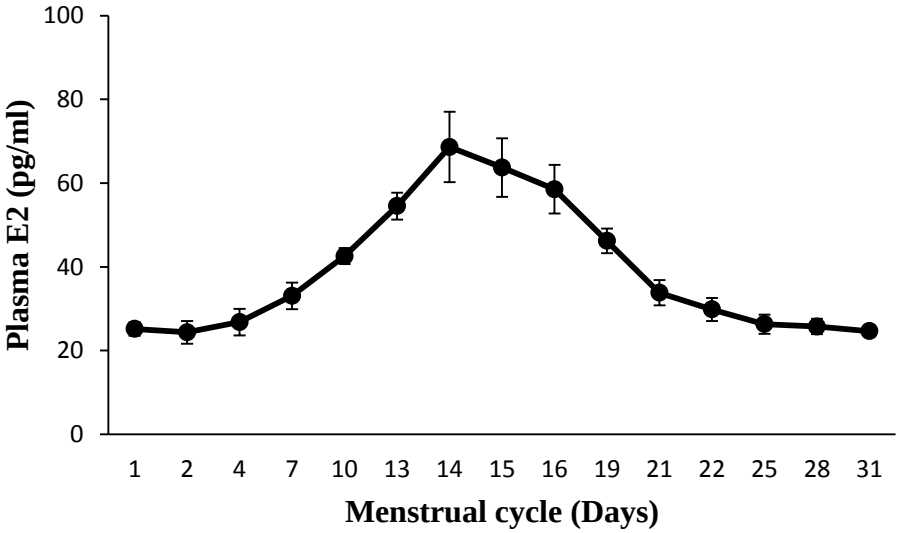


Fig. 6: Baseline profile of plasma estradiol concentration (pg/ml) in female rhesus monkey on different days of the menstrual cycle.

Table 4: Mean and Individual plasma estradiol concentration before and after NPY i.v. injection in female rhesus monkey on day 1 (menstrual phase) of menstrual cycle.

Plasma Estradiol Concentration (pg/ml)						
	Monkeys					
Time (min)	1	2	3	4	5	Mean ± SE
-60	20.10	30.18	25.50	23.30	24.98	24.81 ± 1.64
-45	20.86	31.69	24.45	23.10	25.18	25.06 ± 1.81
-30	21.30	31.22	25.15	23.39	24.93	25.20 ± 1.66
-15	20.31	31.91	25.32	22.42	24.88	24.97 ± 1.96
NPY ⟶ 0	20.00	31.02	25.09	23.03	24.97	24.82 ± 1.80
15	25.70	46.34	31.86	27.01	28.55	31.89 ± 3.76
30	28.70	65.42	32.55	28.59	30.66	37.18 ± 7.10
45	24.50	71.19	28.03	27.45	28.20	35.87 ± 8.85
60	23.28	73.30	26.52	25.13	26.76	35.00 ± 9.60
75	22.57	78.39	25.31	23.36	24.97	34.92 ± 10.88
90	22.60	64.67	24.20	22.59	25.49	31.91 ± 8.21
105	21.99	43.41	25.00	23.58	25.43	27.88 ± 3.93
120	21.93	34.99	25.93	24.25	24.22	26.26 ± 2.27
135	20.82	31.90	25.39	22.98	25.34	25.29 ± 1.86

Effect of single bolus injection of 200 µg of NPY on plasma estradiol concentration in female rhesus monkey during different phases of menstrual cycle

Day 1 (Menstrual Phase) of menstrual cycle

The individual and mean plasma estradiol (E_2) concentration (pg/ml) before and after NPY bolus administration in five female monkeys on day 1 (menstrual phase) of menstrual cycle is given in Table 4. Mean plasma estradiol concentration was recorded for one hour with an interval of 15 minutes before administration of a single bolus intravenous (i.v.) injection of NPY. Plasma estradiol concentration remained more or less similar up to 0 minute (within an hour). Regression analysis of variance showed that mean plasma E_2 concentration was not different significantly during pre-treatment period (b=-0.006±0.059; F (1, 3)=0.012; P=0.921), (Table 4a and Fig. 7a).

At 0 minute, a single bolus i.v. injection of NPY was given and plasma estradiol concentration was recorded after every fifteen minutes for a period of two hours and fifteen minutes. A non-significant increase in plasma estradiol concentration was observed after 15 minutes of NPY bolus injection compared to that at 0 minute ($t_{(8)}$ =1.697; P=0.128). This increase in estradiol concentration continued and it reached its maximum at 30 minutes (37.18±7.10) (pg/ml). Regression analysis of variance showed temporal significant increase in mean plasma estradiol concentration from 0 minute to 30 minutes time (b= 0.6.180±0.512; F(1,1)=145.35; P=0.050), (Table 4b and Fig. 7b). After 30 minutes decrease in plasma E_2 concentration started and more or less plateau was observed from 45 minutes to 75 minutes which ranged between 35.87±8.85 to 34.92±10.88 (pg/ml). A sharp decrease in plasma E_2 concentration was observed from 90 minutes to 135 minutes. Regression analysis of variance showed significant temporal decrease in mean plasma estradiol concentration from 30 minute to 135 minutes time (b=-1.853±0.196; F(1,6)=89.332; P=0.0001), (Table 4c and Fig. 7c).

Table 4a: Regression analysis of variance regarding plasma estradiol concentration (pg/ml) on day 1 (menstrual phase) of menstrual cycle from -60 minute to 0 minutes time in female monkey before single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*p*
Regression	1	0.000	0.0004	0.012	0.921
Residual	3	0.106	0.035		
Total	4	0.106		b=-0.006±0.059	

Table 4b: Regression analysis of variance regarding plasma estradiol concentration (pg/ml) on day 1 (menstrual phase) of menstrual cycle from 0 minute to 30 minutes time female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	76.401	76.401	145.353	0.050
Residual	1	0.526	0.526		
Total	2	76.926		b=6.180±0.512	

Table 4c: Regression analysis of variance regarding plasma estradiol concentration (pg/ml) on day 1 (menstrual phase) of menstrual cycle from 30 minute to 135 minutes time female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	144.291	144.291	89.332	0.0001
Residual	6	9.691	1.615		
Total	7	153.982		b=-1.853±0.196	

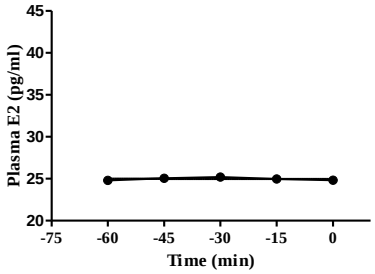


Fig 7a: Regression analysis of variance showed no change in plasma estradiol concentration (pg/ml) on day 1 (menstrual phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

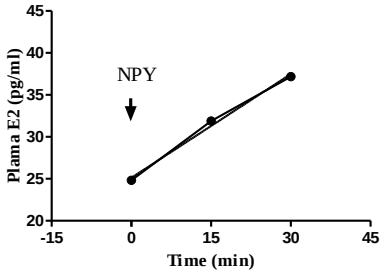


Fig 7b: Regression analysis of variance showed non-significant increase in plasma estradiol concentration (pg/ml) on day 1 (menstrual phase) from 0 minute to 30 minutes in female monkey after single bolus i.v. injection of NPY.

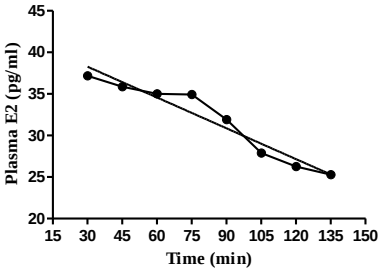


Fig 7c: Regression analysis of variance showed highly significant decrease in plasma estradiol concentration (pg/ml) on day 1 (menstrual phase) from 30 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Day 7 (Follicular Phase) of menstrual cycle

The individual and mean plasma estradiol (E_2) concentration before and after NPY administration in female monkey on day 7 (follicular phase) of menstrual cycle is given in Table 5. Mean plasma estradiol concentration was recorded for one hour with an interval of 15 minutes before administration of NPY single bolus i.v. injection. No significant difference in plasma estradiol concentration was observed from -60 minutes to 0 minutes. Regression analysis of variance showed that mean plasma E_2 concentration was not significantly different during pretreatment period with NPY (b=-0.007±0.059; F(1,3)=0.018; P=0.901), (Table 5a and Fig. 8a).

At 0 minute, a single bolus i.v. injection of NPY was given and plasma estradiol concentration was recorded after every fifteen minutes for a period of two hours and fifteen minutes. A non-significant increase in plasma estradiol concentration 37.01 ±4.03 (pg/ml) ($t_{(8)}$=0.9058; P=0.3915) was observed after 15 minutes of treatment compared to that of at 0 minute. The increase in plasma estradiol concentration continued and it reached its maximum at 60 minutes (43.70±7.24) (pg/ml). Regression analysis of variance showed significant increase in mean plasma estradiol concentration from 0 minute to 60 minutes (b= 2.922±0.4440; F(1, 3)=43.20; P=0.007), (Table 5b,and Fig. 8b. Then gradual decrease was recorded after 60 minutes onwards till the end of the experiment and reached 32.94±3.41 (pg/ml) which was more or less equal to plasma estradiol concentration at 0 minute. Regression analysis of variance showed significant decrease in mean plasma estradiol concentration from 60 minute to 135 minutes (b=-2.061±0.253; F(1,4)=66.356; P=0.001), (Table 5c and Fig. 8c).

Day 15 (Peri-ovulatory Phase) of menstrual cycle

The individual and mean plasma estradiol concentration (pg/ml) before and after NPY administration in five female monkeys on day 15 is shown in Table 6. Mean plasma estradiol level was recorded for one hour with an interval of 15 minutes before the administration of NPY single bolus i.v. injection. No significant difference in plasma estradiol concentration was observed from -60 minutes to 0 minutes. Regression analysis

Table 5: Individual and mean Plasma estradiol concentrations (pg/ml) before and after NPY i.v. injection in female rhesus monkey on day 7 (follicular phase) of menstrual cycle.

Plasma Estradiol Concentration (pg/ml)						
	Monkeys					
Time (min)	1	2	3	4	5	Mean ± SE
-60	24.45	45.04	31.36	29.79	30.79	32.29 ± 3.42
-45	24.42	44.34	32.57	29.42	29.75	32.10 ± 3.33
-30	24.87	43.83	32.85	30.28	30.26	32.42 ± 3.13
-15	24.94	44.19	31.31	29.77	29.85	32.01 ± 3.23
NPY → 0	24.97	44.76	31.65	30.28	29.79	32.29 ± 3.31
15	28.24	52.19	35.22	33.45	35.97	37.01 ± 4.03
30	30.72	52.90	37.87	30.51	48.53	40.10 ± 4.58
45	33.00	54.20	39.43	34.72	55.74	43.42 ± 4.84
60	30.40	51.60	37.87	30.45	68.17	43.70 ± 7.24
75	28.74	51.84	37.36	31.70	45.92	39.11 ± 4.33
90	29.79	51.94	35.55	28.89	43.10	37.85 ± 4.34
105	29.28	51.93	33.83	28.65	37.47	36.23 ± 4.24
120	26.26	48.18	31.65	28.44	33.12	33.53 ± 3.85
135	25.21	45.63	31.24	29.95	32.67	32.94 ± 3.41

Table 5a: Regression analysis of variance regarding plasma estradiol concentration on day 7 (follicular phase) of menstrual cycle from -60 minute to 0 minutes time in female monkey before single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.001	0.001	0.018	0.901
Residual	3	0.105	0.035		
Total	4	0.105		b=-0.007±0.059	

Table 5b: Regression analysis of variance regarding plasma estradiol concentration on day 7 (follicular phase) of menstrual cycle from 0 minute to 60 minutes time in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	85.413	85.413	43.199	0.007
Residual	3	5.932	1.977		
Total	4	91.345		b=2.922±0.444	

Table 5c: Regression analysis of variance regarding plasma estradiol concentration on day 7 (follicular phase) of menstrual cycle from 60 minutes to 135 minutes time female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	74.382	74.382	66.356	0.001
Residual	4	4.484	1.121		
Total	5	78.866		b=-2.061±0.253	

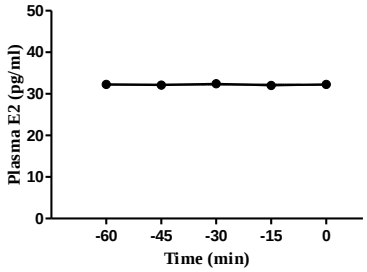


Fig. 8a: Regression analysis of variance showed no change in plasma estradiol concentration (pg/ml) on day 7 (follicular phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

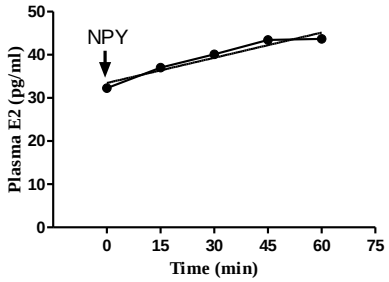


Fig. 8b: Regression analysis of variance showed highly significant increase in plasma estradiol concentration (pg/ml) on day 7 (follicular phase) from 0 minute to 60 minutes in female monkey after single bolus i.v. injection of NPY.

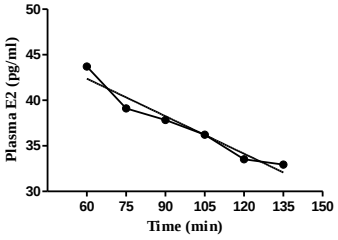


Fig. 8c: Regression analysis of variance showed highly significant decrease in plasma estradiol concentration (pg/ml) on day 7 (follicular phase) from 60 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY

Table 6: Individual and mean plasma estradiol concentration before and after NPY i.v. injection in female rhesus monkey on day 15 (periovulatory phase) of menstrual cycle.

Plasma Estradiol Concentration (pg/ml)						
	Monkeys					
Time (min)	1	2	3	4	5	Mean ± SE
-60	50.22	89.76	53.90	57.87	62.28	62.81 ± 7.03
-45	51.45	89.07	54.26	58.34	63.29	63.28 ± 6.75
-30	49.93	87.48	53.80	57.16	63.28	62.33 ± 6.66
-15	50.44	89.28	54.46	56.42	61.80	62.48 ± 6.95
NPY → 0	51.19	90.26	54.11	56.36	63.28	63.04 ± 7.09
15	58.15	103.01	57.31	66.60	90.34	75.08 ± 9.18
30	61.80	110.87	66.16	70.56	73.85	76.65 ± 8.79
45	58.77	113.33	67.56	71.90	76.33	77.58 ± 9.40
60	54.03	117.24	66.63	76.05	74.91	77.77 ± 10.62
75	60.66	116.02	75.91	84.11	67.36	80.81 ± 9.65
90	52.77	125.28	69.80	76.18	75.03	79.81 ± 12.11
105	54.23	102.32	64.60	67.23	77.27	73.13 ± 8.17
120	50.87	93.39	63.87	67.60	74.89	70.12 ± 7.00
135	50.24	91.29	58.58	60.42	68.33	65.77 ± 7.00

Table 6a: Regression analysis of variance regarding plasma estradiol concentration on day 15 (periovulatory phase) of menstrual cycle from-60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.012	0.012	0.058	0.825
Residual	3	0.603	0.201		
Total	4	0.614		b=-0.0342±0.141	

Table 6b: Regression analysis of variance regarding plasma estradiol concentration (pg/ml) on day 15 (periovulatory phase) of menstrual cycle from 0 minutes to 75 minutes in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	136.833	136.833	9.615	0.036
Residual	4	56.924	14.231		
Total	5	193.757		b=2.796±0.901	

Table 6c: Regression analysis of variance regarding plasma estradiol concentration (pg/ml) on day 15 (periovulatory phase) of menstrual cycle from 75 minutes to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	158.157	158.157	86.390	0.003
Residual	3	5.492	1.831		
Total	4	163.650		b=-3.977±0.427	

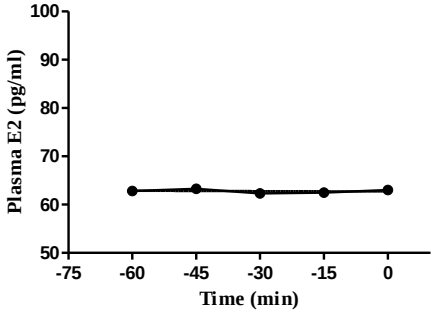


Fig. 9a: Regression analysis of variance showed no change in plasma estradiol concentration (pg/ml) on day 15 (periovulatory phase) from -60 minute to 0 minute in female monkey before single bolus i.v. injection of NPY.

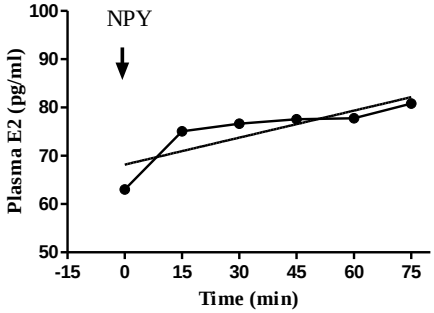


Fig. 9b: Regression analysis of variance showed highly significant increase in plasma estradiol concentration (pg/ml) on day 15 (periovulatory phase) from 0 minute to 75 minutes in female monkey after single bolus i.v. injection of NPY.

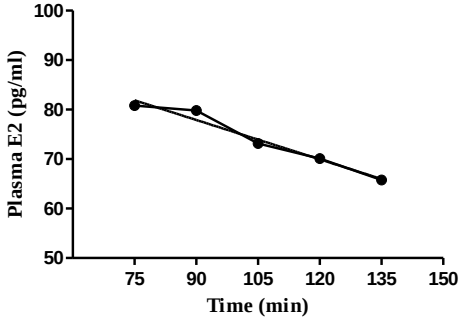


Fig. 9c: Regression analysis of variance showed highly significant decrease in plasma estradiol concentration (pg/ml) on day 15 (periovulatory phase) from 75 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY

of variance showed that mean plasma E_2 concentration was not significantly different during pretreatment period (b=-0.034±0.141; F (1,3)=0.058; P=0.825), (Table 6a and Fig. 9a).

At 0 minute a single bolus i.v. injection of NPY was given and plasma estradiol concentration was recorded after every fifteen minutes for a period of two hours and fifteen minutes. A non-significant rise in mean plasma estradiol concentration was observed after 15 minutes of NPY bolus injection compared to that of 0 minute ($t_{(8)}$ =1.038; P= 0.3297). The increase in plasma estradiol concentration continued and it reached its maximum at 75 minutes (80.81±9.65) (pg/ml). Regression analysis of variance showed significant temporal increase in mean plasma estradiol concentration from 0 minute to 75 minutes (b=2.796±0.901; F(1, 4)=9.615; P=0.036), (Table 6b and Fig. 9b). Then after 75 minute onwards gradual decrease in estradiol concentration after single bolus i.v. injection of NPY recorded and at 135 minutes it was the lowest concentration (65.77±7.0) (pg/ml) which was more or less equal to the concentration that was at zero minute. Regression analysis of variance showed significant temporal decrease in mean plasma estradiol concentration from 75 minute to 135 minutes (b=-3.977±0.427; F (1, 3)=86.390; P=0.003), (Table 6c and Fig. 9c).

Day 21 (Luteal Phase) of menstrual cycle

The individual and mean plasma estradiol concentration (pg/ml) before and after a single bolus i.v. administration of NPY in female monkey on day 21 (luteal phase) of menstrual cycle is shown in Table 7. No significant difference in plasma estradiol concentration was observed from -60 minutes to 0 minutes (one hour) ($p>0.05$) before NPY administration. Regression analysis of variance showed that mean plasma E_2 concentration was not different significantly during pretreatment period (b=-0.04±0.069; F(1,3)=0.409; P=0.568), (Table 7a and Fig. 10a).

At 0 minute a single bolus i.v. injection of NPY was given and plasma estradiol concentration was recorded after every fifteen minutes for a period of two hours and fifteen minutes. Increase in estradiol concentration was observed after 15 minutes of NPY single bolus i.v. injection compared to that of at 0 minute (pg/ml) ($t_{(8)}$ =1.002

P=0.345). The increase in plasma estradiol concentration continued and it reached its maximum at 60 minutes (40.97±3.15) (pg/ml). Regression analysis of variance showed significant temporal increase in mean plasma estradiol concentration from 0 minute to 60 minutes time (b=2.12±0.36; F(1,3)=33.50; P=0.010), (Table 7b and Fig. 10b). Then a gradual decrease was recorded after 60 minutes till 135 minutes which was not significantly different from that at 0 minute ($t_{(at\ 0\ 8)}$ =-0.445 P=0.667). Regression analysis of variance showed significant temporal decrease in mean plasma estradiol concentration from 60 minute to 135 minutes time (b=-1.421±0.146; F(1,4)=94.18; P=0.001), (Table 7c and Fig. 10c).

Table 7: Individual and mean plasma estradiol concentration before and after NPY single bolus i.v. injection in female rhesus monkey on day 21 (luteal phase) of menstrual cycle.

Plasma Estradiol Concentration (pg/ml)						
	Monkeys					
Time (min)	1	2	3	4	5	Mean ± SE
-60	25.63	42.00	30.88	30.65	40.72	33.97 ± 3.16
-45	25.93	41.96	31.53	29.82	41.14	34.08 ± 3.19
-30	26.28	41.17	30.80	30.28	40.77	33.86 ± 3.01
-15	26.31	42.40	30.78	30.72	40.83	34.21 ± 3.14
NPY → 0	25.99	41.62	31.08	29.88	39.84	33.69 ± 3.01
15	29.36	42.98	38.37	35.06	42.33	37.62 ± 2.51
30	28.77	47.85	41.93	37.75	45.79	40.42 ± 3.38
45	30.26	48.23	45.29	38.04	43.05	40.97 ± 3.15
60	31.73	50.77	51.92	36.78	42.04	42.65 ± 3.91
75	31.96	48.83	52.19	36.04	41.08	42.02 ± 3.79
90	34.79	47.61	49.12	34.85	40.01	41.28 ± 3.06
105	31.71	46.73	41.79	33.47	40.86	38.91 ± 2.78
120	30.11	45.91	40.89	32.39	40.15	37.89 ± 2.91
135	28.72	44.79	31.76	31.06	41.92	35.65 ± 3.22

Table 7a: Regression analysis of variance regarding plasma estradiol concentration (pg/ml) on day 21 (luteal phase) of menstrual cycle from -60 minutes to 0 minutes in female monkey before single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.019	0.019	0.409	0.568
Residual	3	0.143	0.048		
Total	4	0.1624		b=-o.o4±0.069	

Table 7b: Regression analysis of variance regarding plasma estradiol concentration (pg/ml) on day 21 (luteal phase) of menstrual cycle from 0 minutes to 60 minutes in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	45.294	45.294	33.501	0.010
Residual	3	4.056	1.352		
Total	4	49.350		b=2.128±0.367	

Table 7c: Regression analysis of variance regarding plasma estradiol concentration on day 21 of (luteal phase) of menstrual cycle from 60 minutes to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	35.364	35.364	94.187	0.001
Residual	4	1.502	0.375		
Total	5	36.866		b=-1.421±0.146	

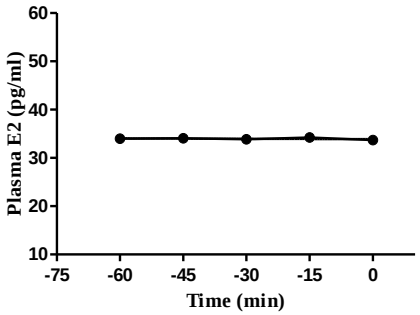


Fig. 10a: Regression analysis of variance showed no change in plasma estradiol concentration (pg/ml) on day 21 (luteal phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

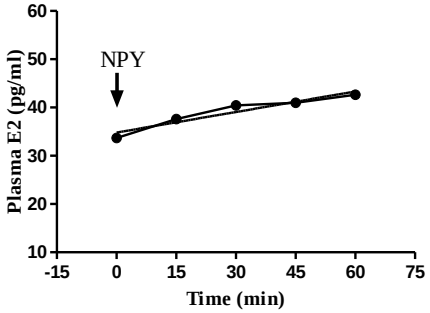


Fig. 10b: Regression analysis of variance showed highly significant increase in plasma estradiol concentration (pg/ml) on day 21 (luteal phase) from 0 minute to 60 minutes in female monkey after single bolus i.v. injection of NPY

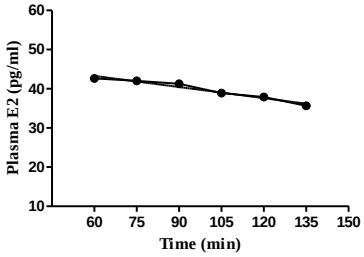


Fig. 10c: Regression analysis of variance showed highly significant decrease in plasma estradiol concentration (pg/ml) on day 21 (luteal phase) from 60 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Progesterone

Plasma Progesterone (P) concentrations in female rhesus monkey during the menstrual cycle

Baseline profile of progesterone during menstrual cycle

Mean basal plasma progesterone (P) profile in the female monkeys during the menstrual cycle is shown in Table 8 and Fig 11. Mean plasma P levels were low at the menstrual phase (day 1-4) compared to all other phases i.e. follicular (day 7-13), (P=<0.019), periovulatory (day 14-16), (P=<0.008) and luteal phase (day 19-31) (P=<0.001). Plasma P level was non-significantly high in periovulatory phase compared to follicular phase whereas significantly low than luteal phase ($t_{(8)}$ =-2.42; P=0.041), (Table 8a).

Effect of single bolus injection of 200µg NPY on plasma progesterone concentration in female rhesus monkeys during different phases of menstrual cycle

Day 1 (Menstrual Phase) of menstrual cycle

The individual and mean plasma progesterone (P) concentration (ng/ml) before and after NPY bolus administration in five female monkeys on day 1 of menstrual phase of menstrual cycle is given in Table 9. Mean plasma Progesterone level was recorded one hour before the injection of NPY with an interval of 15 minutes. Concentration of progesterone remained more or less the same up to 0 minute (within an hour). Regression analysis of variance showed that mean plasma P level was not different significantly during pre-treatment period (b=-0.001±0.003; F (1,3)=0.238; P=0.659), (Table 9a and Fig. 12a).

At 0 minute, NPY single bolus intravenous (i.v.) injection was given and progesterone levels were recorded after every fifteen minutes for a period of two hours and fifteen minutes. A non-significant rise in progesterone concentration was observed after 15 minutes of NPY bolus injection compared to that at 0 minute ($t_{(8)}$ =1.79; P=0.110). Then

Table 8: Individual and mean baseline profile of plasma progesterone concentration (ng/ml) on different days of the menstrual cycle in adult female rhesus monkey.

Plasma Progestrone Concentration (ng/ml)							
Menstrual Cycle	Monkey #						
Days	1	2	3	4	5	Mean	± SE
1	1.07	0.46	1.06	1.56	1.46	1.12 ±	0.19
2	1.18	0.94	1.31	1.34	1.59	1.27 ±	0.11
4	1.20	1.00	1.61	1.47	1.61	1.38 ±	0.12
7	1.25	1.04	1.62	2.43	1.83	1.63 ±	0.24
10	2.36	1.39	1.84	2.47	1.86	1.98 ±	0.20
13	2.45	1.53	2.14	2.28	1.88	2.06 ±	0.16
14	2.45	1.56	2.18	2.32	1.90	2.08 ±	0.16
15	2.46	1.99	2.30	4.35	1.95	2.61 ±	0.45
16	2.87	2.04	2.53	4.30	1.97	2.74 ±	0.42
19	6.97	4.04	4.54	8.47	8.57	6.52 ±	0.96
21	5.34	3.27	4.45	8.21	8.04	5.86 ±	0.98
22	4.77	2.95	4.12	6.71	7.71	5.25 ±	0.86
25	2.91	1.28	4.05	3.10	3.10	2.89 ±	0.45
28	1.23	1.02	3.99	3.04	3.24	2.50 ±	0.59
31	1.18	0.70	2.02	1.98	1.66	1.51 ±	0.25

Table 8a: Mean plasma progesterone concentration (ng/ml) during different phases of menstrual cycle in adult female rhesus monkey.

Plasma Progesterone concentration (ng/ml)			
Menstrual Phase (1-4 Days)	Folicular Phase (7-13 Days)	Periovulatory Phase (14-16 Days)	Luteal Phase (19-31 Days)
1.26 ± 0.13	1.89 ± 0.17	2.48 ± 0.32	4.09 ± 0.58

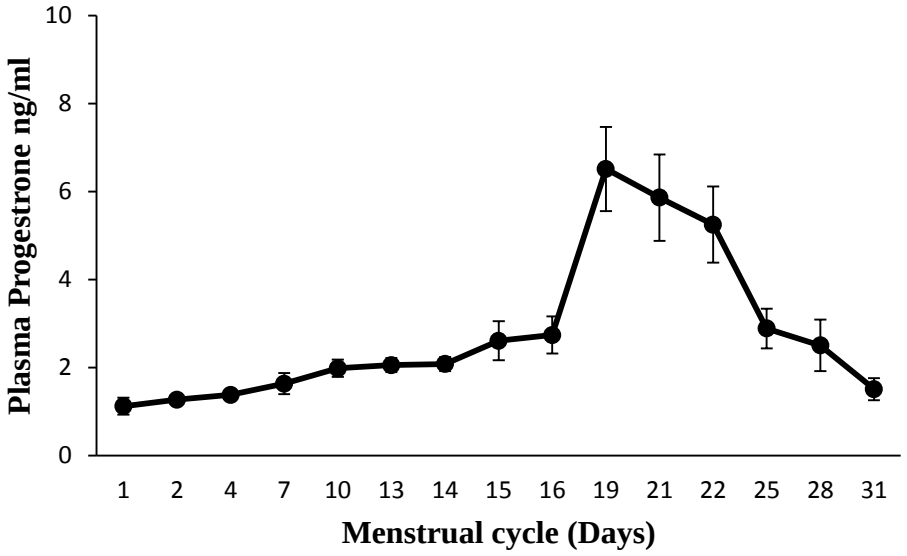


Fig. 11: Baseline profile of plasma progesterone concentration (ng/ml) in female rhesus monkey on different days of menstrual cycle.

Table 9: Individual and mean plasma progesterone concentration before and after NPY single bolus i.v. injection in female rhesus monkeys on day 1 (menstrual phase) of menstrual cycle.

Plasma Progestrone Concentration (ng/ml)						
		Monkey #				
	Time (min)	1	2	3	4	5	Mean ± SE
	-60	1.07	0.49	0.63	1.61	1.50	1.06 ± 0.22
	-45	1.06	0.48	0.65	1.60	1.51	1.06 ± 0.22
	-30	1.05	0.48	0.60	1.60	1.51	1.05 ± 0.23
	-15	1.06	0.49	0.66	1.61	1.51	1.07 ± 0.22
NPY $\longrightarrow$	0	1.05	0.48	0.60	1.61	1.50	1.05 ± 0.23
	15	1.75	1.04	0.98	2.96	3.32	2.01 ± 0.48
	30	2.39	1.33	1.11	3.02	5.78	2.73 ± 0.84
	45	2.51	1.81	1.38	3.61	5.39	2.94 ± 0.72
	60	2.55	2.20	1.63	4.81	5.35	3.31 ± 0.74
	75	2.70	2.45	1.50	4.42	5.33	3.28 ± 0.70
	90	2.50	2.20	1.43	4.41	5.26	3.16 ± 0.72
	105	2.49	2.05	1.44	3.91	5.15	3.01 ± 0.67
	120	2.48	2.20	1.42	3.40	5.14	2.93 ± 0.64
	135	2.91	1.55	1.36	3.02	5.16	2.80 ± 0.68

Table 9a: **Regression analysis of variance regarding plasma progesterone concentration (ng/ml) on day 1 (menstrual phase) of menstrual cycle from -60 minute to 0 minutes time in female monkey before single bolus i.v. injection of NPY**

Spource	*df*	*SS*	*MS*	*F*	*P*
Regression	1	2.1E-05	2.1E-05	0.238	0.659
Residual	3	0.0003	8.7E-05		
Total	4	0.0003		b=-0.001±0.003	

Table 9b: Regression analysis of variance regarding plasma progesterone concentration (ng/ml) on day 1 (menstrual phase) of menstrual cycle from 0 minute to 60 minutes time female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	2.970	2.970	38.174	0.009
Residual	3	0.233	0.078		
Total	4	3.203		b=0.545±0.088	

Table 9c: Regression analysis of variance regarding plasma progesterone concentration (ng/ml) on day 1 (menstrual phase) of menstrual cycle from 60 minute to 135 minutes time female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.201	0.201	177.473	0.0002
Residual	4	0.005	0.001		
Total	5	0.205		b=-0.107±0.008	

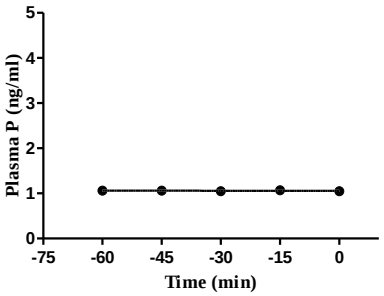


Fig. 12a: Regression analysis of variance showed no change in plasma progesterone concentration (ng/ml) on day 1 (menstrual phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

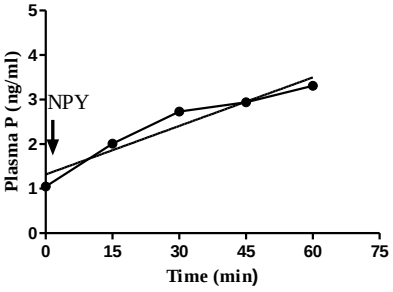


Fig. 12b: Regression analysis of variance showed significant increase in plasma progesterone concentration (ng/ml) on day 1 (menstrual phase) from 0 minute to 60 minutes in female monkey after single bolus i.v. injection of NPY.

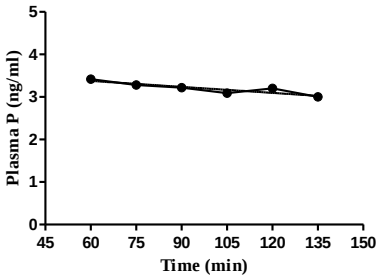


Fig. 12c. Regression analysis of variance showed highly significant decrease in plasma progesterone concentration (ng/ml) on day 1 (menstrual phase) from 60 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

increase in plasma progesterone concentration continued and it reached its maximum at 60 minutes (3.31±0.74 (ng/ml)). This increase is highly significant compared to that at 0 minute ($t_{(8)}$ =2.90; P=0.019). Regression analysis of variance showed significant temporal increase in mean plasma progesterone concentration from 0 minute to 60 minutes time (b=0.545±0.088; F(1,3)=38.174; P=0.009), (Table 10b and Fig. 12b). Then progressive temporal decrease in plasma P concentration was recorded after 60 minutes till the duration of experiment i.e. up to 135 minutes whereas these levels was significantly high compared to baseline plasma progesterone concentration ($t_{(8)}$ =2.44; P=0.040). Regression analysis of variance showed significant temporal decrease in mean plasma progesterone concentration from 60 minute to 135 minutes time (b=-0.107±0.008; F(1,4)=177.47; P=0.0002), (Table 9c and Fig. 12c).

Day 7 (Follicular Phase) of menstrual cycle

The individual and mean plasma progesterone concentration (P) before and after NPY administration in five female monkeys during the follicular phase of menstrual cycle is given in Table 10. Progesterone concentration was recorded from -60 minutes after every 15 minutes for an hour. No significant difference in progesterone concentration was observed from -60 minutes to 0 minutes (within an hour). Regression analysis of variance showed that mean plasma P level was not significantly different during pretreatment period with NPY (b=-0.0162±0.008; F(1,3)=3.300; P=0.167), (Table 10a and Fig. 13a).

At 0 minute, a single bolus i.v. injection of NPY was given and plasma progesterone levels were recorded after every fifteen minute for a period of two hour and fifteen minutes. Increase in plasma progesterone concentration was observed after 15 minutes of treatment compared to that of at 0 minute ($t_{(8)}$=1.365; P=.209). Then significant increase in plasma progesterone concentration continued and reached to maximum at 105 minutes ($t_{(8)}$ =2.559; P=0.033) compared to 0 minute. Regression analysis of variance showed highly significant increase in progesterone concentration from 0 minute to 105 minutes time (b=0.367±0.030; F(1,6)=140.66; P=0.00002), (Table 10b and Fig. 13b).

Table 10: Individual and mean Plasma progesterone concentrations (ng/ml) before and after NPY single bolus i.v. injection in female rhesus monkey on day 7 (follicular phase) of menstrual cycle.

Plasma Progestrone Concentration (ng/ml)							
		Monkey #					
	Time (min)	1	2	3	4	5	Mean ± SE
	-60	1.26	1.06	1.62	2.43	1.84	1.64 ± 0.24
	-45	1.25	1.06	1.61	2.45	1.98	1.67 ± 0.25
	-30	1.24	1.09	1.59	2.35	1.66	1.59 ± 0.22
	-15	1.28	1.06	1.60	2.36	1.69	1.60 ± 0.22
NPY $\longrightarrow$	0	1.26	1.07	1.59	2.39	1.68	1.60 ± 0.23
	15	1.86	1.98	1.75	2.70	1.69	1.99 ± 0.18
	30	1.92	2.11	2.07	2.99	2.06	2.23 ± 0.19
	45	3.76	2.09	2.61	4.60	2.60	3.13 ± 0.46
	60	3.61	2.28	2.35	5.80	2.68	3.34 ± 0.66
	75	4.23	2.35	2.33	5.92	2.30	3.43 ± 0.72
	90	4.85	2.37	2.70	7.14	2.53	3.92 ± 0.92
	105	5.11	2.56	2.74	7.35	2.67	4.08 ± 0.94
	120	2.82	2.47	2.15	5.68	2.80	3.18 ± 0.64
	135	2.82	1.81	2.28	4.71	2.98	2.92 ± 0.49

Table 10a: Regression analysis of variance regarding plasma progesterone concentration on day 7 (follicular phase) of menstrual cycle from -60 minute to 0 minutes time in female monkey before single bolus i.v. injection of NPY

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.003	0.003	3.300	0.167
Residual	3	0.002	0.001		
Total	4	0.005		b=-0.016±0.008	

Table 10b: **Regression analysis of variance regarding plasma progesterone concentration on day 7 (follicular phase) of menstrual cycle of menstrual cycle from 0 minute to 105 minutes time in female monkey after single bolus i.v. injection of NPY.**

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	5.660	5.660	140.665	0.00002
Residual	6	0.241	0.040		
Total	7	5.901		b=0.367±0.030	

Table 10c: Regression analysis of variance regarding plasma progesterone concentration on day 7 (follicular phase) of menstrual cycle from 105 minute to 135 minutes time in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.678	0.678	9.996	0.195
Residual	1	0.068	0.068		
Total	2	0.745		b=0.184±-3.161	

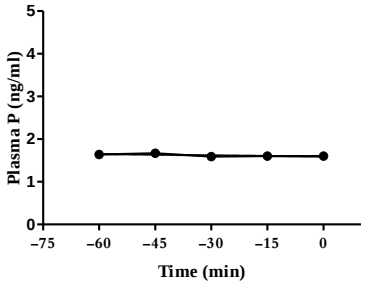


Fig. 13a: Regression analysis of variance showed no change in plasma progesterone concentration (ng/ml) on day 7 (follicular phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

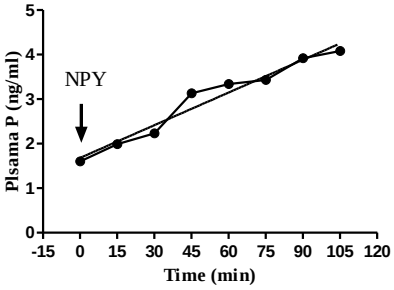


Fig. 13b. Regression analysis of variance showed highly significant increase in plasma progesterone concentration (ng/ml) on day 7 (follicular phase) from 0 minute to 105 minutes in female monkey after single bolus i.v. injection of NPY

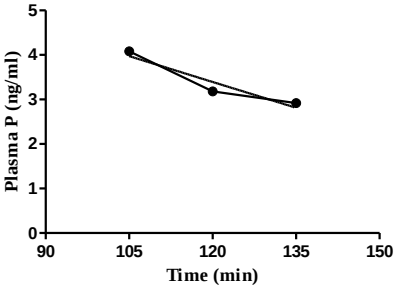


Fig. 13c. Regression analysis of variance showed non-significant decrease in plasma progesterone concentration (ng/ml) on day 7 (follicular phase) from 105 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Then gradual temporal decrease in progesterone concentration was recorded after 105 minutes till the end of experiment i.e.135 minutes, but the progesterone level remained significantly high compared to that at 0 minute (P=<0.05). Regression analysis of variance showed this temporal decrease in progesterone concentration was not significant from 105 to 135 minute (b=0.184±-3.161; F(1,1) = 9.996; P=0.195), (Table 10c and Fig. 13c).

Day 15 (Peri-ovulatory Phase) of Menstrual Cycle

The individual and mean plasma progesterone (P) concentration before and after NPY administration in five female monkeys on day 15 is shown in Table 11. Mean plasma Progesterone level was recorded for one hour before administration of NPY single bolus i.v. injection with an interval of 15 minutes. No significant difference in progesterone concentration was observed from -60 minutes to 0 minutes (within an hour). Regression analysis of variance showed that mean plasma P level was not significantly different during pretreatment period (b=0.006±0.0023; F (1, 3)=9.021; P=0.058), (Table 11a and Fig. 14a.

At 0 minute, a single bolus i.v. injection of NPY was given and plasma progesterone level was recorded after every fifteen minute for a period of two hour and fifteen minutes. Increase in mean progesterone concentration after 15 minutes of NPY bolus injection compared to that of 0 minute ($t_{(8)}$ =0.860; P=0.414). This non-significant increase in plasma progesterone concentration progressively continued till the end of experiment and was maximum at 135 minute ($t_{(8)}$=-2.076; P=0.071). Regression analysis of variance showed highly significant temporal increase in plasma progesterone concentration from 0 minute to 135 minutes (b=0.22±0.035; F(1,8)= 36.334; P=0.0003), (Table 11b and Fig. 14b).

Day 21 (Luteal Phase) of Menstrual Cycle

The individual and mean plasma progesterone (P) concentration (ng/ml) before and after NPY bolus i.v. administration in five female monkeys on day 21 of menstrual cycle

(luteal phase) is shown in Table 12. Non-significant difference in plasma progesterone concentration was observed from -60 minutes to 0 minutes ($p>0.05$). Regression analysis of variance showed that mean plasma P level was not significantly different during pretreatment period ($b=0.004\pm2.11$; $F(1,3)=0.0$; $P= 0.1$), (Table 12a and Fig. 15a).

At 0 minute, a single bolus i.v. injection of NPY was given and plasma progesterone level was recorded after every fifteen minute for a period of two hour and fifteen minutes. Increase in plasma progesterone concentration was observed after 15 minutes of NPY administration compared to that at 0 minute (ng/ml) ($t_{(8)} =0.340$;$P=0.742$). The increase in plasma progesterone concentration continued and it reached its maximum at 30 minutes (6.84 ± 1.150 pg/ml). Regression analysis of variance showed significant temporal increase in mean plasma progesterone concentration from 0 minute to 30 minutes time ($b=0.48\pm0.0220$; $F(1,1)=482.39$; $P=0.029$), (Table 12b and Fig. 15b). Then after 30 minutes gradual decrease was recorded till the end of experiment with slight fluctuation. Regression analysis of variance showed significant temporal decrease in mean plasma progesterone concentration from 30 minute to 135 minutes time ($b=0.135\pm0.021$; $F(1,6)=40.341$; $P=0.0007$), (Table 12c and Fig. 15c).

Table 11: Individual and mean plasma progesterone concentration before and after NPY single bolus i.v. injection in female rhesus monkey on day 15 (periovulatory phase) of menstrual cycle.

Plasma Progestrone Concentration (ng/ml)							
		Monkey #					
	Time (min)	1	2	3	4	5	Mean ± SE
	-60	2.64	1.92	2.28	4.33	1.93	2.62 ± 0.45
	-45	2.69	1.90	2.32	4.30	1.94	2.63 ± 0.44
	-30	2.68	1.89	2.31	4.33	1.93	2.63 ± 0.45
	-15	2.69	2.01	2.31	4.30	1.94	2.65 ± 0.43
NPY	⟶ 0	2.70	1.91	2.36	4.31	1.93	2.64 ± 0.44
	15	3.70	2.40	2.48	7.11	1.99	3.53 ± 0.94
	30	4.39	2.81	4.48	6.89	2.19	4.15 ± 0.82
	45	4.65	2.91	4.75	6.39	2.75	4.29 ± 0.67
	60	5.37	2.80	4.69	6.17	2.77	4.36 ± 0.68
	75	5.20	2.49	4.74	6.61	2.79	4.36 ± 0.77
	90	5.23	2.43	4.82	6.95	2.80	4.45 ± 0.83
	105	5.13	2.58	4.43	7.81	3.08	4.61 ± 0.92
	120	5.03	3.61	4.75	9.39	2.53	5.06 ± 1.17
	135	5.00	4.75	4.17	9.15	2.47	5.11 ± 1.10

Table 11a: Regression analysis of variance regarding plasma progesterone concentration on day 15 (periovulatory phase) of menstrual cycle from-60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.000	0.000	9.021	0.058
Residual	3	0.000	0.000		
Total	4	0.001		b=0.006±0.0023	

Table 11b: **Regression analysis of variance regarding plasma progesterone concentration (ng/ml) on day 15 (periovulatory phase) of menstrual cycle from 0 minutes to 135 minutes in female monkey after single bolus i.v. injection of NPY.**

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	3.849	3.849	36.334	0.0003
Residual	8	0.847	0.106		
Total	9	4.696		b=0.22±0.035	

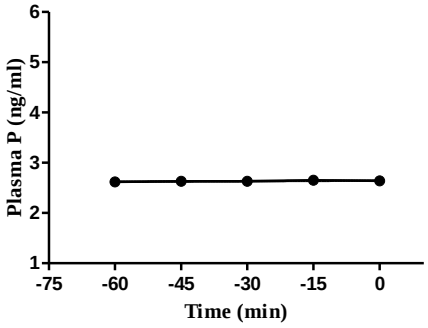


Fig. 14a: Regression analysis of variance showed no change in plasma progesterone concentration (ng/ml) on day 15 (periovulatory phase) from -60 minute to 0 minute in female monkey before single bolus i.v. injection of NPY.

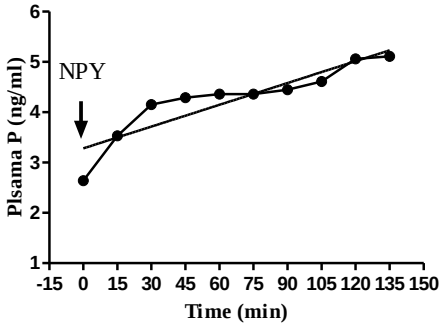


Fig. 14b. Regression analysis of variance showed highly significant increase in plasma progesterone concentration (ng/ml) on day 15 (periovulatory phase) from 0 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Table 12: Individual and mean plasma progesterone concentration before and after NPY single bolus i.v. injection in female rhesus monkey on day 21 (luteal phase) of menstrual cycle.

Plasma Progestrone Concentration (ng/ml)							
		Monkey #					
	Time (min)	1	2	3	4	5	Mean ± SE
	-60	5.32	3.28	4.47	8.21	8.10	5.88 ± 0.98
	-45	5.32	3.25	4.46	8.21	8.00	5.85 ± 0.98
	-30	5.36	3.30	4.43	8.23	8.00	5.86 ± 0.98
	-15	5.35	3.26	4.45	8.20	8.02	5.86 ± 0.98
NPY ⟶	0	5.34	3.27	4.44	8.22	8.09	5.87 ± 0.99
	15	6.35	3.39	4.28	8.38	9.57	6.39 ± 1.17
	30	8.50	3.49	4.71	8.13	9.37	6.84 ± 1.15
	45	5.46	3.45	4.86	7.81	8.17	5.95 ± 0.90
	60	4.43	3.52	5.63	6.68	7.72	5.60 ± 0.75
	75	4.84	3.70	4.79	6.64	7.43	5.48 ± 0.68
	90	4.47	3.24	4.51	6.31	7.28	5.16 ± 0.72
	105	4.03	3.30	4.43	6.34	7.21	5.06 ± 0.73
	120	4.26	3.76	4.50	6.43	7.83	5.36 ± 0.77
	135	5.19	3.35	4.52	7.15	8.20	5.68 ± 0.88

Table 12a: Regression analysis of variance regarding plasma progesterone concentration (ng/ml) on day 21 (luteal phase) of menstrual cycle of menstrual cycle from -60 minutes to 0 minutes in female monkey before NPY single bolus i.v. injection.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.00000	0.000	0.000	1.000
Residual	3	0.0005	0.0002		
Total	4	0.0005		b=0.004±2.11	

Table 12b: Regression analysis of variance regarding progesterone concentration after NPY single bolus injection on day 21 (luteal phase) of menstrual cycle from 0 minutes to 30 minutes in rhesus monkey.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.469	0.469	482.395	0.029
Residual	1	0.001	0.001		
Total	2	0.470		b=0.48±0.0220	

Table 12c: Regression analysis of variance regarding plasma progesterone concentration on day 21 (luteal phase) of menstrual cycle from 30 minutes to 135 minutes in female monkey after NPY single bolus i.v. injection.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.7702	0.7702	40.3415	0.0007
Residual	6	0.1146	0.0191		
Total	7	0.8847		b=0.135±0.021	

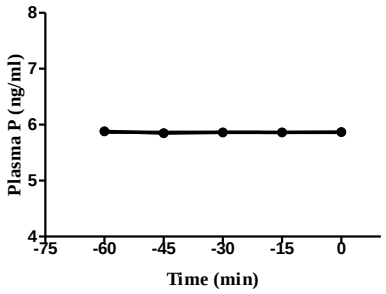


Fig. 15a: Regression analysis of variance showed no change in plasma progesterone (ng/ml) day 21 (luteal phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

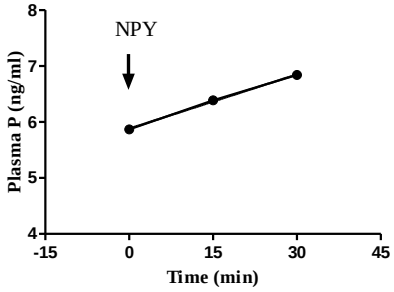


Fig. 15b: Regression analysis of variance showed highly significant increase in plasma progesterone concentration (ng/ml) day 21 (luteal phase) from 0 minute to 30 minutes in female monkey after single bolus i.v. injection of NPY.

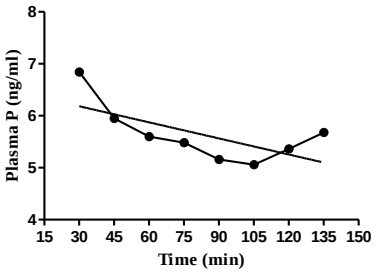


Fig. 15c: Regression analysis of variance showed highly significant decrease in plasma progesterone concentration (ng/ml) day 21 (luteal phase) from 30 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Prolactin

Plasma prolactin (PRL) concentrations in female rhesus monkey during the menstrual cycle

Baseline profile of plasma prolactin during menstrual cycle

Mean basal plasma prolactin (PRL) profile in the female monkeys during the menstrual cycle is shown in Table 13 and Fig 16. Mean plasma PRL levels of menstrual (day 1-4) and peri-ovulatory phases (day 14-16) were significantly high compared to luteal phase (day 19-31) ($t_{(8)}$ =-3 .141; P=0.013) and ($t_{(8)}$ =-2.793; P=0.023) respectively. Follicular phase (day 7-13) plasma prolactin level was non-significantly lower than menstrual ($t_{(8)}$ = 1.866; P=0.098) and peri-ovulatory ($t_{(8)}$ =1.738; P=0.120) phases. The plasma prolactin levels of follicular and luteal phases were not different ($t_{(8)}$ =0.596; P=0.567), (Table 13a).

Effect of single bolus injection of 200 µg NPY on prolactin concentration in female rhesus monkeys during different phases of menstrual cycle

Day 1 (Menstrual Phase) of menstrual cycle

The individual and mean prolactin (PRL) concentration (ng/ml) before and after NPY bolus administration in five female monkeys on day 1 of menstrual phase of menstrual cycle is given in Table 14. Mean prolactin level was recorded one hour before the injection of NPY with an interval of 15 minutes. Concentration of prolactin remained more or less same up to 0 minute (within an hour). Regression analysis of variance showed that mean plasma PRL level was not significantly different during pre-treatment period (b=-0.182±0.088; F (1,3)=4.245; P=0.131), (Table 14a and Fig. 17a).

At 0 minute a single bolus i.v. injection of NPY was given and plasma prolactin concentration was recorded after every fifteen minutes for a period of two hours and fifteen minutes. A non-significant rise in prolactin concentration was observed after 15

Table- 13: Individual and mean baseline profile of plasma prolactin concentration (ng/ml) on different days of the menstrual cycle in adult female rhesus monkey.

Plasma Prolactin Concentration (ng/ml)						
Menstrual Cycle	**Monkeys #**					
Days	**1**	**2**	**3**	**4**	**5**	**Mean ± SE**
1	24.45	22.14	18.96	30.93	29.81	25.26 ± 2.27
2	22.49	26.33	25.21	24.05	23.65	24.35 ± 0.00
4	22.03	25.40	33.14	26.62	28.76	27.19 ± 1.84
7	20.94	22.30	37.15	23.59	23.83	25.56 ± 2.94
10	11.88	34.77	19.02	17.28	23.86	21.36 ± 3.86
13	9.10	15.12	13.50	23.38	27.79	17.78 ± 3.41
14	16.08	22.10	22.79	22.71	24.36	21.61 ± 1.43
15	26.16	32.17	27.93	27.97	23.01	27.45 ± 1.49
16	30.92	26.99	22.74	38.99	20.32	27.99 ± 3.29
19	25.52	25.22	20.06	30.88	27.56	25.85 ± 1.76
21	21.19	20.99	20.67	35.23	21.12	23.84 ± 2.85
22	13.90	14.63	17.13	21.86	13.15	16.13 ± 1.58
25	27.17	12.22	15.40	24.09	11.70	18.12 ± 3.17
28	22.64	14.07	13.32	22.82	18.88	18.35 ± 2.03
31	23.73	14.00	19.34	15.19	18.05	18.06 ± 1.71

Table 13a: Mean plasma prolactin concentration (ng/ml) during different phases of menstrual cycle in adult female rhesus monkey

Plasma Prolactin concentration (ng/ml)			
Menstrual Phase (1-4 days)	**Folicular Phase (7-13 days)**	**Periovulatory Phase (14-16 days)**	**Luteal Phaser (19-31 days)**
25.60 ± 0.83	21.57 ± 2.00	25.68 ± 1.28	20.06 ± 1.56

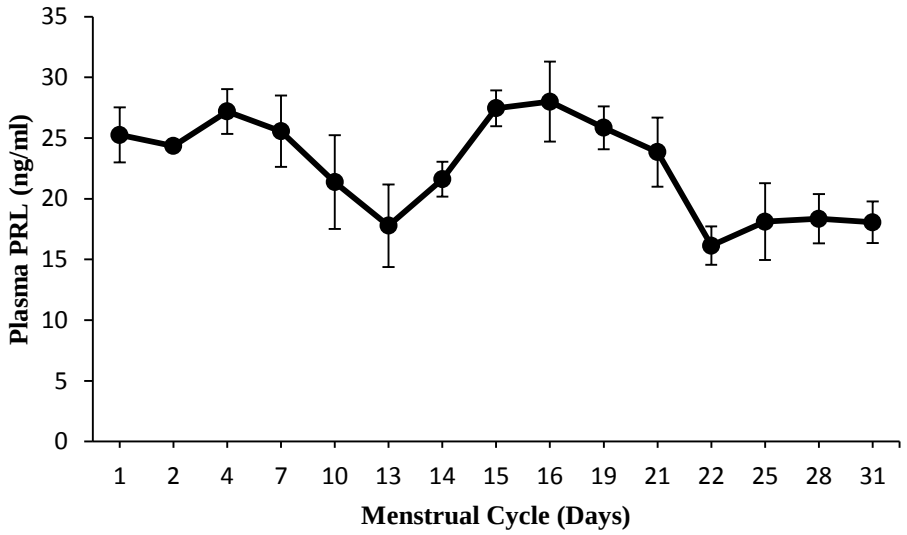


Fig.-16: Mean baseline profile of plasma prolactin in female rhesus monkey on different days during the menstrual cycle.

minutes of NPY bolus injection compared to that at 0 minute ($t_{(8)}$ =2.22; P=0.057). This non-significant increase in prolactin concentration continued and it reached its maximum at 30 minutes. Regression analysis of variance showed temporal increase in mean plasma prolactin concentration from 0 minute to 30 minutes time (b=7.989±2.061; F(1,1)=15.027; P=0.161), (Table 14b and Fig. 17b). After 30 minutes decrease in plasma PRL concentration started and continued progressively till the end of experiment i.e. 135 minutes. Regression analysis of variance showed highly significant temporal decrease in mean plasma estradiol concentration from 30 minute to 135 minutes time (b=-3.143±0.433; F(1,6)=52.579; P=0.0003), (Table 14c and Fig. 17c).

Day 7 (Follicular Phase) of menstrual cycle

The individual and mean prolactin concentration (PRL) before and after NPY administration in five female monkeys during the follicular phase of menstrual cycle is given in Table 15. Plasma prolactin concentration was recorded from -60 minutes after every 15 minutes for an hour. No significant difference in prolactin concentration was observed from -60 minutes to 0 minutes (within an hour). Regression analysis of variance showed that mean plasma PRL level was not significantly different during pretreatment period with NPY (b=-0.063±0.094; F (1,3)=0.459; P=0.547), (Table 15a and Fig. 18a).

At 0 minute, a single bolus i.v. injection of NPY was given and plasma prolactin concentration was recorded after every fifteen minutes for a period of two hours and fifteen minutes. A maximum rise in prolactin concentration was observed after 15 minutes of treatment compared to that at 0 minute ($t_{(8)}$=-1.772; P=.114). After 15 minute abrupt non-significant decrease was started in plasma PRL level which continues progressively till the end of experiment i.e. 135 minutes. Regression analysis of variance showed highly significant temporal decrease in prolactin concentration from 15 minute to 135 minutes (b=-3.095±0494; F(1,7)=39.214; P=0.0004), (Table 15b and Fig. 18b).

Day 15 (Periovulatory Phase) of menstrual cycle

The individual and mean prolactin (PRL) concentration before and after NPY administration in five female monkeys on day 15 is shown in Table 16. Mean plasma

Table 14: Individual and mean plasma prolactin concentration before and after NPY single bolus i.v. injection in female rhesus monkeys on day 1 (menstrual phase) of menstrual cycle.

Plasma Prolactin Concentration (ng/ml)						
	Monkeys #					
Time (min)	1	2	3	4	5	Mean ± SE
-60	24.45	22.07	19.08	30.93	29.88	25.28 ± 2.26
-45	24.46	22.18	18.95	30.75	30.22	25.31 ± 2.29
-30	24.13	22.12	18.86	30.61	30.12	25.17 ± 2.28
-15	23.58	22.13	19.03	28.08	29.32	24.43 ± 1.90
NPY ⟶ 0	23.04	22.21	18.90	30.41	29.51	24.81 ± 2.22
15	43.92	25.37	24.37	43.02	45.18	36.37 ± 4.71
30	61.95	21.73	20.57	59.43	40.28	40.79 ± 8.85
45	54.48	18.38	19.19	47.56	32.65	34.45 ± 7.30
60	39.31	14.83	15.56	44.18	26.47	28.07 ± 6.00
75	37.30	13.53	14.23	40.85	23.06	25.79 ± 5.70
90	34.32	11.86	11.83	34.76	17.85	22.12 ± 5.19
105	28.52	11.55	10.19	26.58	19.60	19.29 ± 3.75
120	26.18	10.86	10.35	26.12	20.21	18.74 ± 3.50
135	21.87	13.60	11.85	24.93	20.62	18.57 ± 2.50

Table 14a: Regression analysis of variance regarding plasma prolactin concentration (ng/ml) on day 1 (menstrual phase) of menstrual cycle from -60 minute to 0 minutes time in female monkey before single bolus i.v. injection of NPY.

Soucrce	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.332	0.332	4.245	0.131
Residual	3	0.235	0.078		
Total	4	0.567		b=-0.182±0.088	

Table 14b: Regression analysis of variance regarding plasma prolactin concentration (ng/ml) on day 1 (menstrual phase) of menstrual cycle from 0 minute to 30 minutes time in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	127.679	127.679	15.027	0.161
Residual	1	8.497	8.497		
Total	2	136.175		b=7.989±2.061	

Table 14c: Regression analysis of variance regarding plasma prolactin concentration (ng/ml) on day 1 (menstrual phase) of menstrual cycle from 30 minute to 135 minutes time in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	415.130	415.130	52.579	0.0003
Residual	6	47.372	7.895		
Total	7	462.501		b=-3.143±0.433	

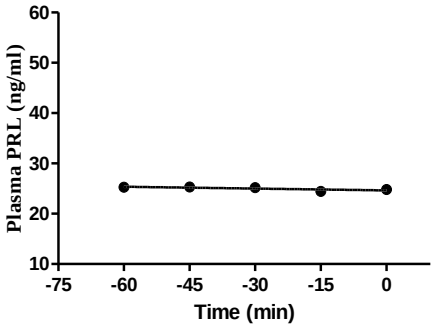


Fig. 17a: Regression analysis of variance showed no change in plasma prolactin concentration (ng/ml) on day 1 (menstrual phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

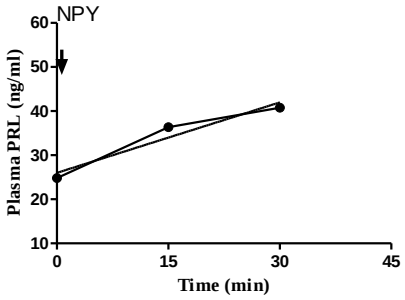


Fig. 17b: Regression analysis of variance showed non-significant increase in plasma prolactin concentration (ng/ml) on day 1 (menstrual phase) from 0 minute to 30 minutes in female monkey after single bolus i.v. injection of NPY.

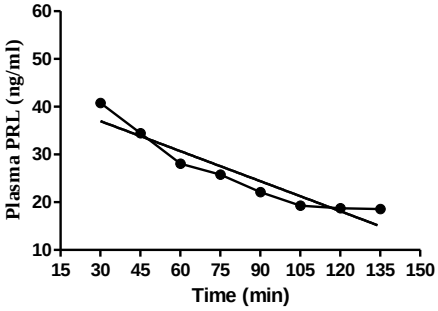


Fig. 17c: Regression analysis of variance showed highly significant decrease in plasma prolactin concentration (ng/ml) on day 1 (menstrual phase) from 30 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Table 15: Individual and mean plasma prolactin concentrations (ng/ml) before and after NPY single bolus injection in female rhesus monkey on day 7 (follicular phase) of menstrual cycle.

Plasma Prolactin Concentration (ng/ml)							
		Monkeys #					
	Time (min)	1	2	3	4	5	Mean ± SE
	-60	21.47	22.40	15.97	24.03	23.98	21.57 ± 1.48
	-45	19.76	22.13	15.45	23.45	23.53	20.86 ± 1.52
	-30	21.98	22.27	14.21	22.87	23.90	21.05 ± 1.74
	-15	20.87	22.45	15.76	23.71	23.76	21.31 ± 1.48
NPY ⟶	0	20.61	22.29	14.36	23.87	24.01	21.03 ± 1.78
	15	58.69	23.43	27.83	27.73	27.05	32.95 ± 6.49
	30	47.45	18.62	24.45	23.83	16.62	26.20 ± 5.52
	45	32.79	17.05	20.90	6.63	15.87	18.65 ± 4.24
	60	26.25	15.42	15.43	4.89	10.43	14.48 ± 3.53
	75	24.77	7.81	12.66	4.27	8.25	11.55 ± 3.6
	90	16.51	7.05	10.11	2.08	8.11	8.77 ± 2.3
	105	14.79	6.49	9.83	2.42	5.79	7.86 ± 2.09
	120	11.45	7.06	7.92	2.36	8.29	7.42 ± 1.47
	135	12.98	7.65	6.22	1.78	8.44	7.42 ± 1.81

Table 15a: Regression analysis of variance regarding plasma prolactin concentration on day 7 (follicular phase) of menstrual cycle from -60 minute to 0 minutes time in female monkey before single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.041	0.041	0.459	0.547
Residual	3	0.267	0.089		
Total	4	0.308		b=-0.064±0.094	

Table 15b: Regression analysis of variance regarding plasma prolactin concentration on day 7 (follicular phase) of menstrual cycle from 15 minute to 135 minutes time in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	574.993	574.993	39.214	0.0004
Residual	7	102.640	14.663		
Total	8	677.633		b=-3.095±0494	

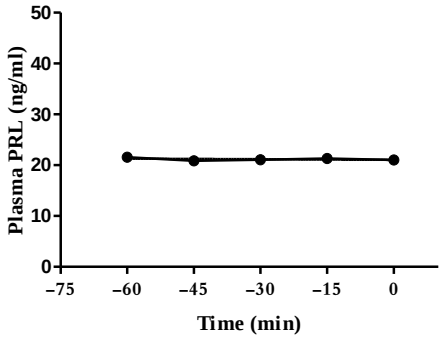


Fig. 18a: Regression analysis of variance showed no change in plasma prolactin concentration (ng/ml) on day 7 (follicular phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

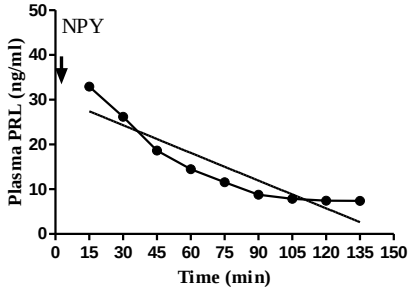


Fig. 18b: Regression analysis of variance showed highly significant decrease in plasma prolactin concentration (ng/ml) on day 7 (follicular phase) from 15 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

prolactin level was recorded for one hour before administration of NPY single bolus i.v. injection with an interval of 15 minutes. No significant difference in prolactin concentration was observed from -60 minutes to 0 minutes (within an hour). Regression analysis of variance showed that mean plasma PRL level was not significantly different during pretreatment period (b=-0.004±0.095; F (1, 3)=0.002; P=0.966), (Table 16a and Fig. 19a).

At 0 minute, a single bolus i.v. injection of NPY was given and plasma prolactin concentration was recorded after every fifteen minutes for a period of two hours and fifteen minutes. A significant and maximum rise in mean plasma prolactin concentration after 15 minutes of NPY bolus injection was observed compared to that of 0 minute ($t_{(8)}$ =-2.557; P=0.033). Then sharp and significant decrease in plasma PRL concentration was recorded from 15 minutes onwards which progressively continued till end of experiment. Regression analysis of variance showed highly significant temporal decrease in plasma prolactin concentration from 15 minute to 135 minutes (b=-2.076±0.452; F(1,7)=21.089; P=0.003), (Table 16b and Fig. 19b).

Day 21 (Luteal Phase) of menstrual cycle

The individual and mean prolactin (PRL) concentration (ng/ml) before and after NPY bolus i.v. administration in five female monkeys on day 21 of menstrual cycle (luteal phase) is shown in Table 17. Non-significant difference in prolactin concentration was observed from -60 minutes to 0 minutes (p>0.05). Regression analysis of variance showed that mean plasma PRL level was not different significantly during pretreatment period (b=-0.015±0.040; F (1,3)=0.151; P= 0.724), (Table 17a and Fig. 20a).

At 0 minute, a single bolus i.v. injection of NPY was given and plasma prolactin concentration was recorded after every fifteen minutes for a period of two hours and fifteen minutes. Decrease in prolactin concentration was observed after 15 minutes of NPY administration compared to that at 0 minute (ng/ml) ($t_{(8)}$ =0.688;P=0.51). The decrease in plasma prolactin level reached to significant level at 45 minutes compared to that at 0 minutes ($t_{(8)}$ =3.119;P=0.014) and continued till the end of experiment.

Regression analysis of variance showed highly significant temporal decrease in mean prolactin concentration from 15 minute to 135 minutes ($b=-1.647\pm0.276$; $F(1,8)=35.586$; $P=0.0003$), (Table 17b and Fig. 20b).

Table 16: Individual and mean plasma prolactin concentration before and after NPY single bolus injection in female rhesus monkey on day 15 (periovulatory phase) of menstrual cycle.

Plasma Prolactin Concentration (ng/ml)						
	Monkeys #					
Time (min)	1	2	3	4	5	Mean ± SE
-60	26.42	31.65	28.52	28.35	22.70	27.53 ± 1.47
-45	25.78	31.86	27.73	27.58	22.60	27.11 ± 1.50
-30	25.73	32.04	27.84	27.97	24.88	27.69 ± 1.24
-15	26.37	32.69	28.82	28.00	22.54	27.68 ± 1.65
NPY ⟶ 0	26.51	32.61	26.76	27.97	22.48	27.26 ± 1.63
15	35.30	41.52	33.47	33.10	27.87	34.25 ± 2.20
30	30.67	35.74	29.41	31.95	22.33	30.02 ± 2.19
45	19.58	32.81	22.69	16.80	19.93	22.36 ± 2.77
60	16.78	30.99	14.91	14.85	20.00	19.51 ± 3.02
75	16.70	21.40	15.25	14.20	19.88	17.49 ± 1.37
90	16.21	14.13	21.76	13.28	19.75	17.03 ± 1.63
105	15.82	10.98	25.62	13.01	19.12	16.91 ± 2.57
120	14.21	10.03	28.60	12.55	18.98	16.88 ± 3.28
135	14.13	9.56	27.61	11.47	18.77	16.31 ± 3.22

Table 16a: Regression analysis of variance regarding plasma prolactin concentration on day 15 (periovulatory phase) of menstrual cycle from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.000	0.000	0.002	0.966
Residual	3	0.269	0.090		
Total	4	0.269		b=0.004±0.095	

Table-16b: Regression analysis of variance regarding plasma prolactin concentration (ng/ml) on day 15 (peri-ovulatory phase) of menstrual cycle from 15 minutes to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	258.745	258.745	21.089	0.003
Residual	7	85.885	12.269		
Total	8	344.630		b=-2.076±0.452	

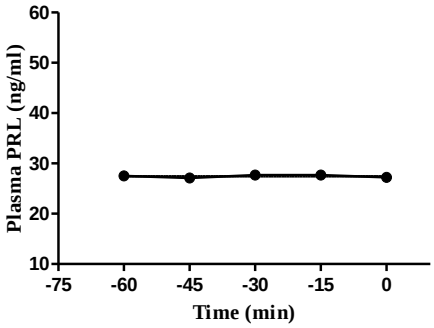


Fig. 19a: Regression analysis of variance showed no change in plasma prolactin concentration (ng/ml) on day 15 (periovulatory phase) from -60 minute to 0 minute in female monkey before single bolus i.v. injection of NPY.

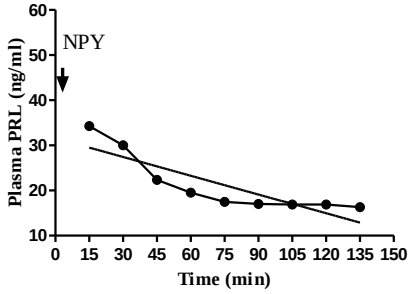


Fig. 19b: Regression analysis of variance showed highly significant decrease in plasma prolactin concentration (ng/ml) on day 15 (periovulatory phase) from 15 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Table 17: Individual and mean plasma prolactin concentration before and after NPY single bolus injection in female rhesus monkey on day 21 (luteal phase) of menstrual cycle.

Plasma Prolactin Concentration (ng/ml)							
		Monkeys #					
	Time (min)	1	2	3	4	5	Mean ± SE
	-60	20.79	21.03	20.30	35.72	21.00	23.77 ± 2.99
	-45	21.79	21.07	20.47	35.58	21.16	24.01 ± 2.90
	-30	21.74	21.78	20.77	34.12	21.05	23.89 ± 2.57
	-15	21.15	20.24	20.84	35.14	21.25	23.72 ± 2.86
NPY	⟶ 0	21.60	20.85	20.99	34.59	21.15	23.84 ± 2.69
	15	17.56	20.09	19.51	32.65	15.46	21.05 ± 3.01
	30	16.43	11.86	18.28	29.56	2.93	15.81 ± 4.34
	45	16.13	11.02	16.24	14.62	2.00	12.00 ± 2.67
	60	15.05	11.74	16.55	12.32	3.86	11.90 ± 2.19
	75	14.17	6.93	15.97	8.00	3.17	9.65 ± 2.37
	90	13.91	6.63	13.75	8.95	2.74	9.20 ± 2.14
	105	12.47	6.17	14.46	7.62	2.34	8.61 ± 2.18
	120	12.44	5.98	12.04	8.63	3.06	8.43 ± 1.79
	135	11.93	7.27	10.86	7.39	4.61	8.41 ± 1.33

Table 17a: Regression analysis of variance regarding plasma prolactin concentration (ng/ml) on day 21 (luteal phase) of menstrual cycle from -60 minutes to 0 minutes in female monkey before single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.002	0.002	0.151	0.724
Residual	3	0.050	0.017		
Total	4	0.052		b=-0.015±0.040	

Table 17b: Regression analysis of variance regarding plasma prolactin concentration (ng/ml) on day 21 (luteal phase) of menstrual cycle from 15 minutes to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	223.894	223.894	35.586	0.0003
Residual	8	50.333	6.292		
Total	9	274.227		b=-1.647±0.276	

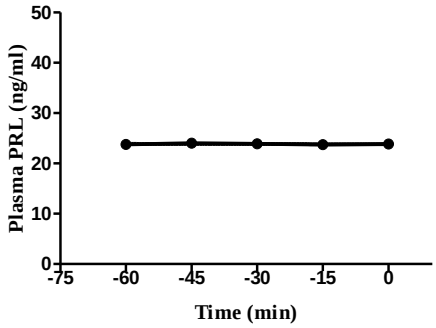


Fig. 20a: Regression analysis of variance showed no change in plasma prolactin concentration (ng/ml) on day 21 (luteal phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY

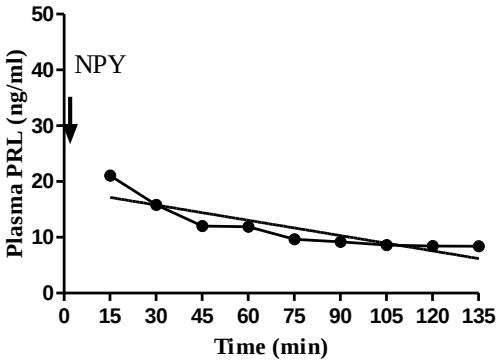


Fig. 20b: Regression analysis of variance showed highly significant decrease in plasma prolactin concentration (ng/ml) on day 21 (luteal phase) from 15 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

Growth Hormone

Plasma Growth Hormone (GH) concentrations in female rhesus monkey during the menstrual cycle

Baseline profile of plasma GH during menstrual cycle

Individual and mean basal plasma growth hormone (GH) profile in the female monkeys during the menstrual cycle is shown in Table 18 and Fig. 21. Mean plasma GH levels were non-significantly low during menstrual phase (day 1-4) compared to follicular (day 7-13) ($t_{(8)}$ =0.814; P=0.439) peri-ovulatory (14-16) ($t_{(8)}$ =0.369; P=0.721) and luteal phases (day 19-31) ($t_{(8)}$ =0.729; P=0.486). The plasma growth hormone level of peri-ovulatory phase was not significantly different from follicular ($t_{(8)}$ =0.476; P=0.646) and luteal ($t_{(8)}$ =0.4129; P=0.690) phases. The plasma GH levels of follicular and luteal phases were not different (Table 18a).

Effect of single injection of 200 µg NPY on plasma growth hormone concentration in female rhesus monkey during different phases of menstrual cycle

Day 1 (Menstrual Phase)

The individual and mean plasma growth hormone (GH) concentration (ng/ml) before and after NPY bolus administration in female monkeys on day 1 of menstrual phase of menstrual cycle is given in Table 19. Mean plasma Growth hormone level was recorded one hour before the injection of NPY with an interval of 15 minutes. The plasma growth hormone level remained more or less the same up to 0 minute (within an hour) before NPY treatment. Regression analysis of variance showed that mean plasma GH level was not different significantly during pre-treatment period (b=-0.012±0.014; F (1,3)=0.599; P=0.495), (Table 19a and Fig. 22a).

At 0 minute, NPY single bolus intravenous (i.v.) injection was given and growth hormone levels were recorded after every fifteen minutes for a period of two hours and

fifteen minutes. Decrease in plasma growth hormone concentration was observed after 15 minutes of NPY bolus injection compared to that at 0 minute ($t_{(8)}$ =1.688; P=0.129). Then decrease in growth hormone concentration continued up-to 45 minutes. Regression analysis of variance showed non-significant temporal decrease in mean plasma growth hormone concentration from 0 minute to 45 minutes time (b=-0.522±0.0204; F(1,2)=6.545; P=0.125), (Table 19b and Fig. 22b). Then gradual increase was recorded after 45 minutes till the end of experiment in plasma GH concentration and remained low compared to that of base line. Regression analysis of variance showed significant temporal increase in mean plasma growth hormone concentration from 45 minute to 135 minutes time (b=0.093±0.018; F(1,5)=26.297; P=0.004), (Table 19c and Fig. 22c).

Day 7 (Follicular Phase) of menstrual cycle

The individual and mean plasma growth hormone (GH) concentration before and after NPY administration in five female monkeys during the follicular phase of menstrual cycle is given in Table 20. Growth hormone concentration was recorded from -60 minutes after every 15 minutes for an hour. Before NPY treatment no significant difference in growth hormone concentration was observed from -60 minutes to 0 minutes (within an hour). Regression analysis of variance showed that mean plasma GH level was not significantly different during pretreatment period with NPY (b=0.007±0.011; F(1,3)=0.409; P=0.568), (Table 20a and Fig. 23a).

At 0 minute, NPY bolus injection i.v. was given and growth hormone levels were recorded after every fifteen minute for a period of two hour and fifteen minutes. A significant decrease in plasma growth hormone concentration was observed after 15 minutes of treatment compared to that of at 0 minute ($t_{(8)}$=2.457; P=.038). This decrease in plasma GH concentration was continued till the end of experiment. Regression analysis of variance showed temporal decrease in growth hormone concentration from 15 minute to 135 minutes time (b=-0.011±0.028; F(1,7)=0.141; P=0.718), (Table 20b and Fig. 23b).

Table 18: Individual and mean baseline profile of plasma growth hormone concentration (ng/ml) on different days of the menstrual cycle in adult female rhesus monkey.

Plasma Growth Hormone Concentration (ng/ml)						
Menstrual Cycle	**Monkeys #**					
Day	**1**	**2**	**3**	**4**	**5**	**Mean ± SE**
1	4.67	2.69	2.53	2.38	6.16	3.68 ± 0.75
2	3.50	2.35	2.61	2.25	6.50	3.44 ± 0.80
4	2.96	2.95	2.75	2.29	6.90	3.57 ± 0.84
7	5.66	3.10	3.86	3.09	6.51	4.44 ± 0.70
10	8.20	3.88	3.62	2.62	6.70	5.00 ± 1.05
13	2.83	2.57	3.57	3.82	5.90	3.74 ± 0.59
14	3.69	3.00	4.71	2.96	4.68	3.81 ± 0.38
15	4.33	1.69	6.92	1.61	4.01	3.71 ± 0.98
16	4.16	3.95	4.63	1.43	7.40	4.31 ± 0.95
19	3.80	3.33	5.23	2.85	6.97	4.44 ± 0.75
21	6.23	2.73	5.89	2.33	4.68	4.37 ± 0.80
22	8.02	2.98	3.42	3.51	3.36	4.26 ± 0.95
25	4.90	2.74	3.29	2.71	8.68	4.46 ± 1.13
28	6.00	2.68	5.70	1.17	7.78	4.67 ± 1.20
31	5.62	1.98	3.35	2.09	7.23	4.05 ± 1.03

Table 18 a: Mean plasma growth hormone concentration (ng/ml) during different phases of menstrual cycle in adult female rhesus monkey.

Plasma Growth Hormone Concentration (ng/ml)			
Menstrual Phase (1-4 Days)	**Folicular Phase (7-13 Day)**	**Periovulatory Phase (14-16 Days)**	**Luteal Phase (19-31 Days)**
3.57 ± 0.78	4.39 ± 0.66	3.95 ± 0.68	4.38 ± 0.48

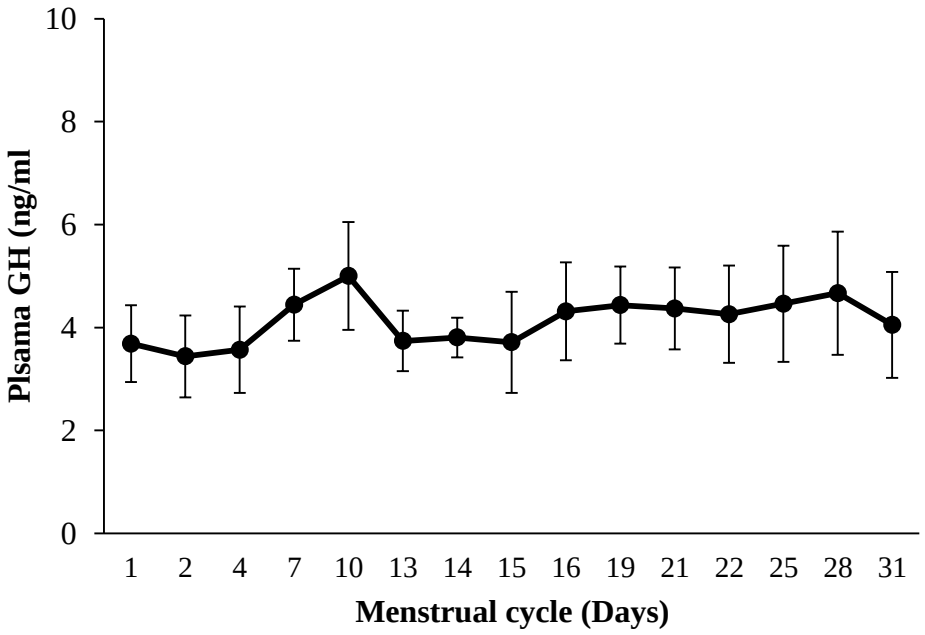


Fig. 21: Mean baseline profile of plasma growth hormone concentration (ng/ml) in female rhesus monkey on different days of menstrual cycle.

Table 19: Individual and mean plasma growth hormone concentration before and after NPY single bolus i.v. injection in female rhesus monkey on day 1 (menstrual phase) of menstrual cycle.

Plsama Growth Hormone Concentration (ng/ml)						
		Monkeys #				
	Time (min)	1	2	3	4	5	Mean ± SE
	-60	4.62	2.60	2.48	2.37	6.13	3.64 ± 0.75
	-45	4.67	2.66	2.78	2.36	6.19	3.73 ± 0.74
	-30	4.51	2.64	2.47	2.36	6.17	3.63 ± 0.75
	-15	4.47	2.62	2.47	2.37	6.17	3.62 ± 0.75
NPY $\longrightarrow$	0	4.48	2.62	2.49	2.38	6.23	3.64 ± 0.75
	15	1.61	2.55	2.02	2.24	3.09	2.30 ± 0.25
	30	1.11	2.44	1.86	2.18	2.96	2.11 ± 0.31
	45	0.91	2.42	1.68	2.17	2.63	1.96 ± 0.31
	60	2.10	2.40	1.67	2.16	2.09	2.08 ± 0.12
	75	2.20	2.45	1.64	2.18	2.19	2.13 ± 0.13
	90	2.55	2.47	1.45	2.21	2.61	2.26 ± 0.21
	105	2.51	2.61	1.78	2.27	2.15	2.26 ± 0.15
	120	2.59	2.65	1.61	2.32	2.25	2.28 ± 0.18
	135	2.61	2.68	3.39	2.35	2.28	2.66 ± 0.20

Table 19a: Regression analysis of variance regarding plasma growth hormone concentration (ng/ml) before NPY single bolus i.v. injection on day 1 (menstrual phase) of menstrual from -60 minute to 0 minutes time.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.001	0.001	0.599	0.495
Residual	3	0.007	0.002		
Total	4	0.008		b=-0.011±0.014	

Table 19b: Regression analysis of variance regarding plasma growth hormone concentration (ng/ml) after NPY single bolus i.v. injection on day 1 (menstrual phase) of menstrual cycle from 0 minute to 45 minutes time.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	1.364	1.364	6.545	0.125
Residual	2	0.417	0.208		
Total	3	1.780		b=-0.522±0204	

Table 19c: Regression analysis of variance regarding plasma growth hormone concentration (ng/ml) after NPY single bolus i.v. injection in female rhesus monkey on day 1 (menstrual phase) of menstrual cycle from 45 minute to 135 minutes time.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.247	0.247	26.297	0.004
Residual	5	0.047	0.009		
Total	6	0.294		b=0.093±0183	

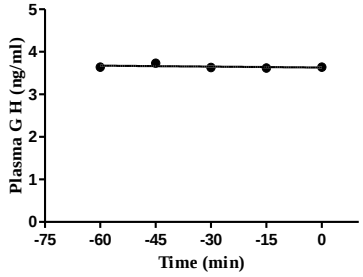


Fig. 22a: Regression analysis of variance showed no change in plasma growth hormone concentration (ng/ml) on day 1 (menstrual phase) from -60 minute to 0 minutes in female rhesus monkey before single bolus i.v. injection of NPY.

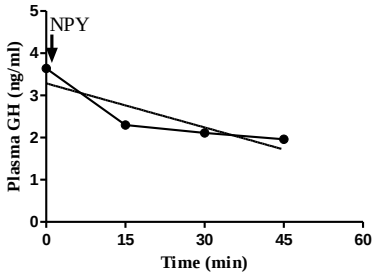


Fig. 22b: Regression analysis of variance showing non-significant decrease in plasma growth hormone concentration (ng/ml) on day 1 (menstrual phase) from 0 minute to 45 minutes in female rhesus monkey after single bolus i.v. injection of NPY.

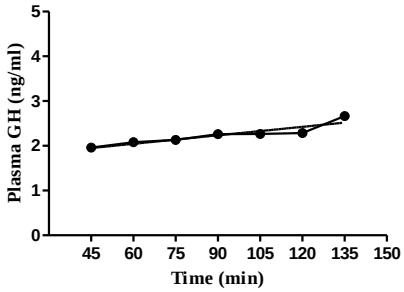


Fig. 22c: Regression analysis of variance showed significant increase in plasma growth hormone concentration (ng/ml) on day 1 (menstrual phase) from 45 minute to 135 minutes in female rhesus monkey after single bolus i.v. injection of NPY.

Table 20: Individual and mean plasma growth hormone concentrations (ng/ml) before and after NPY single bolus i.v. injection in female rhesus monkey on day 7 (follicular phase) of menstrual cycle.

Plasma Growth Hormone Concentration (ng/ml)						
	Monkeys #					
Time (min)	1	2	3	4	5	Mean ± SE
-60	5.55	3.02	3.80	3.05	6.40	4.37 ± 0.69
-45	5.59	2.84	3.79	2.95	6.78	4.39 ± 0.78
-30	5.58	2.71	3.84	2.95	6.63	4.34 ± 0.76
-15	5.60	2.90	3.85	2.86	6.93	4.43 ± 0.80
NPY ⟶ 0	5.61	2.77	3.72	2.86	6.95	4.38 ± 0.82
15	1.78	1.54	3.17	2.38	2.29	2.23 ± 0.28
30	1.02	2.57	3.02	2.44	1.55	2.12 ± 0.36
45	0.76	2.87	2.73	2.23	1.55	2.03 ± 0.39
60	0.36	2.84	2.74	2.21	1.66	1.96 ± 0.45
75	0.29	2.55	2.02	2.32	1.43	1.72 ± 0.40
90	0.26	2.50	1.18	2.20	2.25	1.68 ± 0.42
105	0.31	2.90	0.92	2.19	3.14	1.89 ± 0.55
120	0.96	2.60	1.08	2.19	3.21	2.01 ± 0.44
135	0.73	2.86	1.80	2.23	3.84	2.29 ± 0.52

Table 20a: Regression analysis of variance regarding plasma growth hormone concentration before single bolus i.v. injection of NPY on day 7 (follicular phase) of menstrual cycle from -60 minute to 0 minutes time in female monkey.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.000	0.000	0.409	0.568
Residual	3	0.004	0.001		
Total	4	0.004		b=0.007±0.011	

Table 20b: Regression analysis of variance regarding plasma growth hormone concentration after single bolus i.v. injection of NPY on day 7 (follicular phase) of menstrual cycle from 15 minute to 135 minutes in female monkey.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.007	0.007	0.141	0.718
Residual	7	0.343	0.049		
Total	8	0.350		b=-0.011±0.028	

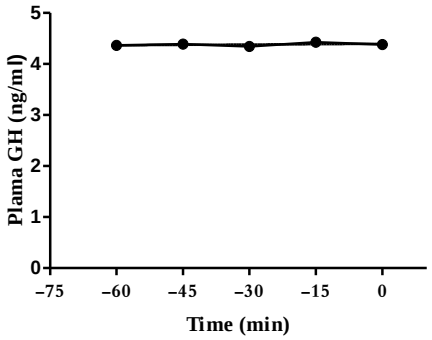


Fig. 23a: Regression analysis of variance showed no change in plasma growth hormone concentration (ng/ml) on day 7 (follicular phase) from -60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

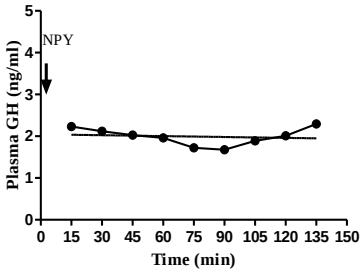


Fig. 23b: Regression analysis of variance showed non-significant decrease in plasma growth hormone concentration (ng/ml) on day 7 (follicular phase) from 15 minute to 135 in female monkey after single bolus i.v. injection of NPY.

Day 15 (Periovulatory Phase) of menstrual cycle

The individual and mean plasma growth hormone (GH) concentration before and after NPY administration in five female monkeys on day 15 is shown in Table 21. Plasma growth hormone level was recorded for one hour before administration of NPY single bolus i.v. injection with an interval of 15 minutes. No significant difference in growth hormone concentration was observed from -60 minutes to 0 minutes (within an hour). Regression analysis of variance showed that mean plasma Growth hormone level was not significantly different during pretreatment period ($b=-0.003\pm0.002$; $F(1, 3)=1.487$; $P=0.310$), (Table 21a and Fig. 24a).

At 0 minute, NPY i.v. bolus injection was given and growth hormone levels were recorded after every fifteen minute for a period of two hour and fifteen minutes. A non-significant decrease in mean growth hormone concentration after 15 minutes of NPY bolus injection was observed which continued with slight fluctuation till the end of experiment. Regression analysis of variance showed temporal decrease in plasma growth hormone concentration from 15 minute to 135 minutes ($b=-0.009\pm0.026$; $F(1,7)= 0.137$; $P=0.722$), (Table 21b and Fig. 24b).

Day 21 (Luteal Phase) of menstrual cycle

The individual and mean plasma growth hormone (GH) concentration ((ng/ml)) before and after NPY bolus i.v. administration in five female monkeys on day 21 of menstrual cycle (luteal phase) is shown in Table 22. Plasma growth hormone level was recorded for one hour before administration of NPY single bolus i.v. injection with an interval of 15 minutes. No significant difference in growth hormone concentration was observed from -60 minutes to 0 minutes ($p>0.05$). Regression analysis of variance showed that mean plasma GH level was not different significantly during pretreatment period ($b=0.057\pm0.023$; $F(1,3)=6.116$; $P=0.09$), (Table 22a and Fig. 25a).

At 0 minute, NPY i.v. bolus injection was given and growth hormone levels were recorded after every fifteen minute for a period of two hour and fifteen minutes. A non-significant decrease in plasma growth hormone concentration was observed after 15

minutes of NPY administration compared to that at 0 minute (ng/ml) ($t_{(8)}$ =0.340;P=0.742). Then slow decrease continued till the end of experiment and was highly significant from 75 minutes onwards compared to that at o minute. Regression analysis of variance showed significant temporal decrease in mean plasma growth hormone concentration from 15 minute to 135 minutes time (b=-0.165±0.038; F(1,7)=18.326; P=0.004), (Table 22b and Fig. 25b).

Table 21: Individual and mean plasma growth hormone concentration before and after NPY single bolus i.v. injection in female rhesus monkey on day 15 (periovulatory phase) of menstrual cycle.

Plsama Growth Hormone Concentration (ng/ml)						
	Monkey #					
Time (min)	1	2	3	4	5	Mean ± SE
-60	4.22	1.66	6.90	1.57	3.98	3.67 ± 0.98
-45	4.24	1.66	6.98	1.56	3.97	3.68 ± 1.00
-30	4.24	1.61	6.94	1.59	3.96	3.67 ± 0.99
-15	4.24	1.67	6.86	1.59	3.94	3.66 ± 0.97
NPY ⟶ 0	4.25	1.65	6.84	1.61	3.95	3.66 ± 0.97
15	2.87	1.38	5.09	1.26	2.66	2.65 ± 0.69
30	2.09	1.34	2.39	1.22	2.86	1.98 ± 0.31
45	2.70	1.50	2.44	1.23	2.26	2.03 ± 0.28
60	2.76	1.54	2.49	1.36	2.21	2.07 ± 0.27
75	2.92	1.53	2.34	1.42	2.44	2.13 ± 0.29
90	2.83	1.61	2.25	1.43	2.66	2.16 ± 0.28
105	2.77	1.68	2.25	1.47	2.81	2.20 ± 0.27
120	3.10	1.70	1.88	1.49	2.93	2.22 ± 0.33
135	3.08	1.71	1.76	1.56	2.98	2.22 ± 0.33

Table 21a: Regression analysis of variance regarding plasma growth hormone concentration before NPY single bolus i.v. injection on day 15 (periovulatory phase) of menstrual cycle from -60 minute to 0 minutes in female monkey.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.0001	0.0001	1.487	0.310
Residual	3	0.0002	0.0001		
Total	4	0.0003		b=-0.003±0.002	

Table 21b: Regression analysis of variance regarding plasma growth hormone concentration after NPY single bolus i.v. injection on day 15 (periovulatory phase) of menstrual cycle from 15 minute to 135 minutes in female monkey.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.006	0.006	0.137	0.722
Residual	7	0.299	0.043		
Total	8	0.305		b=-0.009±0.026	

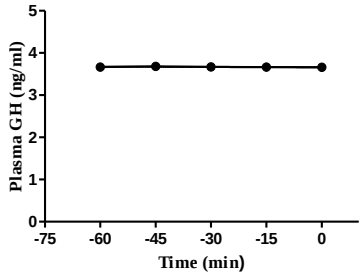


Fig. 24a: Regression analysis of variance showed no change in plasma growth hormone concentration (ng/ml) on day 15 (periovulatory phase) from-60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

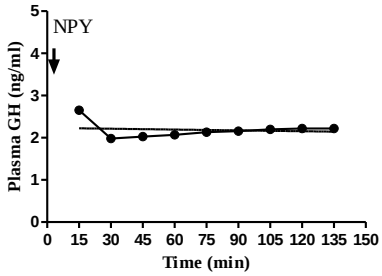


Fig. 24b: Regression analysis of variance showed non-significant decrease in plasma growth hormone concentration (ng/ml) on day 15 (periovulatory phase) from 15 minute to 135 minutes in female rhesus monkey after single bolus i.v. injection of NPY.

Table 22: Individual and mean plasma growth hormone concentration before and after NPY injection in female rhesus monkey on day 21 day (luteal phase) of menstrual cycle.

Plasma Growth Hormone Concentration (ng/ml)						
	Monkeys #					
Time (min)	1	2	3	4	5	Mean ± SE
-60	6.25	2.71	5.40	2.41	4.85	4.32 ± 0.76
-45	6.12	2.71	5.38	2.35	4.85	4.28 ± 0.75
-30	6.28	2.73	5.49	2.40	4.55	4.29 ± 0.76
-15	6.19	2.75	6.27	2.29	4.56	4.41 ± 0.83
NPY ⟶ 0	6.29	2.75	6.90	2.20	4.59	4.55 ± 0.93
15	4.03	1.57	4.97	2.26	3.92	3.35 ± 0.62
30	3.77	1.57	4.76	2.17	2.97	3.05 ± 0.57
45	2.89	1.50	4.28	2.18	2.27	2.62 ± 0.47
60	2.96	1.61	3.50	2.11	2.38	2.51 ± 0.33
75	2.07	1.66	2.21	2.25	2.39	2.12 ± 0.12
90	1.84	1.67	1.15	2.26	2.42	1.87 ± 0.23
105	1.99	1.78	0.72	2.48	2.39	1.87 ± 0.32
120	2.05	2.80	0.59	2.50	2.60	2.11 ± 0.40
135	2.08	2.85	0.59	2.58	2.94	2.21 ± 0.43

Table 22a: Regression analysis of variance regarding plasma growth hormone concentration before NPY single bolus i.v. injection on day 21 (luteal phase) of menstrual cycle from -60 minutes to 0 minutes in rhesus monkey.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	0.033	0.033	6.116	0.090
Residual	3	0.016	0.005		
Total	4	0.049		b=0.057±0.023	

Table 22b: Regression analysis of variance regarding plasma growth hormone concentration after NPY single bolus i.v. injection on day 21 (luteal phase) of menstrual cycle from 15 minutes to 135 minutes in rhesus monkey.

Source	*df*	*SS*	*MS*	*F*	*P*
Regression	1	1.636	1.636	18.326	0.004
Residual	7	0.625	0.089		
Total	8	2.261		b=-0.165±0.038	

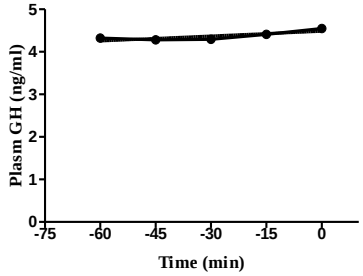


Fig. 25a: Regression analysis of variance showed no change in plasma growth hormone concentration (ng/ml) on day 21 (luteal phase) from-60 minute to 0 minutes in female monkey before single bolus i.v. injection of NPY.

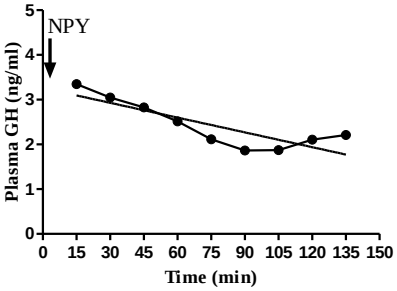


Fig. 25b: Regression analysis of variance showed significant decrease in plasma growth hormone concentration (ng/ml) on day 21 (luteal phase) from 15 minute to 135 minutes in female monkey after single bolus i.v. injection of NPY.

DISCUSSION

Discussion

Neuropeptide Y (NPY) acts at the hypothalamic level to regulate the reproductive function by stimulating the release of GnRH from GnRH neurons. In the present study the possible role of NPY on hormones during different phases of menstrual cycle of adult female rhesus monkey (n=5) was investigated. In addition to this, change in body weight, sex skin colour and baseline profile of plasma estradiol (E2), progesterone (P), prolactin (PRL) and growth hormone (GH) was recorded throughout the menstrual cycle of 31 days.

In earlier literature, effect of NPY on hormones during different phases of menstrual cycle has not been reported in primates. Therefore, this study was designed to determine the effect of 200 µg NPY single bolus intravenous (i.v.) injection on ovarian steroids (estradiol and progesterone) and pituitary hormones (prolactin and growth hormone) was observed on day 1 (menstrual phase), day 7 (follicular phase), day 15 (ovulatory phase) and day 21 (luteal phase) of menstrual cycle.

There were no significant differences in the body weight measured during different phases of the menstrual cycle. However there was progressive increase in sex skin color intensity from menstrual to periovulatory phases. The peak coloration was observed at mid-cycle corresponding to peak in the estradiol levels. In this study the changes observed in sex skin were similar to those reported by Redmond et al. (1975) and Czaja et al. (1977) in rhesus monkey. Then consistent fading in sex skin coloration was observed during the luteal phase (till the end of the menstrual cycle) corresponding to decrease in the level of estrogen.

Estradiol

Baseline profile of estradiol

Baseline profile showed that plasma E_2 concentration was significantly high in follicular and peri-ovulatory phases compared to menstrual and luteal phases ($P<0.001$). In the present study the pattern of changes in plasma E_2 concentration observed during different phases of menstrual cycle, closely resemble to the baboon (Goncharov et al., 1976), and rhesus monkey (Hotchkiss et al., 1971; Bosu et al., 1972; Karsch et al., 1973; Knobil,

1980; Evans and Foltin, 2006; Jahan et al., 2007; Weinbauer et al., 2008). In the present study the estradiol level showed progressive increase from follicular to periovulatory phase in which the estrogen peak was observed. These results are in agreement with the work of Knobil (1974) in rhesus monkey, and Cynomogolus monkey (Stabenfeldt and Hendrickx, 1972), and in human by Thorneycroft et al. (1971); Shah et al. (1999); Jaffe et al. (2000) and Emanuele et al. (2002). However, during the luteal phase plasma estradiol concentration was lower than that observed in human females as reported by Jaffe et al. (2000) and Lacreuse (2006). In the current study no second estradiol peak was observed during luteal phase in the female rhesus monkey. Knobil and Hotchkiss (1988); Lacreuse (2006) also reported that absences of secondary estrogen peak during the luteal phase in monkeys. In this study increased estradiol concentration during late follicular and periovulatory phases was consistent with the findings of Cunningham et al. (1999) and Hileman et al. (2000). Current findings indicated lowest level of estradiol during menstrual phase followed by progressive significant increase during the follicular phase and reached the highest point (maximum) at midcycle (periovulatory phase). Then there was progressive significant (continuously visible) decrease in the plasma estradiol concentration (levels) during the luteal phase of menstrual cycle. The increased secretion of ovarian steroids, especially E_2 during mid-cycle phase was a trigger for the preovulatory events which acted at the pituitary and hypothalamic level (Dailey and Neill, 1981; Cunningham et al., 1999; Hileman et al., 2000). The increase in circulating estradiol concentration at late follicular phase was sufficient to generate the GnRH surge in some spontaneously ovulating species (Moenter et al., 1990; Xia et al., 1992 and Karsch et al., 1997), and in rat which requires the rising levels of estrogen to coincide with a circadian input to generate the LH surge (Legan et al., 1975; Sarkar and Fink, 1980). The pattern of GnRH secretion during the periovulatory phase undergoes a dramatic elevation (Nippoldt et al., 1989; Cunningham, 1999; Flier, 1998) due to high level of plasma estradiol. Some investigators have reported that enhanced GnRH release in mediobasal hypothalamus (MBH) and in third ventricle lasts for several hours and goes with a temporally related rise and fall in plasma LH. These observations suggested

an important, critical, influence of estrogen upon the functioning of mammalian GnRH neurons at hypothalamic level that influences the release of FSH/LH from adenohypophysis, which in turn stimulates the follicular cells of the ovary during early follicular phase to release estrogen. During follicular phase the estrogen level has a negative feedback on hypothalamus to release LH and shows positive feedback at its highest level on the preovulatory GnRH and LH surge. The low level of estrogen during luteal phase is also reported by Lacreuse (2006).

Effect of Neuropeptide Y on plasma estradiol concentration

In the present study the effect of Neuropeptide Y (NPY) on the plasma E_2 concentration during four phases of the menstrual cycle (i.e., day 1 of menstrual, day 7 of follicular, day 15 of periovulatory and day 21 of luteal phase) under different physiological states in rhesus monkey was stimulatory 15 minutes after its administration.

On day 1 (menstrual phase) of menstrual cycle after 15 minutes of NPY administration a non-significant increase in plasma estradiol concentration was observed compared to baseline at zero minute. The maximum increase in plasma E_2 concentration was at 30 minutes followed by significant temporal decrease and attained basal level at 120 minutes. This initial increase in plasma E_2 level may be due to NPY mediated GnRH release from GnRH neurons and then LH/FSH release from gonadotrophs of pituitary in response to GnRH, which in turn stimulated ovarian estradiol release. Previous studies showed that interacerebroventricle (i.c.v.) injection of NPY in ovariectomized rats treated with estrogen and progesterone produces a transient stimulation of LH release (Kalras and Crowley, 1984). Gonadally intact rabbits also display NPY-induced GnRH secretion during push-pull perfusion of the hypothalamus (Khorramo et al., 1987) specifically at the median eminence (Sabatino et al., 1990). NPY also enhanced the release of FSH significantly 15 minutes after i.c.v. injection in male rat (Alexander et al., 1993; Reznikov and McCann, 1993). The rise in estradiol concentration on day 1 of menstrual phase in this study may be due to the stimulatory effect of NPY on granulosa cells. Barreca et al. (1998) found that NPY stimulated the E2 release from human granulosa

cells of the follicles incubated with hCG in the early stage of luteinization and showed direct role in ovarian steroidogenesis. This role of NPY is important in controlling the positive feedback effect of ovarian steroids to stimulate LH release from the pituitary. Whereas, in some other studies it was reported that NPY was not able to stimulate the cultured granulosa cells of rat ovary to release estradiol (Baranowska et al., 1999).

On day 7 (follicular phase) of menstrual cycle a non-significant increase in plasma estradiol concentration was observed 15 minutes after NPY treatment which reached maximum level at 60 minutes followed by significant temporal decrease and plasma estradiol attained the basal level after an hour. The NPY stimulated E2 increase lasted for an hour in follicular phase. It is suggested that due to added release of GnRH and gonadotropins for long duration, mediated by NPY, stimulated the release of estrogen from ovary for longer period of time. NPY stimulated the release of FSH and LH from dispersed perfused anterior pituitary cells of ovariectomized (OVX) rats in dose associated manner, lower doses of NPY (10-7M) stimulated rapid increase in FSH (1.6 fold) and LH (1.5 fold), whereas increase in dose of NPY (10-6 M) there was 2.4 fold increase in FSH and 5 fold in LH (McDonald et al., 1985). Throughout the cycle, NPY accompanies the pulses of GnRH that are intermittently released into the hypophysial portal vasculature (Woller and Terasawa, 1992). NPY not only controls the secretion of GnRH, but NPY also exerts a stimulatory effect on GnRH release in the monkey (Gore et al., 1993; Pau et al., 1995). Estrogen is one of the important determinants of GnRH neuronal functioning and acting as a classic homeostatic feedback molecule between ovary and brain. It is critical in enabling these cells to exhibit fluctuating patterns of biosynthetic and secretory activity for the greater part of the ovarian cycle. Estrogen restrains LH secretion through its “negative feedback” action. This has occurred, in part, through an inhibition of GnRH secretion in several species (Sarkar and Fink, 1980; Caraty et al., 1989; Chongthammakun and Terasawa, 1993), and also involves potent actions of estrogen on the pituitary gonadotrophs (Freeman, 1994; Goodman, 1994; Shupnik, 1996).

On day 15 (periovulatory phase) of menstrual cycle 15 minutes after NPY treatment plasma E2 level increased non-significantly and reached to the maximum level at 75 minutes followed by then significant temporal decrease till the end of experiment and plasma E_2 reached to basal level. Estradiol level remained high for a longer time in this periovulatory phase as compared to other phases. NPY effect in this phase may be more stimulatory on GnRH and gonadotrophs particularly on LH release. Woller and Terasawa (1994) studied the effect of estrogen on the responsiveness of GnRH to NPY stimulation. NPY stimulated GnRH release at doses of 10^{-6} to 10^{-12} M infused into the stalk-median eminence (S-ME) in ovariectomized monkeys primed with or without estrogen using a push-pull perfusion method. GnRH release in a dose-dependent manner, suggested that NPY stimulates GnRH release in the S-ME in the presence or absence of estrogen and that estrogen enhances the responsiveness of the GnRH neurosecretory system to NPY stimulation in the rhesus monkey (Woller and Terasawa, 1994). Numerous studies have shown that NPY augments LH release in proestrus (Bauer-Dantoin et al., 1991; Bauer-Dantoin et al., 1993; Besecke and Levine, 1994; Leupen, 1997) and pentobarbital-blocked, proestrous rats requiring both GnRH and NPY replacement for LH surge of in normal proportions (Bauer-Dantoin et al., 1992). Importantly, this augmentation of LH release cannot occur without a proestrous hormonal environment. NPY augments GnRH-stimulated LH secretion from anterior pituitaries removed from proestrous but not from metestrous rats (Bauer-Dantoin et al., 1993). Immunoneutralization of NPY in the portal circulation greatly attenuates the LH surge (Sutton et al., 1988) and the LH surge is stunted in NPY-knockout mice (Xu et al., 2000). NPY also has no effect on in vivo GnRH-stimulated LH secretion in pentobarbital-treated, metestrous, or ovariectomized (OVX) rats (Bauer-Dantoin et al., 1992). Notably, the action of GnRH on LH release is attenuated in estrogen-primed NPY-knockout mice (Xu et al., 2000). In the present study the increase in plasma E_2 level during periovulatory phase might be responsible for its direct or indirect effect to modulate hypothalamic GnRH neurons to secrete more GnRH in presence of exogenous NPY to enhance the release of LH from pituitary gonadotrophs. Although estrogen-positive feedback at the hypothalamus is known to increase GnRH

release at the time of the LH surge, it has long been recognized that a dramatic increase in pituitary responsiveness to GnRH input on proestrus represents an equal or greater factor in initiation of the LH surge. However, a stimulatory effect of NPY on LH has not been observed consistently in OVX, estrogen treated monkeys (Kaynard et al., 1990). The previous data showed that NPY has stimulatory and inhibitory effect on GnRH release, depending upon the site of NPY infusion within the brain rather than the ovarian steroidal environment (Pau et al., 1995). The NPY has the ability to feedback HPG axis. It has been demonstrated that NPY acts through the neuropeptide Y_1 receptor (Y1R) subtype to augment LH release. Peripheral administration of the selective Y_1R antagonist BIBP3226, a compound that does not cross the blood-brain barrier (Rudolf et al., 1997), attenuates both LH secretion and surges in proestrous rats induced by GnRH and NPY in pentobarbital-blocked, proestrous rats (Leupen et al., 1997). Y_1R expression is also highly dependent on circulating levels of steroid hormones. Y_1R mRNA levels in the hypothalamus increases during the late morning and afternoon of proestrus, when 17ß-estradiol (E2) levels are high and E_2 administration replicates this gain (Xu et al., 2000). In addition, Musso et al. (2000) found that E_2 treatment induces Y_1R gene expression in transfected neuroblastoma-glioma cells through the direct interaction of estrogen receptor (ER) with three hemipalindromic estrogen-responsive elements flanking the Y_1R gene. Dhillon et al. (2009) reported that NPY increase the GnRH gene expression in GT1-7 neurons. The NPY mediated gene expression in GT1-7 neurons was inhibited by NPY Y_1R antagonist BIBP-3226 (Dhillon et al., 2009). Hill et al. (2004) showed that from OVX rat's gonadotroph-enriched cells of pituitary, Y1R mRNA level was elevated when exposed to E2 (Hill et al., 2004). In vitro NPY enhanced the release of LH from these gonadotroph-enriched cells of OVX rats in presence of E_2 suggested that NPY action was mediated by Y1 receptors. Without steroid exposure, this augmentation disappeared, and with progesterone alone, NPY reduced GnRH-induced LH release (Hill et al., 2004). Beseke et al. (1994) reported that NPY significantly increased GnRH release when applied to GT1-7 neurons. Steroid milieu may be responsible for changing the direction of NPY effect on GnRH and LH secretion. Therefore it may be possible that in steroidal

milieu of peri-ovulatry phase, exogenous NPY effect was strong for LH release to stimulate ovarian steroidogenesis for longer duration.

On day 21 (luteal phase) of menstrual cycle 15 minutes after NPY plasma estradiol concentration also increased non-significantly administration and remained high from baseline level for an hour followed by significant temporal decrease leading to baseline level at the end of the experiment. Lowest increase in estradiol concentration during this phase may be due to high concentration of progesterone which down-regulated its own estrogen induced receptors, and its effect enhanced in the presence of NPY or in part may be due to the inability of NPY during luteal to exceed a putative stimulatory threshold of hypothalamic GnRH and LH to affect the release of estradiol.

Progesterone

Baseline profile of plasma progesterone

Baseline profile of plasma progesterone (P) showed that P levels were significantly low during menstrual, follicular and periovulatory phases compared to high plasma progesterone level in luteal phase between day 19–22 ($P<0.01$). There was no significant difference in plasma progesterone (P) concentration between menstrual, follicular, and peri-ovulatory phases. The results of the present research were similar to previous studies in monkeys (Evans and Foltin, 2006; Jahan et al., 2007; Weinbauer et al., 2008) and in women (Jaffe et al., 2000), showing gradual rise in plasma progesterone concentration from late follicular phase to mid-cycle and then mid-luteal progesterone peak during the menstrual cycle. The finding of present study indicated that progesterone concentrations may be important in presence of estradiol to facilitate the LH release from gonadotrophs for steroidogenesis in the ovary. The progressive increase in progesterone concentration during follicular phase may be required for the full expression of the gonadotropin surge at the time of preovulatory LH surge. These results are in concordance with previous findings of Liu and Yen (1983); Batista et al. (1992), showing that administration of a progesterone antagonist at mid-cycle in humans results in a delay or in the abolition of the gonadotropin surge. The rise in LH level may be due to increased activity of GnRH in

the presence of steroids (E_2 and P), on GnRH receptors on gonadotrophs induced by increased level of estradiol during follicular and periovulatory phase which may be decreased after LH surge. Zimmermann et al. (2002), reported that the numbers of GnRH receptors in gonadotrophs increased extensively in several species, with maximum numbers found near ovulation after which these numbers decline sharply post-ovulation (Schneider and Warren, 2006). The high level of progesterone during the luteal phase may result in a significant decrease in GnRH/LH release which in-turn reduced the release of steroid from ovary. Low level of progesterone was during late luteal phase reported by Van et al. (1984), similar to the present findings.

Effect of Neuropeptide Y on plasma progesterone concentration

In the current study on day 1 (menstrual phase) of the menstrual cycle, 15 minutes after a single bolus iv injection of 200µg NPY induced an increase in plasma progesterone which reached maximum level at 60 minutes and followed by significant temporal decreased till the end of the experiment. It is suggested that the increase in plasma P 15 minutes after NPY administration may be due to direct effect of NPY on granulosa cell of ovary and indirectly through FSH/LH release from pituitary gland. Baranowska et al. (1999) documented that NPY stimulated the cultured granulosa cells of rat ovary to release progesterone. On the other hand Barreca et al. (1998) found that NPY treatment did not significantly affect the release of progesterone from human granulosa cells of the follicles incubated with human chorionic gonadotropin (hCG) in the early stage. Hence increase in progesterone concentration may be due to NPY mediated stimulation of GnRH or gonadotrophs of pituitary for release of LH/FSH which in turn acts on ovaries for release of steroids.

On day 7 (follicular phase) of menstrual cycle 15 minutes after NPY administration a non-significant increase in plasma progesterone level with subsequent significant temporal increase till 105 minutes was noted. Then this increase in plasma P followed by a non-significant temporal decrease till the end of experiment. This significant prolonged increase in P in this follicular phase may be due to the release of more FSH/LH in

response to GnRH release mediated by NPY and NPY may have more influence on granulosa cells to release progesterone in high concentration of estradiol. It is also suggested that increase release of progesterone from ovary for longer time may be due to NPY mediated release of GnRH and gonadotropins for long. NPY stimulated the release of FSH and LH from dispersed perfused anterior pituitary cells of OVX rats in dose associated manner, lower doses of NPY (10^{-7} M) stimulated rapid increase in FSH (1.6 fold) and LH (1.5 fold), whereas increase due to high dose of NPY (10^{-6} M) was 2.4 fold in FSH and 5 fold in LH (McDonald et al., 1985). Throughout the cycle, NPY accompanies the pulses of GnRH that are intermittently released into the hypophysial portal vasculature (Woller and Terasawa, 1992). In addition to this Hill et al. (2004) showed that in gonadotroph-enriched cells of pituitary from OVX rats, NPY Y1R mRNA level was elevated when exposed to E2 (Hill et al., 2004). In vitro NPY enhanced the release of LH from gonadotroph-enriched cell of OVX rats in presence of E_2 suggested that NPY action was mediated by Y1 receptors (Hill et al., 2004). Without steroid exposure, this augmentation disappeared, and with progesterone alone, NPY reduced GnRH-induced LH release reported by (Hill et al., 2004). So progesterone level may remain high for longer period during this follicular phase due to high release of GnRH/LH mediated by NPY in high estradiol status.

On day 15 (peri-ovulatory phase) of menstrual cycle NPY 15 minutes after its administration induced an increase in plasma progesterone which is followed by highly significant temporal increase till 135 minutes (i.e. till the end experiment). This continuous increase in P concentration may be due to NPY mediated secretion of GnRH to stimulate LH secretion which in-turn stimulats the more release of steroids. Or during this phase NPY action may be more profound on granulosa cells to release progesterone effective along with estradiol for preovulatory surge of LH from pituitary in a steroidal milieu. Liu and Yen (1993) and Kalra (1993), showed that progesterone has a role in the generation of the normal shape of the estrogen induced LH surge. Horvath et al. (1993) showed that hypothalamic dopamine cells are innervated by NPY axons in the monkeys and suggested that the estrogen induced progesterone receptors containing cells are

involved in mediation of effect of NPY on hypophyseal hormones, including ovarian steroid dependent LH release.

On day 21 (luteal phase) of menstrual cycle 15 minutes after NPY administration a non-significant increase in plasma progesterone followed by significant temporal increase till 30 minutes and then showed a highly significant temporal decrease till 1.5 hours. This increase for a short period in P concentration after NPY treatment is due to low level of LH from gonadotrophs which may be due to down regulation of progesterone receptors by progesterone itself induced by estrogen on GnRH neurons and gonadotrophs. Miyamoto et al. (1993) showed that NPY was most stimulatory to progesterone release 30 minutes after administration in presence of LH and decreased greatly thereafter to control levels, from 60 to 120 minutes during mid-luteal phase. This influence is entirely dependent upon estrogen pre-exposure (Levine, 1997). Lowest increase in plasma P concentration during luteal phase may be due to the inability of NPY to exceed a putative stimulatory threshold of hypothalamic GnRH and LH to affect the release of progesterone from corpus luteum.

Prolactin

Baseline profile of plasma prolactin

In the present study there was no difference in the baseline plasma prolactin levels in the follicular and luteal phases. Quadri and Spies (1976) also reported that the average plasma prolactin concentration during the follicular phase was not significantly different ($P>0.05$) from plasma PRL level in luteal phase in rhesus monkey. In the current study the plasma prolactin level was high during the periovulatory phase compared to follicular and luteal phases. This high level may be due to high level of estradiol which directly or indirectly stimulated the release of prolactin from lactotrophs by decreasing the dopamine synthesis from hypothalamus or by reducing dopamine receptors D2 on the lactotrophs. It is possible that estradiol enhanced the proliferation of lactotrophs and synthesis of prolactin. This high level of prolactin coincides with high release of LH. The prolactin increased in rodent's estrous cycle at the time of LH surge in proestrus (Smith et al.,

1975; Arbogast et al., 1988). In primates, prolactin level was high during late follicular phase compared to early follicular phase (Vekemans et al., 1977) and in periovulatory phase compared to luteal phase in rhesus monkey reported by Evans and Foltin (2006). In this study no plasma PRL peak was observed during luteal phase. It may be possible that high level of progesterone has no stimulatory effect on plasma prolactin release in monkeys. There are contradictory information regarding the effect of progesterone on PRL release as Chen and Meites (1970) reported that in non-human primates progesterone has no effect on prolactin release, whereas Yen and Pan (1998) found stimulatory effect of progesterone on prolactin release and inhibitory effect on prolactin release was reported by Giguere et al. (1982).

Effect of Neuropeptide Y on plasma Prolactin

On day one of the menstrual cycle, non-significant increase in plasma prolactin concentration after 15 minutes of NPY treatment was observed with subsequent temporal non-significant increase till 30 minutes which was followed by temporal significant decrease till the end of experiment. The initial non-significant increase in plasma prolactin concentration may be due to direct effect of NPY on pituitary gland to stimulate the release of plasma prolactin in the presence of steroidal environment. In the current study, short term increase in plasma PRL concentration in response to NPY treatment with subsequent decrease is consistent with result of Kerkerian et al. (1985) who reported short duration stimulation in PRL release after intravenous injection of NPY but not after intraventricular administration of NPY. The gonadal steroids affect the peripheral prolactin level and development of lactotrophs (Pasteel et al., 1972; Herbet et al., 1974). Hill et al. (2004) found that NPY incubated dispersed perfused pituitary cells from randomly cyclic female rat decreased the PRL concentration significantly, whereas NPY increased/no effect PRL secretion from the same cells primed with estrogen. Furthermore, NPY effect to release PRL from perfused rat pituitary cell can be mimicked with a Y1R agonist and blocked by a NPY Y1R antagonist (Hill et al., 2004). On the other hand it can be possible that NPY mediated GnRH release, stimulate the production of Angiotensin II (AII) from the pituitary gonadotrophs which in-turn stimulated the

prolactin release from the lactotrophs by auto or paracrine manner. Kubota et al. (1991) reported that the pituitary cells of young rats released prolactin 20 minutes after perifusion with GnRH and GnRH stimulated release was significantly suppressed by Angiotensin II antagonist (saralasin).

On day 7 (follicular phase) of menstrual cycle, the non-significant increase in plasma prolactin level 15 minutes after NPY administration was observed compared to baseline, followed by highly significant temporal decreased till the end of experiment. The initial rise in plasma prolactin (PRL) concentration during the follicular phase of menstrual cycle may partly be due to an increase in estradiol backdrop. In rats ovariectomy induced decrease in number as well as size of lactotroph and prolactin-secretory granules reported by De Paul et al. (1997), whereas estradiol treatment alter the effect and stimulate the release of prolactin (Chen and Meites, 1970). These findings suggested that NPY may induce release of PRL in presence of ovarian estradiol feedback. Increase in prolactin release from cultured pituitary cells of rat after incubation with NPY was reported by Baranowska et al. (1999). Alexander et al. (1993) reported a significant decrease in plasma prolactin level for longer time from normal and castrated male rats, 30 minute after NPY intraventricular (ivt) injection. NPY initially caused rapid increase after 15 minutes and then subsequent decrease may be by stimulating the dopaminergic neuronal activity. The increase in prolactin concentration after NPY treatment in menstrual and follicular phases was due to endogenous presence of estradiol which initially down regulated the synthesis and release of dopamine with subsequent down regulation of D2 receptors on lactotrophs. As Stoecklin et al. (1999) and Pi et al. (2003) showed that in some tissues prolactin expression and signaling is estrogen sensitive. The subsequent decrease may be due to prolactin high level which controlled its release by short negative feedback which in turn is mediated by NPY. Milenkovic et al. (1990) reported that prolactin via short-loop feedback mechanism affects its release by controlling its own hypothalamic regulation. Increased plasma prolactin levels elevate the hypothalamic dopamine production (Demarest et al., 1981) and in hypothalamo-hypophysial portal

blood dopamine concentration increases as reported by Gudelsky et al. (1980), which inhibit the release of plasma prolactin.

On day 15 (periovulatory phase) of menstrual cycle 15 minutes after NPY treatment, significant and maximum increase in plasma PRL concentration was observed compared to baseline plasma PRL concentration at 0 minute. Estrogen stimulates the release of prolactin in presence of NPY by acting as an antidopaminergic at the lactotroph, because dopamine is a less effective inhibitor for prolactin release from lactotrophs in the presence of estrogen (Raymond et al., 1978). In the periovulatory phase increase in prolactin level may also be due to release of Angiotensin II from gonadotrophs under NPY mediated GnRH release in endogenous high level of estrogen. Kubota et al. (1991) reported that the pituitary cells of young rats released prolactin 20 minutes after perifusion with GnRH and GnRH stimulated release was significantly suppressed by Angiotensin II antagonist (saralasin). Or it may be possible that NPY by acting on through paracrine factors from lactotrophs stimulated the release of prolactin. High expression of NPY Y1 receptor on gonadotrophs perfused pituitary cells but not in whole pituitary cell in presence of estrogen has been reported by Hill et al. (2004). Initial rapid increase in PRL release 15 minutes after NPY injection was followed by a temporal decrease. Increased plasma prolactin level during peri-ovulatory phase may be the cause of rapid decrease by up regulation the dopamine neuron which in turn inhibited the release of plasma prolactin. The activity of dopaminergic neurons increases progressively in response to the prolactin surge release in estradiol environment, which ultimately results in inhibition of secretion of prolactin (Toney et al., 1992).

On day 21 (luteal phase) of menstrual cycle 15 minutes after NPY single bolus injection, non-significant decrease in PRL concentration followed by highly significant temporal decrease till the end of the experiment. This decrease in plasma PRL level during luteal phase may be due to direct effect of high concentration of progesterone in presence of NPY on lactotrophs or indirectly by stimulating PRL inhibitory factor release like dopamine through short negative feedback loop. A decrease in PRL concentration in the

presence of high level of progesterone by direct effect at pituitary level through elevation of intracellular Ca^{++} has been observed by (Giguere et al., 1982; Haymes and Hinkel, 1993). Härfstrand et al. (1987) showed intraventricular (i.v.t.) administration of low doses of Neuropeptide Y reduced the plasma PRL levels in the rats, furthermore these observations suggested that decrease in prolactin level mediated by NPY by changing DA utilization in the tuberoinfundibular DA. However, Watanobe and Tamura, (1996) showed no significant effect on PRL level after 100 µg NPY in bolus injection in patient with prolactinoma. The decrease in PRL level may be due to NPY i.v. administration which acts at median eminence to stimulate release of dopamine into hypophyseal portal blood circulation because it is well known that tuberoinfundibular dopaminergic neurone (TIDA) of arcuate nucleus projected to median eminence in hypothalamus devoid of blood-brain barrier. So TIDA system modulated by NPY may mediate the decrease in plasma PRL and could account for NPY suppressive effect on PRL. This melodramatic reduction in plasma prolactin concentration is most probable due to the inhibitory effects of dopamine on both the synthesis and secretion of prolactin (Yen, 1979; Ben-Jonathan, 1985). Wang et al. (1996) found that NPY in dose dependent manner inhibited the release of PRL from cultured anterior pituitary cells of rats, whereas NPY in combination with dopamine in submaximal concentration gave additive inhibition of prolactin release. Hypothalamic dopamine cells are innervated by NPY axons in the monkeys and it is suggested that the estrogen induced progesterone receptors containing cells are involved in mediation of effect of NPY on hypophyseal hormone, including ovarian steroid hormone dependent LH and PRL release (Horvath et al., 1993). Moreover, on neuroanatomical observations revealed the synaptic connections between TH-positive cells (tyrosine hydroxylate) and neuropeptide Y-positive fibers at the electron microscopic level (Guy and Pelletier, 1988). Hsueh et al. (2002) have shown that intracerebroventricular injection of NPY increases tuberoinfundibular dopaminergic neuronal activity at hypothalamic level and suppressed PRL secretion. These observations undoubtedly revealed the potential of neuropeptide Y as a regulator of prolactin secretion and suggest central site of action. NPY may also influence PRL levels

through its actions both at the hypothalamic and pituitary level. The direct role of NPY on pituitary cell to inhibit the release of prolactin was supported by Wang et al. (1996) who reported that primary cultures of anterior pituitary cells obtained from lactating or ovariectomized estradiol-treated rats, neuropeptide Y causes a concentration-dependent decrease in prolactin secretion.

The findings of present study supported that NPY neurotransmission is important in the control of prolactin secretion in a variety of physiological situations. Taken together, the present data indicates that prolactin response to NPY in an adult female rhesus monkey during the luteal phase of the menstrual cycle is markedly different from that observed in menstrual, follicular and periovulatory phases and that the steroids may overtly influence the NPY dependent prolactin release. Data of present study provides a level of support which can be utilized in future studies keeping the perspective of NPY as an important neuroendocrine modulator.

Growth Hormone

Baseline profile of growth hormone

Baseline profile showed that plasma growth hormone (GH) concentration was slightly high during follicular and luteal phases but not significantly different as compared to menstrual and peri-ovulatory phases. The plasma GH levels were more or less similar throughout the cycle, whereas the studies performed by others showed that during the menstrual cycle the GH level increases with increase in estradiol concentration (Ovesen et al., 1998; Gleeson and Shalet, 2005). Kasa-Vubu et al. (2005) have reported slightly high level of plasma GH during luteal and peri-ovulatory phases of menstrual cycle in humans, whereas Jaffe et al. (2000) reported non-significant high plasma GH level during ovulatory phase. Childs et al. (2005) found low concentration of estradiol stimulated the expression of GH-mRNA, GH protein and biotinylated GHRH binding sites, whereas high concentration have no effect on GH secretion.

Effect of NPY on plasma GH

In the present study in all the four phases of menstrual cycle 15 minutes after NPY administration rapid and substantial decrease in plasma growth hormone levels was observed which continued till the end of experiment i.e. 135 minutes.

On day 1 (menstrual phase) of menstrual cycle non-significant decrease in plasma growth hormone level was observed after 15 minutes of NPY administration followed by non-significant temporal decrease till 45 minutes. Then significant temporal increase in plasma GFH till the end of experiment was recorded. This decrease in plasma GH level may be due to central inhibitory effect of NPY on GH release. Mano-Otagiri et al. (2006) reported decrease expression of GHRH in the presence of NPY. NPY inhibited GH secretion in the male and female rat (Harfstrand et al., 1987; Rettori et al., 1990; Catzeflis et al., 1993). Growth hormone release was reduced by i.c.v. infusion of NPY whereas antibody to NPY increased the GH release in the same animals. A study by Garcia et al. (2004) showed that NPY administration attenuated the GH release from heifer, whereas NPY stimulated the GH release from OVX cows. Carro et al. (1998) found that in fasted rats NPY inhibited the leptin induced GH release. Therefore it may be suggested that NPY effect may be species specific and also depends upon metabolic and steroid milieu. Estradiol stimulated the GH levels in perfused cell of rat's pituitary gland only in absence of NPY but in its presence reduced the GH level non-significantly (Hill et al., 2004). Estrogen up-regulated the secretion of GH in the goat by altering the pattern of neuropeptide release i.e. via stimulating the release of GHRH and decrease in level of NPY (Yonezawa et al., 2011).

On day 7 (follicular phase) of menstrual cycle significant decrease in plasma GH level was observed after 15 minutes of NPY post treatment till the end of experiment. This inhibitory effect of NPY on GH release may be due to its direct effect on the pituitary gland or via hypothalamus. Estrogen stimulated GH release only in the absence of NPY from perfused pituitary cells of randomly cyclic rats (Hill et al., 2004) whereas in presence of NPY had no significant effect on GH release. NPY inhibited the GH release

from sex steroid primed ovariectomized rat and intact male rat (Suzuki et al., 1996). The present investigations are not in concordance with in vitro studies by McDonald (1990) who documented that NPY stimulated the GH release from perfused cell of rat's pituitary in a dose dependent manner. High doses of NPY have significant stimulatory effect on GH release. Result of this study indicates that the effect of NPY on plasma GH release in monkeys is different from the other animals and this could be a species specific phenomenon. NPY i.c.v infusion stimulated GH secretion in sheep (Morrison et al., 2003) and cattle (Thomas et al., 1999; Garcia et. al., 2004). In vitro studies indicated stimulatory role of NPY on GH release from pituitary of goldfish (Peng et al., 1990). NPY stimulates the secretion of GH in humans whereas inhibits its release in rats (Tilemans et al., 1992; Chan et al., 1996). Thus, the multitude of indications favors the NPY inhibitory effect on GH axis in monkeys which could be specie specific. This decrease in growth hormone level may be due to high level of estradiol and NPY which mediated the negative feedback effect of GH on GHRH.

On day 15 (periovulatory phase) of menstrual cycle a non-significant decrease in plasma GH level was observed after 15 minutes of NPY treatment till the end of experiment. The decrease in growth hormone concentration might be due to the central action of NPY or it is possible that high level of GH in this phase inhibits its release by negative feedback effect mediated through NPY. In addition, direct effect of NPY on the anterior pituitary gland may be responsible for reduction of GH release, since NPY reduced the human somatotroph tumor secretion of GH in vitro (Adams et al., 1987) and limited the somatotroph cell proliferation in the rat, possibly by way of gonadotroph-dependent paracrine mechanisms (Tilemans et al., 1992). A sole clinical experiment showed that NPY stimulated the release of GH from 60% patients of prolactinomas (Watanobe and Tamura, 1996). In another study intraventricular injection of low dose (0.5µg) of NPY in OVX rats did not alter the values from those of saline-injected controls; however, the injection of the higher dose of (5.0 µg) of NPY resulted in a sustained reduction in plasma GH concentration that persisted until the end of the experiment (McDonald et al., 1985), whereas intracerebroventricular NPY infusion inhibited the GH release. However

contrasting results reported by Barb et al. (2006a and 2006b) showed that NPY high dose of 100µg in pigs stimulated the release of GH, whereas low doses has no effect. The non-significant decrease after NPY administration in plasma GH level during periovulatory phases may be due high plasma estradiol level.

On day 21 (luteal phase) of menstrual cycle significant decrease was also observed 15 minutes after NPY injection followed by highly significant temporal decrease till the end with slight fluctuation. NPY inhibited the plasma GH release in male and female rat (Harfstrand et al., 1987; Rettori et al., 1990; Catzeflis et al., 1993). In particular, in the intact male rat, as well as in the ovariectomized female rat (with or without sex steroid hormone replacement), i.c.v infusion of NPY inhibited the plasma GH level that lasted for 3-4 hours (Suzuki et al., 1996). In the same study plasma GH level was inhibited in a similar manner when Y_1- or Y_2-receptor agonists were used, suggesting that GH secretion is mediated both by the Y_1- and Y_2-receptor subtypes (Suzuki et al., 1996). Or it may be possible that NPY inhibited the plasma GH release by stimulating somatostatin release or inhibiting GHRH release from hypothalamus. Due to anatomical distribution of NPY neurons of the arcuate region and expression of GH receptor and c-fos gene in these neurons Kamegai et al. (1996) suggested that NPY has stimulatory effects on somatostatin secretion and inhibits the GHRH to suppress the release of GH (Kamegai et al., 1994) and enhances the expression of NPY mRNA in the hypothalamus of hypophysectomized rats (Chan et al., 1996). Or it may be possible that other neuropeptides are involved in the GH regulation.

In this study a significant fall in GH secretion after NPY treatment during different phases may be due to NPY mediated inhibitory action at hypothalamic level by stimulating the somatostatin release or inhibiting the GHRH release to inhibit GH release. NPY can act at hypothalamic level to modulate the GH release, reported by McDonald et al. (1985) and Rottori et al. (1990) and this action may not be mediated via dopamine (DA) (Horvath et al., 1993). Hence a deduction that can be made in relation to the present study is that decrease in GH release may be due to the action of NPY through dopamine

(DA) as results are in concordance with initial rapid increase in plasma PRL release. It is known that the tuberoinfundibular dopaminergic neurons (TIDA) originate in the arcuate nucleus, and their axons project to the median eminence (ME), co-localized with NPY neurons a hypothalamic structure devoid of the blood-brain barrier. Thus intravenous NPY may have acted at the ME and initially inhibited DA release into the hypophyseal portal blood. This mechanism that the tuberoinfundibular DA system may mediate the GH modulation by NPY could account for the stimulatory and inhibitory effects of NPY on Prolactin. This mechanism explains the present data from different phases of menstrual cycle of rhesus monkey, because the decrease in GH secretion by NPY was associated with a concomitant increase in PRL secretion in first three phases just after 15 minutes of NPY administration. However in luteal phase of menstrual cycle decrease in both plasma prolactin and growth hormone level was observed. So it may be possible that NPY effect plasma GH level by some other mechanism. It has been reported by Watanobe et al. (1991); Watanobe and Tamura (1996), that NPY affects the activity of GH-releasing hormone (GHRH) and somatostatin (SS) neurons in the rat. Thus, it may be possible that the inhibition of GH release by NPY is mediated by an alteration in the secretion of GHRH and/or SS. It may also be possible that NPY acts at the pituitary level directly. NPY receptors may appear on the pituitary cells, or a certain humoral factor(s) within the pituitary gland may mediate the action of NPY through paracrine mechanism. In summary, in this study it was observed that intravenous NPY administration inhibited GH secretion in all four phases of menstrual cycle but significant inhibitory effect was on follicular and luteal phase which could be due to the steroidal environment.

CONCLUSION

Conclusion

On the basis of results of present study it is concluded that NPY intravenous (i.v.) single bolus injection has stimulatory effect on the release of steroids (estradiol and progesterone) during all the four phases of menstrual cycle. NPY stimulatory effect increased the plasma estradiol level non-significantly after its administration followed by significant temporal increase for 30 minutes in menstrual phase (day 1), for 75 minutes (longer time) in periovulatory phase (day 15), for 60 minutes in follicular (day 7) and luteal phases (day 21) of menstrual cycle followed by subsequent significant temporal decrease in all the four phases and attained the baseline plasma estradiol level at the end of the experiment (Fig 26a).

NPY i.v. administration also has stimulatory effect on plasma progesterone level in all the four phases of menstrual cycle. Plasma progesterone level was increased after 15 minutes of NPY administration with subsequent significant temporal increase till an hour in menstrual phase, 105 minutes in follicular phase, till the end of experiment (135 minutes) in periovulatory phase and for only 30 minutes in luteal phase followed by subsequent significant temporal decrease in all the four phases but plasma progesterone level remained high compared to baseline, except in luteal phase. Highly significant increase in plasma progesterone was observed during follicular phase but plasma progesterone level remained high for longer duration in periovulatory phase (Fig. 26a).

NPY has stimulatory as well as inhibitory effect on plasma PRL concentration as clearly revealed by the present findings. NPY initially increased plasma prolactin in menstrual phase non-significantly for 30 minutes after its administration with subsequent temporal significant decrease till the end of experiment. Whereas plasma prolactin level increased non-significantly in follicular phase and significantly in periovulatory phase 15 minutes after NPY administration followed by subsequent significant temporal decrease in plasma PRL level till the end of experiment in both the phases. However in luteal phase NPY has only inhibitory effect on plasma PRL. In this phase a non-significant decrease in plasma

PRL concentration 15 minutes after NPY i.v. injection followed by significant temporal decrease till the end of the experiment (Fig. 26b).

NPY has inhibitory effect on plasma growth hormone in all the four phases of menstrual cycle. Plasma Growth hormone concentration in menstrual phase decreased non-significantly after 15 minutes of NPY i.v. administration with subsequent non-significant temporal decrease till 45 minutes, followed by significant temporal increase till the end of experiment but level were lower than baseline level of plasma GH. Whereas plasma GH level decreased significantly in follicular phase and non-significantly in peri-ovulatory phase 15 minutes after NPY i.v. administration followed by non-significant temporal decrease. However in the luteal phases NPY non-significantly decreased the plasma GH level 15 minutes after its administration with subsequent significant temporal decrease till the end of the experiment (Fig. 26c).

It is concluded that NPY by acting as a neuromodulator, neurotransmitter or as neurohormone stimulates or inhibits the release of hormones from the ovary and pituitary gland in the presence of steroidal milieu.

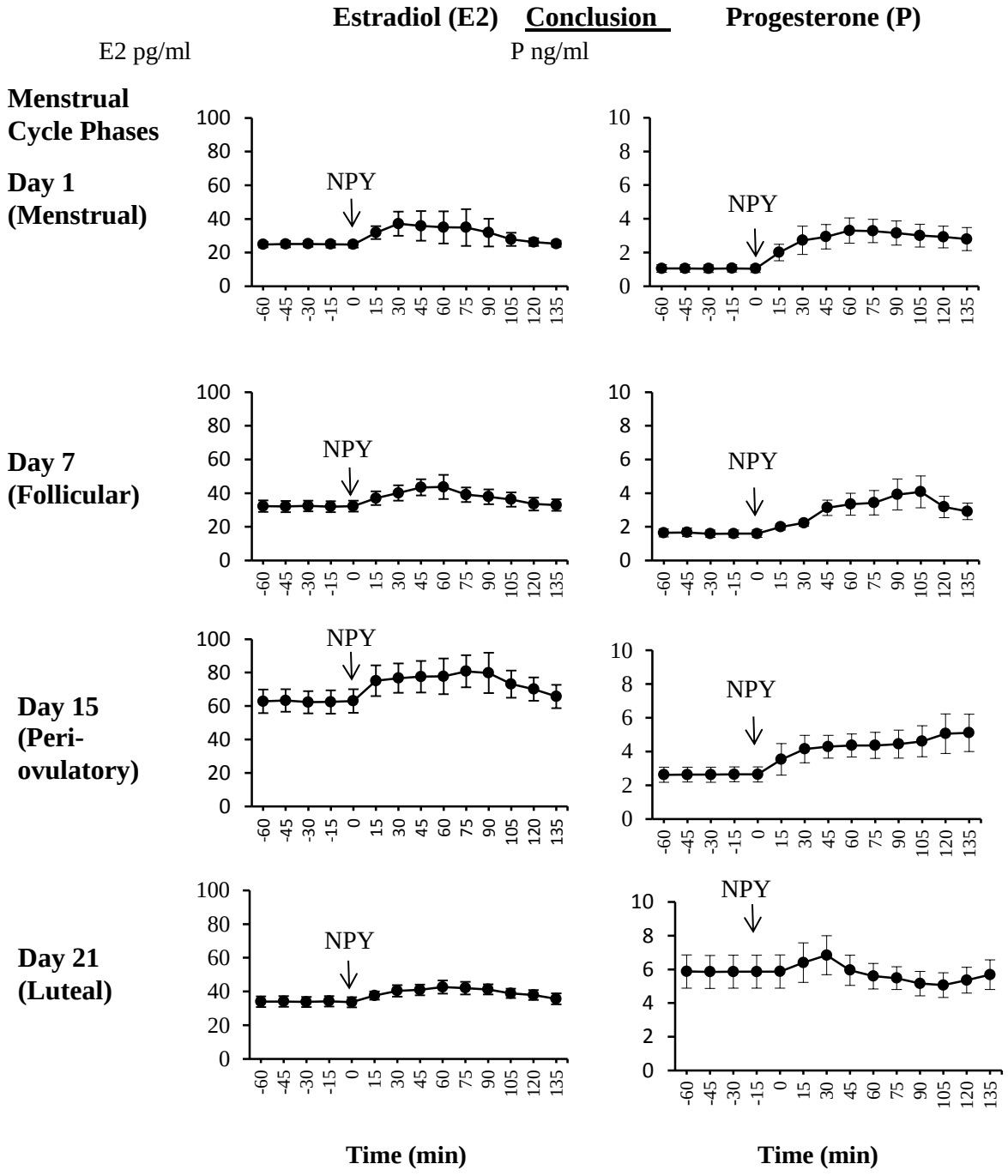


Fig 26a: Mean plasma estradiol (E2) and progesterone (P) concentration before and after 200µg NPY administration during different phases of menstrual cycle. NPY single bolus iv injection has stimulatory effect on plasma E2 and P during all the four phases of menstrual cycle. NPY stimulatory effect increased the plasma E2 level non-significantly 15 minutes after its administration with subsequent significant temporal increase for 30 minutes on day 1 (menstrual phase), for 75 minutes on day 15 (peri-ovulatory phase), for an hour on day 7(follicular) and day 21 (luteal phases) of menstrual cycle, followed by significant temporal decrease in all the four phases and plasma E2 attained the baseline level at the end of the experiment. Whereas non-significantly increase in plasma P level in all four phase 15 minutes after NPY administration followed by significant temporal increase till 60 minutes on day 1 (menstrual), 105 minutes on day 7 (follicular phase), till the end of experiment on day 15 (peri-ovulatory phase) and only for 30 minutes on day 21 (luteal phase) and then followed by non-significant temporal decrease in plasma P level on day 7, significant temporal decrease on day 1 and day 21, but plasma P level remained high on day 15 till the end of experiment.

Conclusion

Prolactin (PRL)

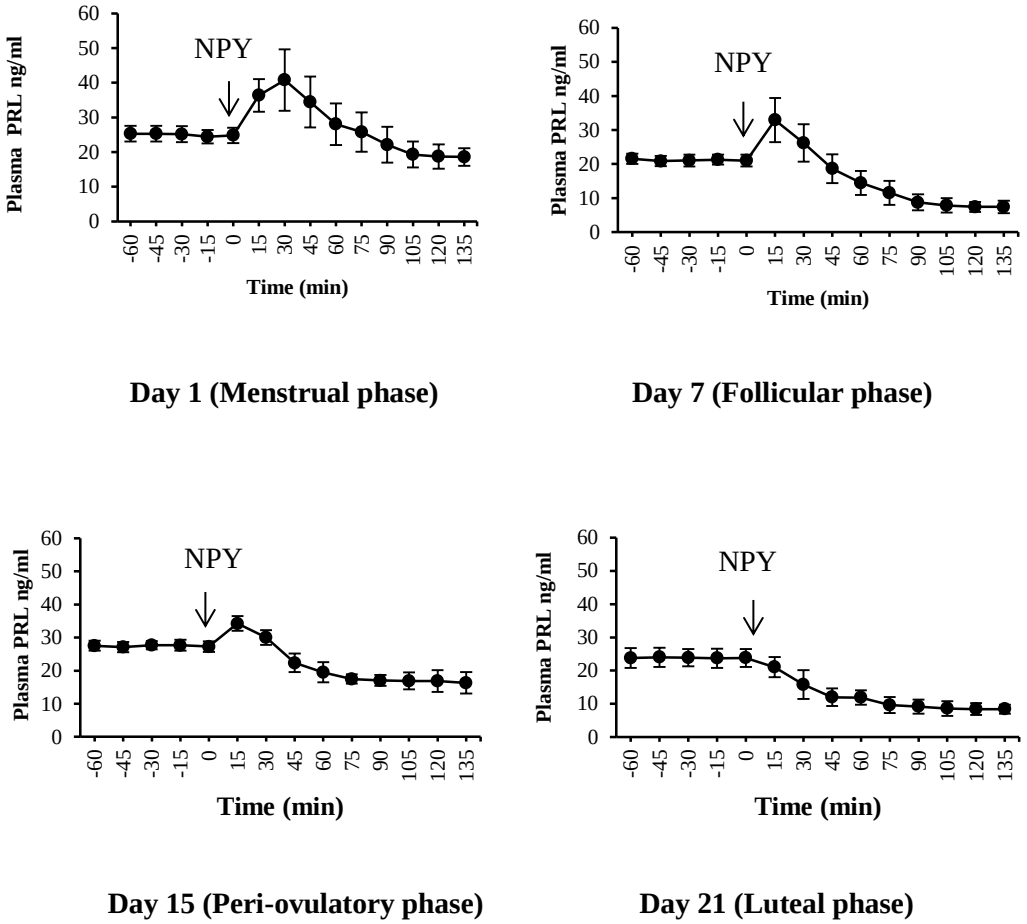
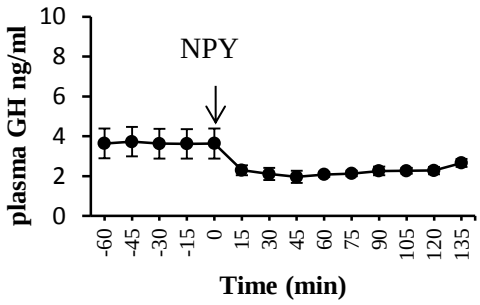


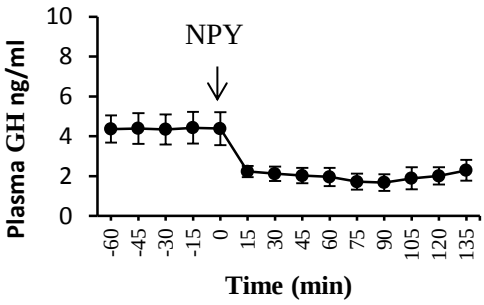
Fig 26b: Mean plasma prolactin (PRL) concentration before and after 200µg NPY administration during different phases of menstrual cycle. NPY single bolus iv injection has stimulatory and inhibitory effect on plasma PRL level during different phases of menstrual cycle. Plasma prolactin concentration on day 1 (menstrual phase) 15 minutes after NPY administration increased non-significantly with subsequent non-significant temporal increase till 30 minutes followed by significant temporal decrease till the end of experiment. Whereas plasma PRL level increased non-significantly on day 7 (follicular phase) and significantly on day 15 (peri-ovulatory phase) 15 minutes after NPY administration compared to baseline level followed by significant temporal decrease till the end of experiment. However on day 21 (luteal phase) a non-significant decrease in plasma PRL level 15 minutes after NPY iv injection compared to baseline level followed by significant temporal decrease till the end of experiment.

Conclusion

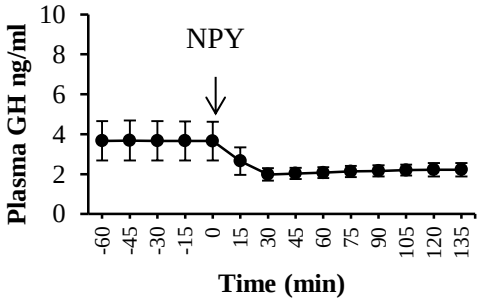
Growth Hormone (GH)



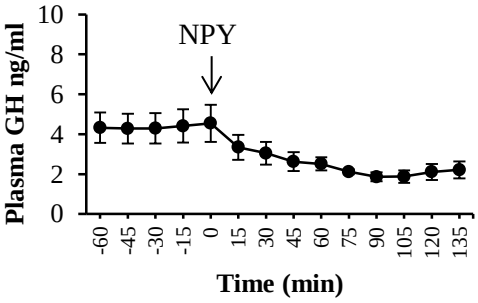
Day 1 (Menstrual phase)



Day 7 (Follicular phase)



Day 15 (Peri-ovulatory phase)



Day 21 (Luteal phase)

Fig 26c: Mean plasma growth hormone (GH) concentration before and after 200µg NPY administration during different phases of menstrual cycle. NPY single bolus iv injection has inhibitory effect on plasma GH level during all the four phases of menstrual cycle. On day 1 (menstrual phase) plasma growth hormone (GH) concentration decreased non-significantly after 15 minutes of NPY administration with subsequent non-significant temporal decrease till 45 minutes followed by significant temporal increase till the end of experiment but level remained low than the baseline plasma GH level. Whereas on day 7 (follicular phase) significant decrease in plasma GH level after 15 minutes of NPY administration followed by non-significant temporal decrease till the end of experiment. However on day 15 (peri-ovulatory phase) and day 21 (luteal phase) plasma growth hormone concentration decreased non-significantly 15 minutes after NPY administration followed by non-significant temporal decrease on day 15 and significant temporal decrease (with slight fluctuation) on day 21 till the end of experiment.

FUTURE PROSPECTS

Future Prospects

In future following studies can be performed to clearly elucidate the role of NPY:

- Effect of NPY Y1 receptor antagonist to elucidate the action of NPY through Y1 receptor during different phases of menstrual cycle on estradiol, progesterone, prolactin, growth hormone, follicle stimulating hormone (FSH) and luteinizing hormone (LH).
- Role of NPY and NPY Y1 antagonist in ovariectomized monkeys to observe the effect of NPY in endogenous absence of steroidal milieu on estradiol, progesterone, prolactin, growth hormone, FSH and LH.
- Role of NPY and NPY Y1 antagonist in ovariectomized steroid primed (both estradiol, progesterone and estradiol plus progesterone) monkeys to observe the steroidal effect on estradiol, progesterone, prolactin, growth hormone, FSH and LH.
- Measuring of plasma NPY level to observe the effect of exogenous administration of NPY.
- Effect of NPY and NPY Y1 during fasting needs to be observed to compare the difference of NPY effect during fed and fasting condition on estradiol, progesterone, prolactin, growth hormone, FSH and LH.
- Effect of different doses of NPY for change in estradiol, progesterone, prolactin, growth hormone, FSH and LH.

REFERENCES

References

Adams EF, Venetikou MS, Woods CA, Lacoumenta S and Burrin JM 1987. Neuropeptide Y directly inhibits growth hormone secretion by human pituitary somatotropic tumors. *Acta Endocrinol* 115:149-154.

Adashi EY and Resnick CE 1987. Prolactin as an inhibitor of granulosa cell luteinization: implications for hyperprolactinemia-associated luteal phase dysfunction. *Fertil Steril* 48: 131-139.

Alexander G. Reznokov, Samuel M. McCann. 1993. Effects of neuropeptide Y on gonadotropin and prolactin release in normal, castrated or flutamide-treated male rats. *Neuroendocrinology* 57(6): 1148-1154.

Allen J, Novotny J, Martin J and Heinrich G 1987. Molecular structure of mammalian neuropeptide Y: Analysis by molecular cloning and computer-aided comparison with crystal structure of avian homologue. *Proc Natl Acad Sci USA* 84: 2532-6.

Allen LG, Crowle WR and Kalra SP 1987. Interactions between neuropeptide Y and adrenergic systems in the stimulation of luteinizing hormone release in steroid-primed ovariectomized rats. *Endocrinology* 121: 1953-1959.

Alsat E, Guibourdenche J, Luton D, Frankenne F and Evain-Brion D 1997. Human placental growth hormone. *Am J Obstet Gynecol* 177: 1526-1534.

Anderson LL, Ford JJ, Klindt J. Molina JR, Vale WW, Rivier J 1991. Growth hormone and prolactin secretion in hypophysial stalk transected pigs as affected growth hormone prolactin releasing and inhibiting factors. *Proc. Soc. Exp. Biol. Med.* 196: 194 –202.

Arbogast LA and Ben Jonathan N 1988. The preovulatory prolactin surge: an evaluation of the role of dopamine. *Endocrinology* 123: 2690-2695.

Arbogast LA, Murai I, and Ben-Jonathan N 1989. Differential alterations in dopamine turnover rates in the stalk-median eminence and posterior pituitary during the preovulatory prolactin surge. *Neuroendocrinology* 49: 525-530.

Arimura A, Dunn JD and Schally AV 1972. Effect of infusion of hypothalamic extracts on serum prolactin levels in rats treated with nembutal, CNS depressants, or bearing hypothalamic lesions. *Endocrinology* 90: 378-383.

Bader R, Bettio A, Beck-Sickinger AG and Zerbe O 2001. Structure and dynamics of micelle-bound neuropeptide Y: Comparison with unligated NPY and implications for receptor selection. *J Mol Bio* 305: 307-329.

Bai FL, Yamano M, Shiotani Y, Emson PC, Smith AD, Powell JF and Tohyama M 1985. An aruato-paraventricular and dorsomedial hypothalamic neuropeptide Y-containing system that lacks noradrenalin in the rat. *Brain Res* 331: 172-175.

Baranowska B, Chmielowska M, Radzikowska M, Borowiec M, Roguski K and Wasilewska-Dziubinska E 1999. Effects of neuropeptide Y (NPY), galanin and vasoactive intestinal peptide (VIP) on pituitary hormone release and on ovarian steroidogensis. *Neuro Endocrinol Lett* 20(6): 385-389.

Barb CR, Hausman GJ and Ramsay TG 2006a. Leptin in farm animals. in: (ed) Castracane VD, Henson MC, Leptin. Endocrinology Update Series. Springer Publishing Group, New York, NY. pp. 263–308.

Barb CR, Kraeling RR, Rampacek GB and Hausman GJ 2006b. The role of neuropeptide Y and interaction with leptin in regulating feed intake and luteinizing hormone and growth hormone secretion in the pig. *Reproduction* 131: 1127-1135.

Barker-Gibb M, Plant TM, White C, Lee PA and Witchel SF 2002a. Genotype analysis of the NPY Y1 and NPY Y5 receptor genes in GnRH-dependent precocious puberty (GnRH-DPP). 84th Annual Meeting of The Endocrine Society, San Francisco, CA, (Abstr), pp. 2-691.

Barnett DK, Bunnell TM, Millar RP and Abbott DH 2006. Gonadotropin-releasing hormone II stimulates female sexual behavior in marmoset monkeys. *Endocrinology*. 147(1): 615-23.

Barreca A, Valli B, Cesarone A, Arvigo M, Balasini M, Battista La Sala G, Garrone S, Minuto F and Giordano G 1998. Effects of the neuropeptide Y on estradiol and progesterone secretion by human granulosa cells in culture. *Fertil Steril* 70(2): 320-5.

Bartke A, Chandrashekar V, Turyn D, Steger RW, Debeljuk L, Winters TA, Mattison JA, Danilovich N, Croson W, Wernsing DR and Kopchick J 1999. Effects of growth hormone overexpression and growth hormone resistance on neuroendocrine and reproductive functions in trangenic and knock-out mice. *Proc Soc Expl Biol Med* 222: 113-123.

Bartke BA, Chandrashekar V and Steger RW 1996. Effects of growth hormone on neuroendocrine function. *Acta Neurbiol Exp* 56: 833-842.

Batista MC, Cartledge TP, Zellmer AW, Merino MJ, Axiotis C, Loriaux DL and Nieman LK 1992. Delayed endometrial maturation induced by daily administration of the antiprogestin RU 486: a potential new contraceptive strategy. *Am J Obstet Gynecol*. 167:60-65.

Batista MC, Cartledge TP, Zellmer AW, Nieman LK, Merriam GR and Loriaux DL 1992. Evidence for a critical role of progesterone in the regulation of the midcycle gonadotropin surge and ovulation. *J Clin Endocrinol Metab.* 74(3):565-70.

Bauer-Dantoin AC, McDonald JK and Levine JE 1991. Neuropeptide Y potentiates luteinizing hormone (LH)-releasing hormone-stimulated LH surges in pentobarbital-blocked proestrous rats. *Endocrinology* 129: 402-408.

Bauer-Dantoin AC, McDonald JK and Levine JE 1992. Neuropeptide Y potentiates luteinizing hormone (LH)-releasing hormone-induced LH secretion only under conditions leading to preovulatory LH surges. *Endocrinology* 131:2946-2952.

Bauer-Dantoin AC, Tabesh B, Norgle JR and Levine JE 1993. RU486 administration blocks neuropeptide Y potentiation of luteinizing hormone (LH)-releasing hormone-induced LH surges in proestrous rats. *Endocrinology* 133: 2418-2423.

Beck B 2005. The arcuate nucleus: its special place in the central networks that regulate feeding behavior. In Nutrient and cell signalling Zempleni J and Dakshinamurti K (ed), NY: Marcel Dekker, New York, pp. 665-699.

Ben-Jonathan N 1980. Catecholamines and pituitary prolactin release. *J Reprod Fertil* 58: 501-512.

Ben-Jonathan N 1985. Dopamine: a prolactin-inhibiting hormone. *Endocr Rev* 6: 564-89.

Ben-Jonathan N and Jo-Wen L 1992. Pituitary lactotrops: Endocrine, paracrine, juxtacrine, and autocrine interactions. *Trends Endocrinol Metab* 3: 254-258.

Ben-Jonathan N, Mershon JL, Allen DL and Steinmetz RW 1996. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. *Endocrin Rev* 17: 639-669.

Ben-Jonathan, N., LaPensee CR and LaPensee EW 2008. What can we learn from rodent? *Endocr. Rev*. 29: 1-41.

Besecke LM and Levine JE 1994. Acute increase in responsiveness of luteinizing hormone (LH)-releasing hormone nerve terminals to neuropeptide-Y stimulation before the preovulatory LH surge. *Endocrinology* 135: 63-66.

Besecke LM, Wolfe AM, Pierce ME, Takahashi JS and Levine JE 1994. Neuropeptide Y stimulates luteinizing hormone-releasing hormone release from super-fused hypothalamic GT1-7 cells. *Endocrinology* 135: 1621-1627.

Bethea CL, Brown NA and Kohama SG 1996. Steroid regulation of estrogen and progestin receptor messenger ribonucleic acid in monkey hypothalamus and pituitary. *Endocrinology* 137 (10): 4372-4383

Bilezikjian LM, Blount AL, Donaldson CJ and Vale WW 2006. Pituitary actions of ligands of the TGF-beta family: Activins and inhibins. *Reproduction* 132:207-215.

Bohnet HG, Dahlen HG, Wuttke W and Schneider HPG 1976. Hyperprolactinaemic anovulatory syndrome. *J Clin Endocrinol Metab* 42: 132-144.

Bole-Feysot C, Goffin V, Edery M, Binart N and Kelly PA 1998. Prolactin (PRL) and its receptor: Actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev* 19: 225-268.

Borman SM, Chaffin CL, Schwinof KM, Stouffer RL and Zelinski-Wooten MB 2004. Progesterone promotes oocyte maturation, but not ovulation, in nonhuman primate follicles without a gonadotropin surge. *Biol Reprod* 71: 366-373

Bosu WTK, Holmdahl TH, Johansson EDB and Gemzell C 1972. Peripheral plasma levels of oestrogens, progesterone and 17α-hydroxyprogesterone during the menstrual cycle of the rhesus monkey. *Acts Endocrinol*. 71: 755-764.

Bronson FH 1985. Mammalian reproduction: an ecological perspective. *Biol Reprod* 32: 1-26.

Brownfield MS, Reid LA, Ganten D and Ganong WF 1982. Differential distribution of immunoreactive angiotensin and angiotensin converting enzyme in rat brain. *Neuroscience* 7: 1759-l769.

Brumstead, J.R. and Riddick, D.H. (1992) Prolactin and the human menstrual cycle. Semin. Reprod. Endocrinol., 10: 220–227.

Burkhoff A, Linemeyer DL and Salon JA 1998. Distribution of a novel hypothalamic neuropeptideY receptor gene and its absence in rat. *Brain Res Mol Brain Res* 53: 311-316.

Butler JA, Sjo¨berg M and Coen CW 1999. Evidence for oestrogen receptor a-immunoreactivity in gonadotropin-releasing hormone-expressing neurones. *J Neuroendocrinol* 11: 331-335.

Cameron IT, Bacon CR, Collett GP and Davenport AP 1995. Endothelin expression in the uterus. *J Steroid Biochem Mol Biol* 53: 209-214.

Cameron JL 1996. Regulation of reproductive hormone secretion in primates by short-term changes in nutrition. *Rev Reprod* 1: 117-26.

Caraty A, Locatelli A and Martin GB 1989. Biphasic response in the secretion of gonadotrophin-releasing hormone in ovariectomized ewes injected with oestradiol. *J endocrinol* 123(3): 375-382.

Carro E, Seoane LM, Senaris R, Considine RV, Casanueva FF and Dieguez C 1998. Interaction between leptin and neuropeptide Y on in vivo growth hormone secretion. *Neuroendocrinology* 68: 187-191.

Carter DA, Pennington JM and Whitehead SA 1982. In-vivo and in-vitro effects of domperidone on the release of prolactin and LH in male and female rats. *J Reprod Fertil* 64:191-197.

Catzeflis C, Pierroz DD, Rohner-Jeanrenaud F, Rivier JE, Sizonenko PC and Aubert ML 1993. Neuropeptide Y administered chronically into the lateral ventricle profoundly inhibits both the gonadotropic and the somatotropic axis in intact adult female rats. *Endocrinology* 132: 224-234.

Chabot JG, Enjalbert A, Pelletier G, Dubois PM, and Morel G 1988. Evidence for a direct action of neuropeptide Y in the rat pituitary gland. *Neuroendocrinology* 47: 511-517.

Chan YY, Steiner RA and Clifton DK 1996. Regulation of hypothalamic neuropeptide-Y neurons by growth hormone in the rat. *Endocrinology* 137: 1319-1325.

Chase CC, Kirby CJ, Hammond AC, Olson TA and Lucy MC 1998. Patterns of ovarian growth and development in cattle with a growth hormone receptor deficiency. *J Anim Sci* 76: 212-219.

Chen CL and Meites J 1970. Effects of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. *Endocrinology* 86: 503-505.

Cheng CK and Leung PC 2005. Molecular biology of gonadotropin-releasing hormone (GnRH)-I, GnRH-II, and their receptors in humans. *Endocr Rev* 26: 283-306.

Chennazhi KP and Nayak NR 2009. Regulation of angiogenesis in the primate endometrium: vascular endothelial growth factor. *Semin Reprod Med* 27: 80-89.

Childs GV, Iruthayanathan M, Akhter N, Unabia G and Johnson BH 2005. Bipotential Effects of Estrogen on Growth Hormone Synthesis and storage in vitro. *Endocrinology* 146(4): 1780-1788.

Cho BN, Suh YH, Yoon YD, Lee CC and Kim K 1993. Progesterone inhibits the estrogen-induced prolactin gene expression in the rat pituitary. *Mol Cell Endocrinol* 93: 47-52.

Chongthammakun S and Terasawa E 1991. Negative and positive feedback effects of estradiol on LHRH release occur in pubertal rhesus monkeys. Proceedings of the 73rd Annual Meeting of The Endocrine Society, Washington DC, Abstr, p. 39.

Chongthammakun S and Terasawa E 1993. Negative feedback effects of estrogen on luteinizing hormone-releasing hormone release occur in pubertal, but not prepubertal, ovariectomized female rhesus monkeys. *Endocrinology* 132(2): 735-43.

Chronwall BM 1985. Anatomy and physiology of the neuroendocrine arcuate nucleus. *Peptides* 6 (Suppl. 2): 1-11.

Chronwall BM, DiMaggio DA, Massari VJ, Pickel VM, Ruggiero DA and O'Donohue TL 1985. The anatomy of neuropeptide Y containing neurons in the rat brain. *Neuroscience* 15: 1159-1181.

Ciofi P, Fallon JH, Croix D, Polak JM and Ramu GT 1991. Expression of neuropeptide Y precursor-immunoreactivity in the hypothalamic dopaminergic tubero-infundibular system during lactation in rodents. *Endocrinology* 128: 823-834.

Colak M, Shimizu T, Matsunaga N, Murayama C, Nagashima S, Kataoka M, Kawashima C, Matsui M, van Dorland HA, Bruckmaier RM and Miyamoto A 2011. Oestradiol enhances plasma growth hormone and insulin-like growth factor-I concentrations and increased the expression of their receptors mRNAs in the liver of ovariectomized cows. *Reprod Domest Anim* 46(5): 854-61.

Conlon JM, Bjenning C and Hazon N 1992. Structural characterization of neuropeptide Y from the brain of the dogfish, Scyliorhinus canicula. *Peptides* 13(3): 493-7.

Conn PM and Crowley WF Jr 1994. Gonadotropin-releasing hormone and its analogs. *Ann Rev Med* 45:391–405.

Conn PM, Crowley WF Jr. 1991. Gonadotropin-releasing hormone and its analogues. *N Engl J Med.* 324 (2): 93-103.

Cooke NE, Ray J, Watson MA, Estes PA, Kuo BA and Liebhauber SA 1988. Two distinct species of human growth hormone variant mRNA in the human placenta predict the expression of novel growth hormone proteins. *J Biol Chem*. 263: 9001-9006.

Cooke PS and Naaz A 2004. Role of estrogens in adipocyte development and function. *Exp Biol Med* (Maywood); 229(11): 1127-1135.

Crowley WR, Hassid A and Kalra SP 1987. Neuropeptide Y enhances the release of luteinizing hormone (LH) induced by LH-releasing hormone. *Endocrinology* 120 (3): 941-945.

Crowley WR, Shah GV, Carroll BL, Kennedy D, Dockter ME and Kalra SP 1990. Neuropeptide-Y enhances luteinizing hormone (LH)-releasing hormone- induced LH release and elevations in cytosolic Ca2+ in rat anterior pituitary cells: evidence for

involvement of extracellular Ca2+ influx through voltage-sensitive channels. *Endocrinology* 127: 1487-1494.

Cunningham MJ, Clifton DK and Steiner RA 1999. Leptin's actions on the reproductive axis: perspectives and mechanisms. *Biol Reprod* 60(2): 216-222.

Czaja JA, Robinson JA, Eisele SG, Scheffler G and Goy RW 1977. Relationship between sexual skin colour of female rhesus monkeys and midcycle plasma levels of oestradiol and progersterone. *J Reprod Fertil* 49: 147-150.

Dailey RA and Neill JD 1981. Seasonal variation in reproductive hormones of rhesus monkeys: Anovulatory and short luteal phase menstrual cycles. *Biol Reprod* 25: 560-567.

Danger JM, Leboulenger F, Guy J, Tonon MC, Benyamina M, Martel JC, Saint-Pierre S, Pelletier G and Vaudry H 1986. Neuropeptide Y in the intermediate lobe of the frog pituitary acts as an alpha-MSH-release inhibiting factor. *Life Sci* 39: 1183-1192.

Danger JM, Tonon MC, Jenks BG, Saint-Pierre S, Martel JC, Fasolo A, Breton B, Quirion R, Pelletier G and Vaudry H 1990. Neuropeptide Y: localization in the central nervous system and neuroendocrine functions. *Fundam Clin Pharmacol*. 4(3): 307-40.

Danilovich N, Bartke A and Winters TA 2000. Ovarian follicle apoptosis in bovine growth hormone transgenic mice. *Biol Reprod* 62: 103-107.

Darendeliler F, Hindmarsh PC, Preece MA, Cox L and Brook CGD 1990. Growth hormone increases rate of pubertal maturation. *Acta Endocrinol* 122: 414-416.

Date Y, Murakami N, Kojima M, Kuroiwa T, Matsukura S, Kangawa K and Nakazato M 2000. Central effects of a novel acylated peptide, ghrelin, on growth hormone release in rats. *Biochem Biophys Res Commun* 275: 477-480.

Davis SR, Smith JF and Gluckman PD 1990. Effects of growth hormone injections on ovulation rate in ewes. *Reproduction, Fertil Dev* 2: 173-178.

de Boer JA, Schoemaker J and Van der Veen EA 1997. Impaired reproductive function in women treated for growth hormone deficiency during childhood. *Clin Endocrinol* 46: 681-689.

De Paul AL, Pons P, Aoki A and Torres AI 1997. Heterogeneity of pituitary lactotrophs: immunocytochemical identification of functional subtypes. *Acta Histochem* 99: 277-289.

Decuypere E, Huybrechts LM, Kuhn ER, Tixier-Boichard M and Merat P 1991. Physiological alterations associated with the chicken sex-linked dwarfing gene. *Crit Rev Poult Biol* 2: 191-221.

Demarest KT, Mckay DW, Riegle GD, and Moore KE 1981. Sexual differences in tuberoinfundibular dopamine nerve activity induced by neonatal androgen exposure. *Neuroendocrinology* 32: 108-113.

Demura R, Ono M, Demura H, Shizume K and Oouchi H 1982. Prolactin directly inhibits basal as well as gonadotropin-stimulated secretion of progesterone and estradiol in the human ovary. *J Clin Endocrinol Metab* 54: 1246-1250.

Denamur R, Martinet J and Short R 1973. Pituitary control of the ovine corpus luteum. *J Reprod Fertil* 32: 207-220.

Dhillon SS and Belsham DD 2011. Estrogen inhibits NPY secretion through membrane-associated estrogen receptor (ER)-α in clonal, immortalized hypothalamic neurons. *Int J Obes Lond* 35(2): 198-207.

Dhillon SS, Gingerich S and Belsham DD 2009. NeuropeptideY induces gonadotropin-releasing hormone gene expression directly and through conditioned medium from mHypoE-38 NPY neurons. *Regul Pept* 156: 96-103.

Dhillon WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, McGowan BM, Amber V, Patel S, Ghatei MA and Bloom SR 2005. Kisspeptin-54 stimulates the hypothalamic-pituitary-gonadal axis in human males. *J Clin Endocrinol Metab* 90(12): 6609-6615.

Dor J, Ben-Shlomo I and Lunenfeld B 1992. Insulin-like growth factor-I (IGF-I) may not be essential for ovarian follicular development: evidence from IGF-I deficiency. *J Clin Endocrinol Metab* 74: 539-542.

Ebling FJ 2005. The neuroendocrine timing of puberty. *Reprod* 129: 675-683.

Economos K, Macdonald PC and Casey ML 1992. Endothelin-1 gene expression and protein biosynthesis in human endometrium: potential modulator of endometrial blood flow. *J Clin Endocrinol Metab* 74: 14-19.

Eisenhauer KM, Chun SY, Billig H and Hsueh AJ 1995. Growth hormone suppression of apoptosis in preovulatory rat follicles and partial neutralization by insulin-like growth factor binding protein. *Biol Reprod* 53: 13–20.

ElMajdoubi M, Sahu A, Ramaswamy S and Plant TM 2000. Effects of orchidectomy on levels of the mRNAs encoding gonadotropin-releasing hormone and other hypothalamic peptides in the adult male rhesus monkey (*Macaca mulatta*). *J Neuroendocrinol* 12: 167-176.

Emanuele, MA., Wezwman, F., Nicholas V. and Emanuele MD 2002b Alcohol effects on female reproductive function. *Alcohol Res Health* 26: 274-281

Ericsson A, Schalling M, McIntyre KR, Lundberg J M, Larhammar D, Seroogy K, Hökfelt T, and Persson H 1987. Detection of neuropeptide Y and its mRNA in megakaryocytes: enhanced levels in certain autoimmune mice. *Proc Natl Acad Sci USA* 84(16): 5585-5589.

Eser D, Romeo E, Baghai TC, di Michele F, Schule C, Pasini A, Zwanzger P, Padberg E and Rupprecht R 2006 Neuroactive steroids as modulators of depression and anxiety. *Neuroscience* 138: 1041-1048.

Evans SM and Foltin RW 2006b. Pharmacokinetics of repeated doses of intravenous cocaine across the menstrual cycle in rhesus monkeys. *Pharmacol. Biochem. Behav.* 83: 56-66.

Everitt BJ and Hökfelt T 1989. "The coexistence of neuropeptide-Y with other peptides and amines in the central nervous system," in Neuropeptide Y, Mutt V, Fuxe K, Hökfelt T and Lundberg JM (eds). Raven Press; New York, pp. 61-71.

Everitt BJ, Hokfelt T, Terenius L, Tatemoto K, Mutt V and Goldstein M 1984. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience* 11: 443-462.

Ewald DA, Sternweis PC and Miller RJ 1988. Guanine nucleotide-binding protein Go-induced coupling of neuropeptide Y receptors to Ca2+ channels in sensory neurons. *Proc Natl Acad Sci USA* 85: 3633-3637.

Ferin M 1996. The menstrual cycle: an integrative view. In: Reproductive Endocrinology, Surgery, and Technology. (eds) Adashi EY, Rock JA, Rosenwaks Z. Raven Press NY. chapter 6: 103.

Ferin M 1999. Stress and the reproductive cycle. *J Clin Endocrinol Metab*. 84(6):1768-1774.

Ferin M 2008. The Hypothalamic-Hypophyseal-Ovarian Axis and the Menstrual Cycle. *Reprod neuroendocrinol* ISSN: 1756-2228.

Fetissov SO, Kopp J and Hokfelt T 2004. Distribution of NPY receptors in the hypothalamus. *Neuropept* 38: 175-188.

Flier JS 1998. What's in a name ? In search of leptin's physiologic role. *J Clin Endocrinol Metab*. 83: 1407-1413.

Franchimont P, Dourcy C and Legros JJ 1976. Prolactin levels during the menstrual cycle. *Clin Endocrinol* 5: 643.

Fraser HM, Groome NP and McNeilly AS 1999. Follicle-stimulating hormone-inhibin B interactions during the follicular phase of the primate menstrual cycle revealed by

gonadotropin-releasing hormone antagonist and antiestrogen treatment. *J Clin Endocrinol Metab* 84: 1365-1369.

Fraser MO, Pohl CR and Plant TM 1989. The hypogonadotropic state of the prepubertal male rhesus monkey (*Macaca mulatta*) is not associated with a decrease in hypothalamic gonadotropin-releasing hormone content. *Biol Reprod* 40: 972-980.

Freeman ME 1993. Neuropeptide Y: a unique member of the constellation of gonadotropin-releasing hormones. *Endocrinology* 133: 2411-2412.

Freeman ME 1994. Neuropeptide Y: a unique member of the constellation of gonadotropin-releasing hormones. *Endocrinology* 133: 2411-2412.

Freeman ME, Kanyicska B, Lerant A and Nagy G 2000. Prolactin: Structure, function, and regulation of secretion. *Physiol Rev* 80: 1523-1631.

Freeman ME, Kanyicska B, Lerant A and Nagy G 2000. Prolactin:structure, function, and regulation of secretion. *Physiol. Rev.* 80: 1523-1631.

Freitas KC, Brito VN, Arnhold IJ, Mendonca BB and Latronico AC 2003. Molecular analysis of two distinct candidate genes (GABRA1 and NYP-Y1) that could be implicated in the human puberty timing. 85th Annual Meeting of The Endocrine Society, Philadelphia, *PA, USA, Abstr* OR8-5.

Furuta M, Funabashi T and Kimura F 2001. Intracerebroventricular Administration of Ghrelin Rapidly Suppresses Pulsatile Luteinizing Hormone Secretion in Ovariectomized Rats. Biochem. Biophys. Res. Commun 288: 780–785.

Furuta M, Funabashi T and Kimura IF 2001. Intracerebroventricular Administration of ghrelin rapidly suppresses pulsatile luteinizing hormone secretion in ovariectomized rats. *Biochem Biophys Res Commun* 288: 780-785.

Fuxe H, Agnati LF, and Harfstrand A 1989. Studies on the neurochemical mechanisms underlying the neuroendocrine actions of neuropeptide Y. In: Neuropeptide Y, (ed) Mutt V, Hokfelt T, Fuxe K, and Lundberg JM, New York: Raven. pp. 115-136.

Gahete MD, Duran-Prado M, Luque RM, Martinez-Fuentes AJ, Quintero A, Gutierrez-Pascual E, Cordoba-Chacon J, Malagon MM, Gracia-Navarro F and Castano JP 2009. Understanding the multifactorial control of growth hormone release by somatotropes: lessons from comparative endocrinology. *Ann N Y Acad Sci* 1163: 137-153.

Garcia de Yebenes E, Li S, Fournier A, St-Pierre S and Pelletier G 1995. Involvement of the Y2 receptor subtype in the regulation of prolactin gene expression by neuropeptide Y in the male rat. *Neurosci Lett* 190: 77-80

Garcia MR, Amstalden M, Keisler DH, Raver N, Gertler A and Williams GL 2004. Leptin attenuates the acute effects of centrally administered neuropeptide Y on somatotropin but not gonadotropin secretion in ovariectomized cows. *Domestic Anim Endocrinol* 26: 189-200.

Ghosh PK, Debeljuk L, Wagner TE and Bartke A 1991. Effect of immunoneutralization of neuropeptide Y on gonadotropin and prolactin secretion in normal mice and in transgenic mice bearing bovine growth hormone gene. *Endocrinology* 129: 597-602.

Giguere V, Meunier H, Veilleux R and Labrie F 1982. Direct effects of sex steroids on prolactin release at the anterior pituitary level: interactions with dopamine, thyrotropin-releasing hormone, and isobutylmethylxanthine. *Endocrinology* 111: 857-862.

Gilbertson J, Kirkwood RN and Thacker PA 1991. Timing of growth hormone injections and reproduction in gilts. *Can J Anim Sci* 71: 717-723.

Girling JE and Rogers PA 2005. Recent advances in endometrial angiogenesis research. *Angiogenesis* 8: 89-99.

Gleeson HK and Shalet SM 2005. GH responsiveness varies during the menstrual cycle *Eur J Endocrinol* 153: 775–779.

Goldsmith PC, Cronin MJ and Weiner RI 1979. Dopamine receptor sites in the anterior pituitary. *J. Histochem. Cytochem*. 27:1205–1207.

Gomez JM, Loir M and Le Gac F 1998. Growth hormone receptors in testis and liver during the spermatogenetic cycle in rainbow trout (Oncorhynchus mykiss). *Biol Reprod* 58: 483–491.

Goncharov N, Aso R, Cekan Z, Panchalia N and Diczfalusy E 1976. Hormonal changes during the menstrual cycle of the baboon (Papio hamad ryas). *Acta Endocrinol* 82: 396-342.

Goodman AL and Hodgen GD 1983. The ovarian triad of the primate menstrual cycle. *Rec Prog Horm Res* 39: 1-73.

Goodman RL 1994. The neuroendocrine control of the ovine estrous cycle. In: Knobil E, Neill JD, (eds). The Physiology of Reproduction. Raven Press, New York. pp. 659-709

Gore AC, Mitsushima D and Terasawa E 1993. A possible role of neuropeptide Y in the control of the onset of puberty in female rhesus monkeys. *Neuroendocrinology* 58: 23-34.

Gore AC, Windsor-Engnell BM and Terasawa E 2004. Menopausal increases in pulsatile gonadotropin-releasing hormone release in a non-human primate (*Macaca mulatta*). *Endocrinology* 145: 4653-4659.

Gottsch ML, Clifton DK and Steiner RA 2006. Kisspepeptin-GPR54 signaling in the neuroendocrine reproductive axis. *Mol Cell Endocrinol* 254-255, 91-96.

Grattan DR and Kokay IC 2008. Prolactin: a pleiotropic neuroendocrine hormone. J *Neuroendocrinol* 20: 752-763.

Gray TS and Morley JE 1986. Neuropeptide Y: anatomical distribution and possible function in mammalian nervous system. *Life Sci* 38: 398-401.

Gruber CJ, Tschugguel W, Schneeberger C and Huber JC 2002. Production and actions of estrogens. *N Engl J Med* 346(5): 340-352.

Gudelsky GA and Porter JC 1980. Release of dopamine from tuberoinfundibular neurons into pituitary stalk blood after prolactin or haloperidol administration. *Endocrinology* 106: 526-529.

Guy J and Pelletier G 1988. Neuronal interactions between neuropeptide Y (NPY) and catecholaminergic systems in the rat arcuate nucleus as shown by dual immunocytochemistry. *Peptides* 9: 567-570.

Guy J, Li S and Pelletier G 1988. Studies on the physiological role and mechanism of action of neuropeptide Y in the regulation of luteinizing hormone secretion in the rat. *Regul Pept* 23: 209-216.

Hahn TM, Breininger JF, Baskin DG and Schwartz MW 1998. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1: 271-272.

Hamada Y, Schlaff S, Kobayashi Y, Santulli R, Wright and Wallach E 1980. Inhibitory effect of prolactin on ovulation in the in-vitro perfused rabbit ovary. *Nature* 285: 161-163.

Han S.K, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, Clifton DK, Steiner RA and Herbison AE 2005. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of Puberty. *J Neurosci*. 25(49): 11349-11356.

Harfstrand A, Eneroth P, Agnati L and Fuxe K 1987. Further studies on the effects of central administration of neuropeptide Y on neuroendocrine function in the male rat: relationship to hypothalamic catecholamines. *Regul Pept* 17: 167-179.

Hauger RL, Aguilera G, Baukal AJ and Catt KJ 1982. Characterization of angiotensin II receptors in the anterior pituitary gland. *Mol cell endocrinol* 25: 203-212.

Haymes AA and Hinkle PM 1993. Activation of protein kinase C increases Ca21 sensitivity of secretory response of GH3 pituitary cells. *Am J Physiol Cell Physiol* 264: 1020-1028.

Henry M, Ghibaudi L, Gao J and Hwa JJ 2005. Energy metabolic profile of mice after chronic activation of central NPYY1, Y2, or Y5 receptors. *Obes Res* 13: 36-47.

Herbert DC and Hayashida T 1974. Histological identification and immunochemical studies of prolactin and growth hormone in the primate pituitary gland. *Gen. Comp. Endocrinol*. 24: 381-397.

Hileman SM, Pierroz DD and Flier JS 2000. Leptin, nutrition, and reproduction: timing is everything. *J Clin Endocrinol Metab* 85(2): 804-7.

Hill JW, Urban JH, Xu M and Levine JE 2004. Estrogen Induces Neuropeptide Y (NPY) Y1 receptor gene expression and responsiveness to NPY in gonadotrope-enriched pituitary cell cultures. *Endocrinology* 145(5): 2283-2290.

Holliday ND, Michel MC and Cox HM 2004. NPY receptor subtypes and their signal transduction. In: Michel MS, (ed). Handbook of experimental pharmacology. Vol 162. Springer-Verlag , Berlin, pp. 45-73.

Holzbanct M and Racke K 1985. The dopaminergic innervation of the intermediate lobe and of the neural lobe of the pituitary gland. *Med. Biol.* 87:63-97.

Horton RJ, Francis H and Clarke IJ 1989. Seasonal and steroid-dependent effects on the modulation of LH secretion in the ewe by intracerebroventricularly administered beta-endorphin or naloxone. *J Endocrinol* 122: 509-517.

Horvath TL, Shanabrough M, Naftolin E and Leranth C 1993. Neuropeptide Y innervation of estrogen-induced progesterone receptor-containing dopamine cells in the monkey hypothalamus: a triple labeling light and electron microscopic study. *Endocrinology* 133: 405-414.

Hotchkiss J and Knobil E 1994. The menstrual cycle and its neuroendocrine control. In: Knobil E, Neill JD, (eds). The physiology of reproduction. New York: Raven Press; 711-733.

Hotchkiss J, Atkinson LE and Knobil E 1971. Time course of serum estrogen and luteinizing hormone (LH) concentration during the menstrual cycle of the rhesus monkey. *Endocrinology* 89: 177-183.

Hrabovszky E, Kallo I, Szlavik N, Keller E, Merchenthaler I and Liposits ZS 2007. Gonadotropin-releasing hormone neurons express estrogen receptor β. *J Clin Endocrinol Metab* 92: 2827-2830.

Hrabovszky E, Steinhauser A, Barabas K, Shughrue PJ, Petersen SL, Merchenthaler I and Liposits Z 2001. Estrogen receptor-β immunoreactivity in luteinizing hormone-releasing hormone neurons of the rat brain. *Endocrinology* 142: 3261-3264.

Hsueh YC,Cheng SM and Pan JT 2002. Fasting stimulates tuberoinfundibular dopaminergic neuronaol activity and inhibits prolactin secretion in oestrogen-primed ovariectomized rats: involvement of orexin A and neuropeptide Y. J *Endocrinol* 14(9):745-752.

Ilondo MM, Vanderschueren-Lodeweyckx M, Vlietnick R, Pizarro M, Malvaux P, Eggermont E and Eeckels R 1982. Plasma androgens in children and adolescents. Part II. A longitudinal study in patients with hypopituitarism. *Hor Res* 16: 78-95.

Jaffe CA, Ocampo-Lim B, Guo W, Krueger K, Sugahara I, DeMott-Friberg R and Barkan AL 2000. Growth hormone secretory dynamics over the menstrual cycle. *Endocr J* 47(5): 549-556.

Jahan S, Jalali S and Shami SA 2007. Neuroendocrine regulation of prolactin in adult female rhesus monkeys during different phases of the menstrual cycle: Role of Neuroexcitatory Amino Acid (NMA). *Am J Primatol* 69: 395-406.

Joseph NT, Aquilina-Beck A, Macdonald C, Decatur WA, Hall JA, Kavanaugh SI and Sower SA 2012. Molecular cloning and pharmacological characterization of two novel GnRH receptors in the lamprey (Petromyzon marinus). *Endocrinology* 153: 3345-3356.

Kalra SP 1986. Neural circuitry involved in the control of LHRH secretion: a model for preovulatory LH release. (Ed) Martini L, Ganong WR, Frontiers in Neuroendocrinology, Raven Press, New York, pp. 31-75.

Kalra SP 1986. Neural circuitry involved in the control of LHRH secretion: a model for preovulatory LH release. In: Frontiers in Neuroendocrinology, (ed) Martini L and Ganong WR, New York Raven, vol. 9, pp. 31-75.

Kalra SP 1993. Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge. *Endocr Rev* 14: 507-538.

Kalra SP and Crowley WR 1984 Norepinephrine-like effects of neuropeptide Yon LH release in the rat. *Life Sci* 35: 1173-1176.

Kalra SP and Crowley WR 1992. Neuropeptide Y: A novel neuroendocrine peptide in the control of pituitary hormone secretion, and its relation to luteinizing hormone. In: Frontiers in Neuroendocrinology, Ganong WF and Martini L (eds). New York Raven, pp. 1-46.

Kamegai J, Minami S, Sugihara H, Hasegawa O, Higuchi H and Wakabayashi I 1996. Growth hormone receptor gene is expressed in neuropeptide Y neurons in hypothalamic arcuate nucleus of rats. *Endocrinology* 137: 2109-2112.

Kamegai J, Minami S, Sugihara H, Higuchi H and Wakabayashi I 1994. Growth hormone induces expression of the c-los gene on hypothalamic neuropeptide Y and somatostatin neurons in hypophysectomized rats. *Endocrinology* 135: 2765-2771.

Kanayama G, Amiaz R, Seidman S and Pope HGJ 2007. Testosterone supplementation for depressed men: current research and suggested treatment guidelines. *Exp Clin Psychopharmacol* 15: 529-538.

Karsch FJ, Bowen JM, Caraty A, Evans NP and Moenter SM. 1997. Gonadotropinreleasing hormone requirements for ovulation. *Biol Reprod* 56: 303-309.

Karsch FJ, Dierschke DJ, Weick RF, Yamaji T, Hotchkiss J and Knobil E 1973. Positive and negative feedback control by estrogen of luteinizing hormone secretion in the rhesus monkey. *Endocrinology* 92:799-804.

Kasa-Vubu JZ, Dimaraki EV and Young EA 2005. The pattern of growth hormone secretion during the menstrual cycle in normal and depressed women. *Clin Endocrinol* 62: 656-660.

Kauppila A, Leinonen P, Vihko R and Ylostalo P 1982. Metoclopramide-induced hyperprolactinemia impairs ovarian follicle maturation and corpus luteum function in women. *J Clin Endocrinol Metab* 54: 955-960.

Kaynard AH, Pau KY, Hess DL and Spies HG 1990. Third-ventricular infusion of neuropeptide Y suppresses luteinizing hormone secretion in ovariectomized rhesus macaques. *Endocrinology* 127: 2437-2444.

Kaynard AH, Pau KYE, Hess DL and Spies HG 1990. Gonadotropin-releasing hormone and norepinephrine release from the rabbit mediobasal and anterior hypothalamus during the mating-induced luteinizing hormone surge. *Endocrinology* 127: 1176-1185.

Kerkerian L and Pelletier G 1986. Effects of monosodium L-glutamate administration on neuropeptide Y-containing neurons in the rat hypothalamus. *Brain Res* 369: 388-390.

Kerkerian L, Guy J, Lefevre G and Pelletier G 1985. Effects of Neuropeptide Y (NPY) on the release of anterior pituitary hormones in the rat. *Peptides* 6: 1201-1204.

Khorram O, Pau KY and Spies HG 1987. Bimodal effects of neuropeptide Y on hypothalamic release of gonadotropin-releasing hormone in conscious rabbits. *Neuroendocrinol* 45: 290-7.

Knobil E 1974. On the control of gonadotropin secretion in the rhesus monkey. *Recent Prog Horm Res* 30: 1-46.

Knobil E 1980. The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res* 36: 53–88

Knobil E and Hotchkiss J 1988 The menstrual cycle and its neuroendocrine control. In: The Physiology of Reproduction, (ed) Knobil E, Neill J, Ewing LL, Greenwald GS, Markert CL and Pfaff DW, Raven Press, New York, pp. 1971-1994.

Knobil E, Plant TM, Wildt L, Belchetz PE and Marshall G 1980. Control of the rhesus monkey menstrual cycle: permissive role of hypothalamic gonadotropin-releasing hormone. *Science.* 207(4437): 1371-1373.

Konturek SJ, Konturek JW, Pawlik T, Brzozowski T 2004. Brain-gut axis and its role in the control of food intake. *J Physiol Pharmacol* 55: 137-54.

Korach KS 2000. Estrogen receptor knock-out mice: molecular and endocrine phenotypes. *J Soc Gynecol Investig* 7(Suppl 1): S16-7.

Krsmanović LZ, Stojilković SS, Merelli F, Dufour SM, Virmani MA and Catt KJ 1992.Calcium signaling and episodic secretion of gonadotropin-releasing hormone in hypothalamic neurons. *PNAS* 89: 8462-8466.

Kubota T, Kamada S and Aso T 1991. The role of angiotensisn in paracrine interaction between rat anterior pituitary cells. *Nihon Sanka Fujinka Gakkai Zasshi* 43(1): 80-84.

Kuiper GG, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S and Gustafsson JA 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 138(3): 863-70.

Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B and Gustafsson JA 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 139(10): 4252-63.

Kuiper GG, Shughrue PJ, Merchenthaler I and Gustafsson JA 1998. The estrogen receptor subtype: a novel mediator of estrogen action in neuroendocrine systems. *Front Neuroendocrinol* 19: 253-286.

Labrie F 2003. Extragonadal synthesis of sex steroids: intracrinology. *Ann Endocrinol* 64(2): 95-107.

Lacreuse A 2006. Effects of ovarian hormones on cognitive function in nonhuman primates. *Neuroscience* 138(3): 859-867.

Langer G, Ferin M and Sachar E 1978. Effect of haloperidol and L-dopa on plasma prolactin in stalk-sectioned and intact monkeys. *Endocrinology* 102: 367-370.

Larhammar D 1996. Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. *Regul Pept* 62: 1-11.

Larhammar D and Salaneck E 2004. Molecular evolution of NPY receptor subtypes. *Neuropeptides* 38: 141-151.

Larhammar D, Blomqvist AG and Soderber C 1993. Evolution of neuropeptide Y and its related peptides. *Comp Biochem Physiol* C 106 (3): 743-752.

Legan SJ, Coon GA and Karsch FJ 1975. Role of estrogen as initiator of daily LH surges in the ovariectomized rat. *Endocrinology* 96: 50-56.

Leupen SM, Besecke LM and Levine JE 1997. Neuropeptide Y Y1-receptor stimulation is required for physiological amplification of preovulatory luteinizing hormone surges. *Endocrinology* 138: 2735-2739.

Levine JE 1997. New concepts of the neuroendocrine regulation of gonadotropin surges in rats. *Biol Reprod* 56:293-302.

L'Hermite M and Robyn C 1972. Prolactine hypophysaire humaine: detection radio-immunologique et taux au cours de la grossesse. Ann Endocrinol (Paris) 33(4):357–360.

Li C, Chen P and Smith MS 1999. Morphological evidence for direct interaction between arcuate nucleus neuropeptide Y (NPY) neurons and gonadotropin releasing hormone (GnRH) neurons and the possible involvement of NPY Y1 receptors. *Endocrinology* 140: 5382-5390.

Lin KC, Kawamura N, Okamura H and Mori T 1980. Inhibition of ovulation, steroidogenesis and collagenolytic activity in rabbits by sulpride-induced hyperprolactinaemia. *J Reprod Fertil* 83: 611-618.

Liu JH and Yen SSC 1983. Induction of midcycle gonadotropin surge by ovarian steroids in women: a critical evaluation. *J Clin Endocrinol Metab* 57: 797-802.

Liu X, Andoh K, Yokota H, Kobayashi J, Abe Y, Yamada K, Mizunuma H and Ibuki Y 1998. Effects of growth hormone, activin, and follistatin on the development of preantral follicle from immature female mice. *Endocrinology* 139: 2342-2347.

Lucy MC 2012. Growth hormone regulation of follicular growth. *Reprod, Fertil Dev*. 24: 19-28.

Macleod RM 1976. Regulation of prolactin secretion. In: Martini L and Ganong WI., (eds) Frontiers in neuroendocrinology. New York: Raven Press; 1976: pp. 169-194.

Maeda K, Ohkura S, Uenoyama Y, Wakabayashi Y, Oka Y, Tsukamura H and Okamura H 2010. Neurobiological mechanisms underlying GnRH pulse generation by the hypothalamus. *Brain Res.* 1364: 103-115.

Mano-Otagiri A, Nemoto T, Sekino A, Yamauchi N, Shuto Y, Sugihara H, Oikawa S and Shibasaki T 2006. Growth hormone-releasing hormone (GHRH) neurons in the arcuate nucleus (Arc) of the hypothalamus are decreased in transgenic rats whose expression of ghrelin receptor is attenuated: Evidence that ghrelin receptor is involved in the up-regulation of GHRH expression in the arc. *Endocrinology* 147(9): 4093-103.

Mathias JR and Clench MH 1998. Relationship of reproductive hormones and neuromuscular disease of the gastrointestinal tract. *Dig Dis*. 16(1): 3-13.

McCann SM, Rettori V, Milenkovic L, Mcdonald JK, Riedel M, and Aguila C 1989. The role of neuropeptide Y in the control of anterior pituitary hormone release in the rat. In Neuropeptide Y, Karolinksa Institute Nobel Conference Series, (ed) Mutt V. Fuxe K, Hokfelt T and Lundberg JM, Raven Press. New York. pp. 215-228.

McCann SM, Taleisnik S and Friedman HM 1960. LH-releasing activity in hypothalamic extracts. *Proc Soc Exp Biol Med*. 104: 432-43.

McDonald JK 1990. Role of neuropeptide Y in reproductive function. *Ann NY Acad Sci* 611: 258-272.

McDonald JK, Lumpkin MD and Depaloi LV 1989. Neuropeptide Y suppresses pulsatile secretion of luteinizing hormone in ovariectomized rats: possible site of action. *Endocrinology* 125: 186-191.

McDonald JK, Lumpkin MD, Samson WK and McCann SM 1985 Neuropeptide Y affects secretion of luteinizing hormone and growth hormone in ovariectomized rats. *Proc Natl Acad Sci USA* 82: 561-564.

McEwen BS and Alves SE 1999. Estrogen actions in the central nervous system. *Endocr Revs*. 20: 279-307.

McHenry R, 2008. The Structure and Dynamics of Human Neuropeptide Y. http://maptest.rutgers.edu/drupal/?q=node/249

McNatty KP, Sawers RS and McNeilly AS 1974. A possible role for prolactin in control of steroid secretion by the human Graafian follicle. *Nature* 250: 653-655.

McNeilly AS 1993. Lactational amenorrhea. *Endocrinol Metab Clin N Am* 22: 59-72

McNeilly AS, Glasier A, Jonassen J and Howie P 1982. Evidence of direct inhibition of ovarian function by prolactin. *J Reprod Fertil* 65: 559-569.

Menashe Y, Sack Y and Mashinach S 1991. Spontaneous pregnancies in two women with Laron-type dwarfism: are growth hormone and circulating insulin-like growth factor mandatory for induction of ovulation? *Hum Reprod* 6: 670-671.

Messinis IE 2006. Ovarian feedback, mechanism of action and possible clinical implications. *Hum Reprod* 12: 557–571.

Messinis IE 2006. Ovarian feedback, mechanism of action and possible clinical implications. *Hum Reprod* 12: 557-571.

Michel MC 2004. Neuropeptide Y and related peptides. In: Starke K, Br FI, (eds), Handbook of experimental pharmacology. New York: Springer. pp. 555-592.

Michel MC, Beck-Sickinger A, Cox H, Doods HN, Herzog H, Larhammar D, Quirion R, Schwartz T and Westfall T 1998. XVI International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. *Pharmacol Rev* 50:143-150.

Milenkovic L, Parlow AF, and Mccann SM 1990. Physiological significance of the negative short-loop feedback of prolactin. *Neuroendocrinology* 52: 389-392.

Millar RP 2005. GnRHs and GnRH receptors. *Anim Reprod Sci* 88: 5-28.

Misra S, Murthy KS, Zhou H and Grider JR 2004. Coexpression of Y1, Y2, and Y4 receptors in smooth muscle coupled to distinct signaling pathways. *J Pharmacol Exp Ther* 311: 1154-1162.

Miyamoto A, Brückmann A, von Lützow H and Schams D 1993. Multiple effects of neuropeptide Y, substance P and vasoactive intestinal polypeptide on progesterone and oxytocin release from bovine corpus luteum in vitro. *J Endocrinol* 138(3): 451-8.

Mizuno M, Gearing M, and Terasawa EI 2000. The role of neuropeptide Y in the progesterone-induced luteinizing hormone-releasing hormone surge in vivo in ovariectomized female rhesus monkey. *Endocrinology* 141(5): 1772-1779.

Moenter SM, Caraty A, Karsch FJ 1990. The estradiol-induced surge of gonadotropin-releasing hormone in the ewe. *Endocrinology* 127: 1375-1384.

Moenter SM, DeFazio AR, Pitts GR and Nunemaker CS 2003. Mechanisms underlying episodic gonadotropin-releasing hormone secretion. *Front Neuroendocrinol* 24: 79-93.

Mol JA, Van Garderen E, Rutteman GR and Rijnberk A 1996. New insights in the molecular mechanism of progestin-induced proliferation of mammary epithelium: induction of the local biosynthesis of growth hormone (GH) in the mammary glands of dogs, cats and humans. *J Steroid Biochem Mol Biol* 57: 67-71.

Morgan K, Conklin D, Pawson AJ, Sellar R, Ott TR and Millar RP 2003. A transcriptionally active human type II gonadotropin-releasing hormone receptor gene homolog overlaps two genes in the antisense orientation on chromosome 1q.12. *Endocrinology* 144(2): 423-36.

Morishige WK and Rothchild 1974. Temporal aspects of the regulation of corpus luteum function by luteinizing hormone, prolactin and placental luteotrophin during the first half of pregnancy in the rat. *Endocrinology* 95: 260-274.

Morrison CD, Daniel JA, Hampton JH, Buff PR, McShane TM, Thomas MG and Keisler DH 2003 Luteinizing hormone and growth hormone secretion in ewes infused intracerebroventricularly with neuropeptide Y. *Domest Anim Endocrinol* 24: 69-80.

Musso R, Maggi A and Eva C 2000. 17-estradiol stimulates mouse neuropeptide Y-Y (1) receptor gene transcription by binding to estrogen receptor in neuroblastoma cells. *Neuroendocrinology* 72: 360 -367.

Naor Z 2009. Signaling by G-protein-coupled receptor (GPCR): studies on the GnRH receptor. *Front Neuroendocrinol.* 30(1): 10-29.

Navarro CE, Saeed SA, Murdock C, Martinez-Fuentes AJ, Arora KK, Krsmanovic LZ and Catt KJ 2003. Regulation of cyclic adenosine 3-5-monophosphate signaling and pulsatile neurosecretion by Gi-coupled plasma membrane estrogen receptors in immortalized gonadotrophin-releasing hormone neurons. *Mol.Endocrinol* 17: 1792-1804.

Neill JD 1974. Prolactin: Its secretion and control. In: Knobil, E.; Sawyer, W. H., (eds), Handbook of physiology, section 7: endocrinology vol. 4. Washington: *Am Physiol Soc*; pp. 469-488.

Neill JD 1980. Neuroendocrine regulation of prolactin secretion. In: Martini, L.; Ganong, W. F., (ed), Frontiers in neuroendocrinology, Raven Press; New York. pp. 129-155.

Nippoldt TB, Reame NE, Kelch RP and Marshall JC 1989. The roles of estradiol and progesterone in decreasing luteinizing hormone pulse frequency in the luteal phase of the menstrual cycle. *J Clin Endocrinol Metab.* 69(1): 67-76.

Ovesen P 1998. Synergistic effects of growth hormone and insulin-like growth factor-I on differentiation and replication of cultured human luteinized granulosa cells. *Acta Obstet Gynecol Scan* 77: 487-491.

Ovesen P, Ingerslev J, Orskov H and Ledet T 1994. Effect of growth hormone on steroidogenesis, insulin-like growth factor-I (IGF-I) and IGF-binding protein-1 production and DNA synthesis in cultured human luteinized granulosa cells. *J Endocrinol* 140: 313-319.

Ovesen P, Vahl N, Fisker S, Veldhuis JD, Christiansen JS and Jorgensen JO 1998. Increased pulsatile, but not basal, growth hormone secretion rates and plasma insulin-like growth factor I levels during the preovulatory interval in normal women. *J Clin Endocrinol Metab*. 83:1662-1667.

Parker SL, Carroll BL, Kalra SP, St-Pierre S, Fournier A and Crowley WR 1996b. Neuropeptide Y Y2 receptors in hypothalamic neuroendocrine areas are upregulated by estradiol and decreased by progesterone cotreatment in the ovariectomized rat. *Endocrinology* 137: 2896-2900.

Pasteels JL, Gauesset, P, Danguy A, Ectors F, Nicoll CS and Varavudhi P 1972. Morphology of the lactotropes and somatotropes of man and rhesus monkeys. *J. Clin. Endocr*. 34: 959-67.

Pasteels LL, Gausset P, Danguy A and Ectors F 1972. Immunofluorescent studies on prolactin and the pituitary. In Prolactin and Carcinogenesis. 4th Tenovus Workshop. Boyns AR and Griffiths K, (ed). Alpha Omega Alpha Publishing, Cardiff, pp. 128-136.

Pau K-YF, Berria M, Hess DL and Spies HG 1995. Hypothalamic site-dependent effects of neuropeptide Y on gonadotropin-releasing hormone secretion in rhesus macaques. *J Neuroendocrinol* 7: 63-67.

Peng C, Huang Y and Peter RE 1990. Neuropeptide Y stimulates growth hormone secretion and gonadotropin release from the goldfish pituitary in vivo. *Neuroendocrinology* 52: 28-34

Pi X, Zhang B, Li J and Voogt JL 2003. Promoter usage and estrogen regulation of prolactin receptor gene in the brain of the female rat. *Neuroendocrinology* 77: 187–197.

Plant TM 2001. Neurobiological bases underlying the control of the onset of puberty in the rhesus monkey: a representative higher primate. *Front Neuroendocrinol* 22: 07-139.

Plant TM 2006. The role of KiSS-1 in the regulation of puberty in higher primates. *Eur J Endocrinol* 155: S11–S16.

Plant TM 2008 Hypothalamic control of the pituitary-gonadal axis in higher primates: key advances over the last two decades. *J Neuroendocrinol* 20: 719-726.

Plant TM and Barker-Gibb ML 2004. Neurobiological mechanisms of puberty in higher primates. *Hum Reprod* 10: 67-77.

Plant TM and Shahab M 2002. Neuroendocrine mechanisms that delay and initiate puberty in higher primates. *Physiol Behav* 77: 717-722.

Plotsky PM, Gibbs DM and Neill JD 1978. Liquid Chromatographic-Electrochemical Measurement of Dopamine in Hypophysial Stalk Blood of Rats. *Endocrinology* 102(6): 1887-1894

Pope HGJ and Brower KJ 2000. Anabolic-androgenic steroid abuse.In: Sadock BJ, Sadock VA (ed). *Comprehensive Textbook of Psychiatry*/VII. Lippincott Williams and Wilkins: Philadelphia, PA, pp. 1085-1095.

Pope HGJ, Kouri EM and Hudson JI 2000. The effects of supraphysiologic doses of testosterone on mood and aggression in normal men: a randomized controlled trial. *Arch Gen Psychiatry* 57: 133-140.

Quadri SK and Spies HG 1976. Cyclic and diurnal patterns of serum prolactin in the rhesus monkey. *Biol Reprod* 14: 495-501.

Quesnel H 1999. Localization of binding sites for IGF-I, insulin and GH in the sow ovary. *J Endocrinol* 163: 363-372.

Ramaswamy S, Seminara SB, Pohl CR, DiPietro MJ, Crowley WF and Plant TM 2007. Effect of continuous intravenous administration of human metastin 45-54 on the neuroendocrine activity of the hypothalamic-pituitary-testicular axis in the adult male rhesus monkey (*Macaca mulatta*). *Endocrinology* 148 (7): 3364-3370.

Raymond V, Beaulieu M, Labrie F and Boissier J 1978. Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. *Science* 200: 1173-1175.

Reddy DS 2003. Pharmacology of endogenous neuroactive steroids. *Crit Rev Neurobiol* 15: 197-234.

Redmond DE JR, Murphy DL, Baulu J, Ziegler MG and Lake CR 1975. Menstrual cycle and ovarian hormone effects on plasma and platelet monoamine oxidase (MAO) and plasma dopamine-beta-hydroxylase (DBH) activities in the rhesus monkey. *Psychosom Med.* 37(5): 417-28.

Rettori V, Milenkovic L, Aguila MC and McCann SM 1990. Physiologically significant effect of neuropeptide Y to suppress growth hormone release by stimulating somatostatin discharge. *Endocrinology* 126: 2296-2301.

Rettori V, Milenkovic L, Riedel M and McCann SM 1990. Physiological role of neuropeptide Y (NPY) in control of anterior pituitary hormone release in the rat. *Endocrinol Exp* 24: 37-45.

Reznikov AG and McCann SM 1993. Effects of neuropeptide Y on gonadotropin and prolactin release in normal, castrated or flutamide-treated male rats. *Neuroendocrinology* 57(6): 1148-1154.

Richards JS and Williams JL 1976. Luteal cell receptor content for prolactin (PRL) and luteinizing hormone (LH). Regulation by LH and PRL. *Endocrinology* 99: 1571-1581.

Richardson DW, Goldsmith LT, Pohl CR, Schallenberger E and Knobil E 1985. The role of prolactin in the regulation of the primate corpus luteum. *J Clin Endocrinol Metab* 60: 501-504.

Roa J and Herbison AE 2012. Direct regulation of GnRH neuron excitability by arcuate nucleus POMC and NPY neuron neuropeptides in female mice. *Endocrinology* 153 (11): 5587-99.

Roy D, Angelini NL and Belsham DD 1999. Estrogen directly respresses gonadotropin-releasing hormone (GnRH) gene expression in estrogen receptor-alpha (ERα) and ER-beta (ERβ) expressing GT1-7 GnRH neurons. *Endocrinology* 140: 5045-5053.

Rudolf K, Eberlein W, Engel W, Beck-Sickinger A, Wittneben H and Doods H 1997. BIBP3226, a potent and selective neuropeptide Y Y1-receptor antagonist. Structure-activity studies and localization of the human Y1 receptor binding. In: Grundemar L, Bloom S (eds), Neuropeptide Y and Drug Development. San Diego: Academic Press. pp. 175-190

Rupprecht R 2003. Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. *Psychoneuroendocrinology* 28: 139-168.

Rupprecht R and Holsboer DF 1999a. Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci* 22: 410-416.

Rupprecht R and Holsboer DF 1999b. Neuropsychopharmacological properties of neuroactive steroids. *Steroids* 64: 83-91.

Rupprecht R, Rammes G, Eser D, Baghai TC, Schüle C, Nothdurfter C, Troxler T, Gentsch C, Kalkman HO, Chaperon F, Uzunov V, McAllister KH, Bertaina-Anglade V, La Rochelle CD, Tuerck D, Floesser A, Kiese B, Schumacher M, Landgraf R, Holsboer F and Kucher K 2009. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science* 325 (5939): 490-493.

Sabatino FD, Collins P and McDonald JK 1990. Investigation of the effects of progesterone on neuropeptide Y-stimulated luteinizing hormone-releasing hormone secretion from the median eminence of ovariectomized and estrogen-treated rats. *Neuroendocrinology* 52(6): 600-607.

Sahu A, Crowley WR and Kalra SP 1995. Evidence that hypothalamic neuropeptide Y gene expression increases before the onset of the preovulatory LH surge. *J Neuroendocrinol* 7: 291-6.113.

Sahu A, Kalra SP, Crowley WR and Kalra PS 1988b. Evidence that NPY-containing neurons in the brainstem project into selected hypothalamic nuclei: implication in feeding behavior. *Brain Res* 457: 376-378.

Salamonsen LA, Marsh MM and Findlay JK 1999. Endometrial endothelin: regulator of uterine bleeding and endometrial repair. *Clin Exp Pharmacol Physiol* 26: 154-157.

Sarkar DK and Fink G 1980. Luteinizing hormone releasing factor in pituitary stalk plasma from long-term ovariectomized rats: effects of steroids. *J Endocrinol* 86: 511-524.

Scarbrough K, Jakubowski M, Levin N, Wise PM and Roberts JL 1994. The effect of time of day on levels of hypothalamic proopiomelanocortin primary transcript, processing intermediate and messenger ribonucleic acid in proestrous and estrous rats. *Endocrinology* 134: 555-561

Schneider LF and Warren MP 2006. Functional hypothalamic amenorrhea is associated with elevated ghrelin and disordered eating. *Fertil Steril* 86: 1744-1749.

Schumacher M, Guennoun R, Ghoumari A, Massaad C, Robert F, El-Etr M, Akwa Y, Rajkowski K and Baulieu EE 2007. Novel perspectives for progesterone in hormone replacement therapy, with special reference to the nervous system. *Endocr Rev* 28: 387-439.

Shah N, Alo J, Evans WS and Veldhui JG 1999. Time Mode of Growth Hormone (GH) entry into the bloodstream and steady-state plasma GH concentrations, rather than sex, estradiol, or menstrual cycle stage, primarily determine the GH elimination rate in healthy young women and men. *Clin endocrinol metab* 84(8): 2862-2869

Shahab M, Balasubramaniam A, Sahu A and Plant TM 2003. Central nervous system receptors involved in mediating the inhibitory action of neuropeptide Y on luteinizing hormone secretion in the male rhesus monkey (*Macaca mulatta*). *J Neuroendocrinol* 15: 965-970.

Shaikh AA, Naqvi RH and Shaikh SA 1987. Concentrations of oestradiol-17beta and progesterone in the peripheral plasma of the cynomolgus monkey (*Macaca fascicularis*) in relation to the length of the menstrual cycle and its component phases. *J Endocrinol* 79: 1-7.

Shin SH, Papas S and Obonsawin MC 1987. Current status of the rat prolactin releasing factors. *Can. J. Physiol. Pharmacol.* 65: 2036 –2043.

Shivers BD, Harlan RE, Morrell JI and Pfaff DW 1983. Absence of oestradiol concentrationin cell nuclei of LHRH-immunoreactive neurones. *Nature* 304: 345-347.

Shughrue PJ, Lane MV and Merchenthaler I 1997. Regulation of progesterone receptor messenger ribonucleic acid in the rat medial preoptic nucleus by estrogenic and antiestrogenic compounds: an in situ hybridization study. *Endocrinology* 138: 5476-5484.

Shull JD and Gorski J 1984 Estrogen stimulates prolactin gene transcription by a mechanism independent of pituitary protein synthesis. *Endocrinology* 114: 550-1557.

Shull JD and Gorski J 1989 Estrogen regulation of prolactin gene transcription in vivo: paradoxical effects of 17beta-estradiol dose. *Endocrinology* 124: 279-85.

Shupnik MA 1996. Gonadotropin gene modulation by steroids and gonadotropin-releasing hormone. *Biol Reprod* 54: 279-286.

Simpson ME, MarxW, Becks H and Evans HM 1944. Effect of testosterone propionate on the body weight and skeletal system of hypophysectomized rats. Synergism with growth hormone. *Endocrinology* 35: 309-316.

Singh H, Griffith RW, Takahashi A, Kawauchi H, Thomas P and Stegeman JJ 1988. Regulation of gonadal steroidogenesis in Fundulus heteroclitus by recombinant salmon growth hormone and purified salmon prolactin. *Gen Comp Endocrinol* 72: 144-153.

Sirotkin AV, Makarevic AV, Kotwica J, Marnet PG, Kwon HB and Hetenyi L 1998. Isolated porcine ovarian follicles as a model for the study of hormone and growth factor action on ovarian secretory activity. *J Endocrinol* 159: 313-321.

Smith CG, Almirez RG, Berenberg J and Asch RH 1983. Tolerance develops to the disruptive effects of THC on the primate menstrual cycle. *Science* 219:1453-1455.

Smith MS, Freeman ME and Neill JD 1975. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology* 96: 219-226.

Smith SR, Ckhetri MK, Johanson J, Radfar N and Migeon CJ 1975. The pituitary-gonadal axis in men with protein-calorie malnutrition. *J Clin Endocrinol Metab* 41: 60-69.

Sokolowski MB 2003. NPY and the regulation of behavioral development. *Neuron*. 39 (1): 6–8.

Stabenfeldt GH and Hendrickx AG 1972. Progesterone levels in the bonnet monkey (*Macaca radiata*) during the menstrual cycle and pregnancy. *Endocrinology* 91: 614-619.

Stanhope R, Albanese A, Hindmarsh P and Brook CGD 1992. The effects of growth hormone therapy on spontaneous sexual development. *Hor Res* 38: 9-13.

Steele MK 1992. The role of brain angiotensin II in the regulation of luteinizing hormone and prolactin secretion. *Trends Endocrinol Metab* 3: 295-301.

Stewart AJ, Katz AA, Millar RP and Morgan K 2009. Retention and silencing of prepro-GnRH-II and type II GnRH receptor genes in mammals. *Neuroendocrinol* 90: 416-432.

Stoecklin E, Wissler M, Schaetzle D, Pfitzner E and Groner B 1999. Interactions in the transcriptional regulation exerted by Stat5 and by members of the steroid hormone receptor family. *J Steroid Biochem Mol Biol* 69: 195-204.

Stouffer RL 2003. Progesterone as a mediator of gonadotrophin action in the corpus luteum: beyond steroidogenesis. *Hum Reprod*. 9: 99-117.

Suganuma N, Kikkawa F, Narita O and Tomoda Y 1988 Changes in Serum Prolactin Levels During the Normal Menstrual Cycle. Basel: Karger.

Sun L, Philipson LH and Miller RJ 1998. Regulation of K and Ca channels by a family of neuropeptide Y receptors. *J Pharmacol Exp Ther* 284: 625-632.

Sutton SW, Toyamas TT, Otto S and Plotsky PM 1988. Evidence that neuropeptide Y (NPY) released into the hypophysial-portal circulation participates in priming gonadotrophs to the effects of gonadotropin releasing hormone (GnRH). *Endocrinology* 123: 1208-1210.

Suzuki N, Okada K, Minami S and Wakabayashi I 1996. Inhibitory effect of neuropeptide Y on growth hormone secretion in rats is mediated by both Y1-and Y2-receptor subtypes and abolished after anterolateral deafferentation of the medial basal hypothalamus. *Regul Pept* 65: 145-151.

Tapanainen J, Martikainen H, Voutilainen R, Orava M, Ruokonen A and Ronnberg L 1992. Effect of growth hormone administration on human ovarian function and steroidogenic gene expression in granulosa-luteal cells. *Fertil Steril* 58: 726-732.

Tatemoto K 1982. Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proc Natl Acad Sci USA* 79: 2514-2518.

Tena-Sempere M 2006. GPR54 and kisspeptin in reproduction. *Hum Reprod* 12 (5): 631-639.

Thomas MG, Gazal OS, Williams GL, Stanko RL and Keisler DH 1999. Injection of neuropeptide Y into the third cerebroventricle differentially influences pituitary secretion of luteinizing hormone and growth hormone in ovariectomized cows. *Domest Anim Endocrinol* 16: 159-169.

Thorneycroft IH, Mishell OR, Stones SC, Kharma KM and Nakamura RM 1971. The relation of serum 17-hydroxyprogesterone and estradiol- 17f1-levels during the human menstrual cycle. *Am J Obstet Gynecol* 111:947-51.

Thorsell A and Heilig M 2002. Diverse functions of neuropeptide Y revealed using genetically modified animals. *Neuropeptides* 36: 182-193.

Tilemans D, Andries M and Denef C 1992. Luteinizing hormone-releasing hormone and neuropeptide Y influence deoxyribonucleic acid replication in three anterior pituitary cell types. Evidence for mediation by growth factors released from gonadotrophs. *Endocrinology* 130: 882-894.

Titolo D, Cai F and Belsham DD 2006. Coordinate regulation of neuropeptide Y and agouti-related peptide gene expression by estrogen depends on the ratio of estrogen receptor (ER) alpha to ERbeta in clonal hypothalamic neurons. *Mol Endocrinol* 20: 2080-2092.

Toney TW, Pawsat DE, Fleckenstein AE, Lookingland KJ and Moore KE 1992. Evidence that prolactin mediates the stimulatory effects of estrogen on tuberoinfundibular dopamine neurons in female rats. *Neuroendocrinology* 55: 282-289.

Tong Y, Simard J, Labrie C, Zhao HF, Labrie F and Pelletier G 1989. Inhibitory effect of androgen on estrogen-induced prolactin messenger ribonucleic acid accumulation in the male rat anterior pituitary gland. *Endocrinology* 125: 1821-1828.

Treiser SL and Wardlaw SL 1992. Estradiol regulation of proopiomelanocortin gene expression and peptide content in the hypothalamus. *Neuroendocrinology.* 55(2): 167-73.

Trott JF, Vonderhaar BK, Hovey RC 2008. Historical perspectives of prolactin and growth hormone as mammogens, lactogens and galactagogues—Agog for the future! *J Mammary Gland Biol Neoplasia* 13: 3-11.

Tsutsui K 2010. Phylogenetic aspects of gonadotropin-inhibitory hormone and its homologs in ertebrates. *Ann N Y Acad Sci.* 1200: 75-84.

Vekemans M, Delvoye P, L'hermite M and Robyn C 1977. Serum prolactin levels during the menstrual cycle. *J Clin Endocrinol Metab* 44: 989-993.

Veldhuis JDAND Johnson ML1990. A review and appraisal of deconvolution methods to evaluate in vivo neuroendocrine secretory events. *J Neuroendocrinol*. 2: 755-771.

Vezzani A, Sperk G and Colmers WF 1999. Neuropeptide Y: emerging evidence for a functional role in seizure modulation. *Trends in Neurosciences* 22: 25-30.

Wade DT, Wood VA and Hewer RL 1985. Recovery after stroke-the first 3 months. *J Neurol Neurosurg Psychiatry* 48: 7-13.

Wade GN and Jones JE 2004. Neuroendocrinology of nutritional infertility. *Am J Physiol Regul Integr Comp Physiol.* 287: R1277-R1296.

Wade GN, Schneider JE and Li HY 1996. Control of fertility by metabolic cues. *Am J Physiol Endocrinol Metab.* 270: E1–19.

Wang J, Ciofi P and Crowley WR 1996. Neuropeptide Y suppresses prolactin secretion from rat anterior pituitary cells: evidence for interactions with dopamine through inhibitory coupling to calcium entry. *Endocrinology* 137: 587-594.

Ward DR, Dear FM, Ward IA, Anderson SI, Spergel DJ, Smith PA and Ebling FJ 2009. Innervation of gonadotropin-releasing hormone neurons by peptidergic neurons conveying circadian or energy balance information in the mouse. PLoS ONE 4 (4): e5322.

Watanobe H and Takebe K 1992. A further study on the stimulatory effect of peptide histidine methiolfine on growth hormone secretion in acromegaly: a dose-related study and a comparison with vasoactive intestinal peptide. *Neuropeptides* 23: 115-119.

Watanobe H and Takebe K 1992. Evidence that neuropeptide Y secretion in the median eminence increases prior to the luteinizing hormone surge in ovariectomized steroid-primed rats: estimation by push-pull perfusion. *Neurosci Lett* 146: 57-59.

Watanobe H and Tamura T 1996. Stimulation by neuropeptide Y of growth hormone secretion in prolactinoma in vivo. *Neuropeptides* 30: 429-432.

Watanobe H, Sasaki S and Takebe K 1991. Failure to confirm a growth hormone-releasing activity of corticotropin-releasing hormone in acromegaly: comparison with the effects of other hypothalamic hormones. *Acta Endocrinol* 125: 487-490.

Watanobe H, Sasaki S, Sone K and Takebe K 1991. Paradoxical response of growth hormone to peptide histidine methionine in acromegaly: comparision with the effects of thyrotropin releasing hormone and vasoactive intestinal peptide. *J Clin Endocrinol Metab* 72: 982-985.

Weinbauer GF, Niehoff M, Niehaus M, Srivastav S, Fuchs A, Esch EV and Cline MJ 2008. Physiology and endocrinology of the ovarian cycle in macaques. *Toxicol Pathol.* 36(7S): 7-23.

Weiner RI and Martinez de la Escalera G 1993. Pulsatile release of gonadotrophin releasing hormone (GnRH) is an intrinsic property of GT1 GnRH neuronal cell lines. *Hum Reprod.* 8: 13-17.

White RB, Eisen JA, Kasten TL and Fernald RD 1998. Second gene for gonadotropin releasing hormone in humans. *Proc Natl Acad Sci U S A* 95 (1): 305-9.

Widdowson, PS, Buckingham R and Williams G 1997. Distribution of [Leu31,Pro34]NPY-sensitive, BIBP3226-insensitive [125I]PYY(3-36) binding sites in rat brain: possible relationship to Y5 NPY receptors. *Brain Res.* 778 (1): 242-250.

Wilson ME, Gordon TP, Rudman CG and Tanner JM 1989. Effects of growth hormone on the tempo of sexual maturation in female rhesus monkes. *J Clin Endocrinol Metab* 68: 29-38.

Winters SJ and Moore JP 2007. Paracrine control of gonadotrophs. *Semin Reprod Med* 25: 379-387.

Woldbye DPD, Larsen PJ, Mikkelsen JD, Klemp K, Madsen TM and Bolwig TG 1997. Powerful inhibition of kainic acid seizures by neuropeptide Y via Y5-likereceptors. *Nature Med* 3:761-764

Woller MJ and Terasawa E 1991. Infusion of Neuropeptide Y into the stalk–median eminence stimulates in vivo release of luteinizing hormone releasing hormone in gonadectomized rhesus monkeys. *Endocrinology* 128: 1144-1150

Woller MJ and Terasawa E 1992. Estradiol enhances the action of neuropeptide Y on in vivo luteinizing hormone-releasing hormone release in the ovariectomized rhesus monkey. *Neuroendocrinology* 56: 921-925.

Woller MJ and Terasawa E 1994. Changes in pulsatile release of neuropeptide-Y and luteinizing hormone (LH)-releasing hormone during the progesterone induced LH surge in rhesus monkeys. *Endocrinology* 135: 1679-1686.

Woller MJ, McDonald JK, Reboussin DM and Terasawa E 1992. Neuropeptide Y is a neuromodulator of pulsatile luteinizing hormone-releasing hormone release in the gonadectomized rhesus monkey. *Endocrinology* 130: 2333-2342.

Xia L, Van Vugt D, Alston EJ, Luckhaus J and Ferin M 1992. A surge of gonadotropin-releasing hormone accompanies the estradiol-induced gonadotropin surge in the rhesus monkey. *Endocrinology* 131: 2812-2820.

Xu M, Hill JW and Levine JE 2000. Attenuation of Luteinizing Hormone Surges in Neuropeptide Y Knockout Mice. *Neuroendocrinology* 72: 263-271.

Xu M, Urban JH, Hill JW and Levine JE 2000. Regulation of hypothalamic neuropeptide Y Y1 receptor gene expression during the estrous cycle: role of progesterone receptors. *Endocrinology* 141 (9): 3319-3327.

Yen SH and Pan JT 1998. Progesterone advances the diurnal rhythm of tuberoinfundibular dopaminergic neuronal activity and the prolactin surge in ovariectomized, estrogen-primed rats and in intact proestrous rats. *Endocrinology* 139: 1602–1609.

Yen SSC 1997. Studies of the role of dopamine in the control of prolactin and gonadotropin secretion in humans. In: (ed) Fuxe K, Hokfelt T, Luft RCentral regulation of the endocrine system. New York' Plenum Press; NY. pp. 387-416.

Yewade NG, Andrew W, Horacio JN and Sally R 2009. Estrogen Regulation of Gene Expression in GnRH neurons. *Mol Cell Endocrinol*. 303(1-2): 25-33.

Yonezawa T, Mogi K, Li JY, Sako R, Manabe N, Yamanouchi K and Nishihara M 2011. Effect of estrogen on growth hormone pulsatility in peripheral blood and Neuropeptide profiles in the cerebrospinal fluid of goats. *J Reprod Dev* 57 (2): 280-287.

Yoshimura Y, Iwashita M, Karube M, Oda T, Akiba M, Shiokawa S, Ando M, Yoshinaga A and Nakamura Y 1994. Growth hormone stimulates follicular development

by stimulating ovarian production of insulin-like growth factor-I. *Endocrinology* 135: 887-894.

Yoshimura Y, Jinno M, Oda T, Shiokawa S, Oshinaga A, Hanya I, Akiba M and Nakamura Y 1994. Prolactin inhibits ovulation by reducing ovarian plasmin generation. *Biol. Reprod.* 50: 1223-1230.

Yoshimura Y, Nakamura Y, Koyama N, Iwashita M, Adachi T and Takeda Y 1993. Effects of growth hormone on follicle growth, oocyte maturation, and ovarian steroidogenesis. *Fertil Steril* 59: 917-923.

Yoshimura Y, Nakamura Y, Koyama N, Iwashita M, Adachi T and Takeda Y 1993. Effects of growth hormone on follicle growth, oocyte maturation, and ovarian steroidogenesis. *Fertil Steril* 59: 917-923.

Yoshimura Y, Nakamura Y, Yamada M, Ando M, Ubukata Y, Oda T and Suzuki M 1991. Possible contribution of prolactin in the process of ovulation and oocyte maturation. *Horm Res*. 35 (Suppl 1): 22-32.

Zachmann M 1992. Interrelations between growth hormone and sex hormones-physiology and therapeutic consequences. *Horm Res* 38: 1-8.

Zeleznik AJ 2001. Follicle selection in primates: "many are called but few are chosen". *Biol Reprod* 65(3): 655-659.

Zeleznik AJ and Pohl CR 2006. Control of follicular development, corpus luteum function, the maternal recognition of pregnancy, and the neuroendocrine regulation of the menstrual cycle in higher primates. In: Neill JD, (ed) Knobil and Neill's Physiology of Reproduction. Louis, MO: Elsevier, St, pp.2449-2510.

Zelinski-Wooten MB, Hutchison JS, Hess DL, Wolf DP and Stouffer RL 1998. A bolus of recombinant human follicle stimulating hormone at midcycle induces periovulatory events following multiple follicular development in macaques. *Hum Reprod* 13: 554-560.

Zhaohui Z, Jingzhu Z, Guipeng D, Xuesong W, Yuanming Z, Yinping W, Yugui C 2012. Role of neuropeptide Y in regulating hypothalamus-pituitary-gonad axis in the rats treated with electro-acupuncture. *Neuropeptides* 46(3):133-9.

Zhaohui Z, Yugui C, Yuanming Z, Xuesong W, Xiaobing J, Zhice X, Guipeng D, Qianle T and , Yue J 2007. Effect of acupuncture on pubertal development of rats and rabbits at different developmental stages. *Neuropeptides* 41: (4) 249-261.

Zimmermann RC, Xiao E and Bohlen P 2002. Administration of antivascular endothelial growth factor receptor 2 antibody in the early follicular phase delays follicular selection and development in the rhesus monkey. *Endocrinology* 143(7):2496-502.

Zinder O and Dar DE 1999. Neuroactive steroids: their mechanism of action and their function in the stress response. *Acta Physiol Scand* 167: 181-188.

Zukowska Z, Pons J, Lee E and Li LJ 2003 Neuropeptide Y: a new mediator linking sympathetic nerves, blood vessels and immune system? *Can J Physiol Pharmacol* 81: 89-94.