CHAPTER V

DISCUSSION

1. Basal values of thyroid and reproductive hormones during pre-treatment cycle

Among 7 selected adult female monkeys, the animals show the last 2 pretreatment menstrual cycles length of 26-35 days which are within the normal range (27-35 days) of the colony (Varavudhi *et al.*, 1982) and other reports of the same species elsewhere : Saldarini *et al.*, (1972) 20-46 days; Dukelow *et al.*, (1979) 26-36 days; Shaikh *et al.*, (1978) 22-37 days and Yoshida *et al.*, (1982) 25-35 days. The classical patterns of LH, FSH, E_2 and P in cynomolgus monkeys (Goodman *et al.*, 1977; Varavudhi and Yodyingyuad, 1980; Varavudhi *et al.*, 1982 ; Yoshida, 1982) resemble strongly that reported for the rhesus monkeys (Kerber *et al.*; 1969, Goodman and Hodgen, 1983) and human (Abraham *et al.*; 1972, Sherman and Korenman, 1975; Saxena *et al.*, 1974; 1976; Laufer *et al.*, 1982). During 31 day menstrual cycle, the levels of P rise above 1 ng/ml for about 15 days prior to the onset of menstruation with peak values (7-8 ng/ml) observed during the mid-luteal phase (day 7) and declined to less than 1 ng/ml for 1-4 days before the next menstruation in cynomolgus monkeys (Goodman *et al.*, 1977). In this study, the levels of E_2 were 47.70 $\pm$ 29.96 pg/ml on D3 and then rose

significantly to the level of 143.61 ± 42.02 pg/ml on D10 of the follicular phase but the levels declined to 72.45 ± 11.87 pg/ml during expected luteal phase (D23) of the cycle. Meanwhile, greater level of P (6.24 ng/ml) is detected on the expected day of active luteal phase (D23) but consistently low during D3 and D10 of follicular phase. In women, intermittent serum LH, E_2 and P measurements on day 2,8 of follicular phase, day 14-16 of mid cycle and day 20, 25 of luteal phase are capable of detecting ovulation in normal menstraul cycle (Sxaena *et al.*, 1976). A normal ovulatory cycle is defined as the cycle which i) has serum LH peak higher than 150 ng/ml (LER-907) ii) has a mid luteal (D20 - D25) serum P levels of at least 3 ng/ml and iii) has a mid cycle (D12 - D17) or mid luteal (D20 - D25) serum E_2 levels of at least 150 pg/ml in women (Stroll *et al.*, 1970; Reeves and Diczfulusy, 1971; Abraham *et al.*, 1972; Saxena *et al.*, 1974; Guerrero *et al.*, 1976). Evidence indicated that serum levels of P and E_2 during pre-treatment cycle of each monkey was comparable with the normal menstrual pattern in women and may further suggested that all of these pretreatment cycles should have ovulation with normal corpus luteum. Similarly, the levels of T3, T4, fT4 and TSH in the expected follicular and luteal phases of pre-treatment cycle in these monkeys are not differed significantly as previously found in human with normal menstrual cycle (Standeven, 1969; Weeke and Hansen, 1975;

Hegedus *et al.*, 1986; Rasmussen *et al.*, 1989).Although, marked alteration in thyroid size could be recognized during menstrual cycle(Hegedus *et al.*,1986; Rasmussen *et al.*,1989) with elevated levels of serum thyroglobulin and TSH in women (Rasmussen *et al.*, 1989). Furthermore, the serum levels of TBG are not differed significantly when compared to those in follicular and luteal phases. In spite of only one immediate pre-treatment cycle with intermittent measurement of these hormones are performed in order to minimize blood loss during a long-term experiment, day to day measurements of these hormones including thyroglobulin and volume of the thyroid gland may need to identify whether this monkeys have similar cyclic variations of thyroid function.

In agreement with previous reports of PRL profile during menstrual cycle in cynomolgus monkeys (Varavudhi *et al.*, 1982; Tangpraprutigul *et al.*, 1987), the levels of PRL in the follicular (D3, D10) and luteal phases (D23) of the cycles are not different significantly. Furthermore, the mean serum PRL levels during the follicular phase (D5 - D8) are not statistically different from those during the luteal phase (D19 - D22) of normal 28-30 days of cycle in rhesus monkeys (Quadri and Spies, 1976). Contrary to the monkey reports, serum levels of PRL in women follow a similar patterns to that of endogenous estrogens during menstrual cycles and that PRL secretion is enhanced after exogenous administration of synthetic E_2 (Delvoye *et al.*, 1973, Franchimont, 1976). On the other hand, the changes

in PRL levels as compare with those of other reproductive hormones such as FSH, LH, E_2 and P during the menstrual cycle, obviously, do not allow either the establishment nor the precise definition of the ultimate role of PRL in the control of follicular maturation of ovulation or of formation and function of the corpus luteum (McNeilly *et al.*, 1982).

2. MMI-induced hypothyroidism

With regard to an inhibition of thyroid hormones synthesis at the thyroid glandular level, MMI was selected to induce the hypothyroidism in this study. The drug has considerable advantages over other anti-thyroid drugs including i) has long plasma half-life (5 - 5.5 hrs by HPLC) ii) has high bioavailability (93% by oral)(Kampmann and Hansen, 1981; Jansson *et al.*, 1985), iii) has no effect on the peripheral conversion of T4 to T3 in blood (Christensen *et al.*, 1969; Saberi *et al.*, 1975; Laurberg *et al.*, 1985), iv) the clinical effects of MMI are related to its concentration in the thyroid gland rather than in the plasma (Lazarus *et al.*, 1975) and maintained high level over 20 hrs in adult man (Jansson *et al.*, 1983a).

Initiation and maintenance dosages of MMI for treatment thyrotoxicosis are 30-60 mg and 5-20 mg/day respectively in man (Greer *et al.*, 1965; Green 1986; Shiroozu *et al.*, 1986). However, the rhesus monkeys appeared to be considerably less sensitive to antithyroid

drug including MMI than human (McGinty and Wilson, 1949). This monkeys require 5-10 times more drug to produce thyroidal inhibitory effects. In addition, normal heart rate of adult cynomolgus monkeys is 172-180 / min which is considerably much greater than normal rate in adult human (Hartley *et al.*, 1984). Hence, the optimal dosage (per body weight) of MMI required in this monkey should be 5 folds greater than normally use in human. In this study, MMI in the dose of 10 mg/day is chosen for initial treatment dose of monkeys which had body weigh range between 3.5 - 5.0 kgs followed by 5.0 and 2.5 mg/day as maintenance dose. However, there were no published reports of MMI force-fed daily for induction of hypothyroidism in non-human primates except the one case reported by Ren *et al.*, 1988 when they gave MMI (0.0125% w/v) in drinking water for a period of 12 weeks. Although overwhelming reports in non-primates by adding MMI into drinking water were published; adding 0.025% MMI in drinking water for 7 weeks is capable of inducing hypothyroidism in rats (Morreale de Escobar *et al.*, 1989; Mooradian, 1990). As compared to an oral dosing of MMI 10 mg/day in this study, adding MMI in drinking water may not be precise enough since dose will vary with the volumn of water taken by each animal.

The basal values of T3, T4, fT4 and TSH obtained on D3 of pre-treatment cycle are within the normal range reported in this macaqe (Smallrigde *et al.*, 1981, Kamis,

1982; Ren *et al.*,1988). It should be noted that the levels of T3, T4 and fT4 in all 7 monkeys fluctuated fairly widely prior to treatment, but were effectively and consistently lowered by MMI until they reached the state of severe hypothyroidism. It is noted that TSH levels had small oscillations during this period. This phenomena may be accounted for early inhibition of iodination process in thyroid gland which itself has some intrinsic ability (autoregulation) to adapt to iodine deficiency and independently of the pituitary TSH (Ingbar, 1985;, Gregerman, 1986; Slaunwhite, 1988). Both T3 and T4 have direct effects on thyroid gland both by inhibiting adenyl cyclase activity and interfering with its coupling process (Takasu *et al.*, 1974; Gafni *et al.*,1975; Yu *et al.*,1976). In this case, iodide depletion from prevented binding to tyrosine of MMI effects, therefore, is associated with an enhanced autoregulatory response (Ingbar, 1985). Available data in this study are capable of established MMI-induced hypothyroidism and characterizing typical patterns of major hormonal changes during MMI treatment, however, different individual time-response of thyroid hormones to MMI suppression was evidenced. The levels of fT4 declined rapidly and ranged between 0.03-0.45 ng/dl during severe hypothyroidism which confirmed by greater TSH (35-53 mIU/L), lower T3 (<80 ng/dl) and T4 (< 2.0 ug/dl). The required time reaching severe hypothyroidism in this study after treatment with 10 mg/day MMI ranged between 9-20 weeks. Similarly, hyperthyroidism patients treated with

single daily dose of 30 mg MMI required approximately 12 weeks to reduce serum thyroid hormone levels to be within normal range (Greer *et al.*, 1965; Shiroozu *et al.*,1986). In adult male and female cynomolgus monkeys feeding with 0.0125% MMI in drinking water, reduced T3 and T4 which in accorded with elevated TSH levels (> 50 mIU/L) were not achieved until after treatment for 12 weeks (Ren *et al.*, 1988). But in the rats which appear to be sensitive to antithyroid drug as compared to the monkey (Takayama *et al.*,1986), the time need to induce hypothyroid state was shorter and appeared to be within 7 weeks after treatment with 0.025 % MMI in drinking water (Mooradian, 1990). The significant clinical features show during severe hypothyroidism as lethargy, soft tissue edema particularly both eye lids, galactorrhea, amenorrhea and anorexia which corresponded with lower T3, T4, fT4 and greater TSH levels. Although, hypothyroidism has been seldom reported in primate, the clinical and laboratory findings in these monkeys are compatible with established syndromes of hypothyroidism in man (Tachman and Guthrie, 1984; Ingbar, 1985; Slaunwhite, 1988), and congenital hypothyroidism in chimpanzee (Miller, 1983). Thyroid hormones, therefore, may acutely regulate TSH release by suppressing TRH secretion or by inducing a decrease in the thyrotrope's sensitivity to TRH in man (Larsen, 1982) and monkeys (Belchetz *et al.*, 1987). Nevertheless, available data obtained permit to be considered fT4 is a significant

parameter for assessment the state of hypothyroidism which could be confirmed the effects by further using TSH, T3 and T4 measurement as described in the congenital hypothyroidism in chimpanzee (Miller, 1983). Moreover, availability of fT4 levels in serum is accepted as one of the most useful parameters of thyroid hormone action at cellular targets (Ekins, 1978; 1982; 1990). During severe hypothyroidism, both profound lower T3 and T4 may elucidate that both were suppressed by MMI at the thyroid gland level and not being peripherally conversed effects from drug (Larsen, 1982; Ingbar, 1985). It seems likely that strong suppression of MMI 10 mg/day during hypothyroidism is capable of inducing nearly completed inhibition of iodination process, subsequently, very low T3 and T4 produce from the thyroid gland. Although only 20 % of amount of T3 is synthesized and released from the thyroid gland but the most 80 % come from peripheral conversion of T4 (Larsen, 1982; Ingbar, 1985). From this point, it may be suggested that T4 is capable of converting T3 during severe hypothyroidism as normally as occur in the physiological state. Rapid increase in TSH levels during early period of severe hypothyroidism and decrease suddenly in late period as represented in fig 17-23 clearly indicated that the trigger from thyroid hormones to TSH release is very rapid response (Larsen, 1982). During severe hypothyroidism, consistently higher TSH levels may probably due to this hormone has slower degradation rate but with higher rate of secretion (Odell

et al.,1967). For fT4, it is widely accepted that its absolute concentration is depened on the relative proportion of both TBG and T4 (Woeber, 1987). Most severe hypothyroidism periods were followed by a striking compensation of T3, T4, and fT4 concurrently with response to reduced TSH levels. Four out of seven monkeys (no. 77, 87, 63, and 78) were unable to detect the compensatory period. It is possible that partial removal of MMI inhibition from of 10 mg/day to 0.5 mg/day may lead to relaxation of the thyroid which in turn results in partial recovery of thyroid hormones synthesis,subsequently, enhances in peripheral conversion of T3 and absolute fT4 concentrations. Conversely, these thyroid hormones turn to trigger the TSH regulation resulting in decreased serum levels of this hormone therafter. The compensatory period is proportionate to the degree of hormone inhibition (Kaptein *et al.*,1980). However, some compensatory period (particularly no. 77, 87, 63, and 78) may be occured in a relatively short time during a weekly interval bleeding and unable to detect.

During the period of prolonged maintenance with daily dose of 2.5 mg MMI, adaptive homeostasis of thyroid hormone is recurrent obviously as evidenced as the plateau levels of thyroid hormone during mild hypothyroidism . Meanwhile, strongly adaptive homeostasis is occured and demonstrated the second hypothyroidism state in the monkey no. 101. Additionally, the serum T3 ranged within normal

pretreatment value (110-260 ng/dl) while the fT4 and T4 were below the normal lower limit (0.50-1.10 ng/dl and 2-6.20 ug/dl, respectively) during mild hypothyroidism. The levels of TSH ranged between 4-19 mIU/L. The animals showed recovery signs of severe hypothyroidism. These evidences were agreed with Lambardi *et al.* (1986) reported in subclinical (mild) hypothyroid patients which defined as normal thyroid hormone levels with normal or slightly increased basal TSH levels and absence of clinical signs of hypothyroidism. Eventhough T3 is metabolically more active than T4 and has a small pool in the body. But T3 has a faster turnover rate (t 1/2 B 13 hrs) and is found mainly in intracellular fluid. Conversely, T4 has a large pool in the body, a low turnover rate (t 1/2 B 29.5 hrs) and manily found in the circulation. Within the pretreatment value of T3 during mild hypothyroidism may be indicated the preservation of active metabolic T3.

It is concluded that MMI is very potent antithyroid drug inducing various degree of hypothyroidism. Four states of hypothyroidism in cynomolgus monkeys are accomplished by varying daily dosage of MMI and are also first report . These conditions are available for further study the influences of these states upon the ovarian function and related reproductive endocrinology in non-human primate.

3. Patterns of TSH and PRL secretion during MMI-induced hypothyroidism.

The present evidences showed a positive correlation between serum TSH and PRL during the state of severe hypothyroidism, in accorded with the previous reports on woman (Onishi *et al.*, 1977; Honbo *et al.*,1987). Increase in serum TSH and PRL levels may mediate by feedback-induced TRH secretion (Onishi *et al.*,1977; Honbo *et al.*,1978; Feek *et al.*, 1980; Tuomisto, 1985; Foord *et al.*, 1986) and by inhibited hypothalamic dopamine secretion (Scanlon *et al.*, 1977; Feek *et al.*,1980). Dopamine and TRH appear to play a mutually antagonistic role in the control of TSH and PRL secretion in human (Besses *et al.*, 1975; Burrow *et al.*, 1977; Scanlon *et al.*,1977; Foord *et al.*, 1986) and rats (Ranta *et al.*, 1977). It is of interesting that rapid increase following decrease in TSH levels always accompanied by a rise and then a fall of serum PRL levels in the early and late severe hypothyroidism peroids, respectively. Moreover, reduced TSH levels also followed by a decrease in PRL levels during the compensatory period. These indicate the existence of a common regulator stimulating TRH and inhibitory dopamine of both TSH and PRL secretion. In contrast, the negative and significant correlation between TSH and PRL during mild hypothyroidism and recovery period point out that both hormones are not paralleled in response. Earlier reports claimed that thyroid hormones

per se are responsible for the blunt response of PRL secretion to TRH (Rapoport *et al.*, 1973; Refetoff *et al.*, 1974; Yamachi, 1974). In agreement with Yamaji (1974), the dissociation of TSH and PRL levels in these states may be delineated that TSH is more susceptible to inhibition by thyroid hormones than that of PRL. In addition, difference responses of both PRL and TSH to three dopamine antagonists (metoclopramide, chlorpromazine and sulpiride) were reported and lead to the possible different neuroregulatory pathway (Kikuoka *et al.*,1982).

It is noted that the galactorrhea could be detected during severe hypothyroidism in all seven monkeys. It is emphasized that hyperprolactinemia results the development of breast and formation of milk in the acini (Archer, 1980, McNeilly, 1986). However, an increase in PRL concentrations during hypoestrogenaemia in the state of severe hypothyroidism seemed likely lower than those found in the initiation of lactation. It may be suggested that E_2 is one of modulating factors of PRL secretion.

4. TBG profile patterns during MMI-induced hypothyroidism and MMI-withdrawal peroid.

Strong fluctuations of TBG were seen in all monkeys during the early period of MMI treatment prior to reach the state of severe hypothyroidism. It is likely that fluctuations of TBG levels were the consequence of

thyroid hormone occillations during earlier period of MMI suppression. Among the most sensitive monkey to MMI treatment, no.33, it was noted that the levels of TBG tended to be low concurrently with sudden decrease in both T4 and fT4 levels. In the fact of this point, total T3 and T4 rise and subsequently fall in concert with TBG levels, therefore, TBG levels must relate to both the levels of free thyroid hormones and their delivery to site of action (De Nayer, 1982). According to the theoretical equation of single standard mass of action, the fT4 is directly related to the total T4 but inversely related to TBG at the equilibrium state (Robbin and Johnson, 1979). During severe hypothyroidism, the animals showed both unchanged trend and increased trend in serum TBG levels. Clearly, difference of TBG response to the lower T4 and fT4 levels affected considerably from the variation of early suppression of thyroid hormones synthesis by MMI, depending on the individual thyroid gland 's autoregulation. However, this phenomena could be elucidated that deficiency in thyroid hormones result in increment of unoccupied binding sites of TBG (De Nayer, 1982 , Woeber, 1986). These evidences were in agreement with Inada and Stering (1967) who reported that 6 out of 11 patients with hypothyroidism had elevated TBG capacities with markedly diminished fT4 fraction. Furthermore, hyperthyroidism in man is often accompanied by a decrease in TBG, whereas in hypothyroidism, the mean TBG levels show a slight increase above normal values

(Gershengorn *et al.*, 1980). Marked increase in TBG concentrations in the different times of severe hypothyroidism, particularly in monkey no. 101, may affected from thyroid hormone deficiency. In thyroidectomized rhesus monkeys, a 21 % increase in serum TBG concentration is reported and further confirmed by decrease in TBG levels after administration of substitute treatment with T4 (Glinoer *et al.*,1979) which leads to decrease in unoccupied binding sites of TBG which subsequently shift to increase in fT4 concentration (Woeber, 1986). It has been estimated that 70 % of T4 is carried by TBG and about one-fourth of the TBG molecules contain T4 (Robbin and Edelhoch, 1986). Under physiological condition, the average proportion of fT4 is approximately 0.03% of the total T4, in turn, this fT4 proportion yields the concentration of fT4 in blood (Woeber, 1986). Obviously, unchanged trend of TBG fluctuated within pretreatment value in 5 monkeys indicated the subsequent shift of TBG toward the equilibrium state (Ekins, 1984; Woeber, 1986; Chopra, 1986).

Most studies in TBG are done in rhesus monkeys, hence, still lacking reports in cynomolgus monkeys from other laboratories. It is noticed that the normal values of TBG in rhesus monkeys are 2-3 times higher than those in cynomolgus monkeys as determined by RIA method. The basal values of TBG in cynomolgus monkeys are 9.37-13.01

ug/ml whereas the value in rhesus monkeys are 23-27 ug/ml (Glinoer *et al.*,1979; Suwanprasert *et al.*,1989; 1990). In this study, TBG was measured using heterogenous RIA and based on the principle that large protein molecule like as TBG has many antigenic determinant which could bind both T4 and anti- TBG simultaneously (Chan and Aucock, 1980). This method claim to measure the'functionally active' sites on TBG and therefore reflect the concentration of TBG (De Nayer, 1982). Although parallelism exists between cynomolgus TBG and human TBG in this RIA system gave good reproducibility both of within and of between assay variation (6.06 and 7.03%, respectively). Normal cynomolgus sera from free-ranging monkeys and captive monkeys in the primate research unit were tested and showed to be similar values, ranging 8.8-10.9 ug/ml and 8-12 ug/ml, respectively (unpublished data). Moreover, greater values of TBG were found ranging 10-18 ug/ml in captive pregnant monkeys. Repeated measurement in sera captive from male *Macaca assumensis* exhibited 7.9-9.9 ug/ml. Controversial data reported from rhesus monkeys leading to distinguish from those in cynomolgus monkeys may come from using homogenous rhesus RIA. Furthermore, the half-life of T4 is longer in rhesus monkeys (2.7 days) than that in cynomolgus monkeys (1.23 days) (Glinoer *et al.*,1979; Smallridge *et al.*,1981). The shorter T4 half-life in cynomolgus monkeys might suggest difference in serum protein binding between these two closely related

macaques (Smallridge *et al.*,1981).

From this study, it is concluded that lower T4 leads to relative higher unsaturated binding sites of TBG in blood, hence, an increase in the capacity of TBG with disproportionate decreased fT4 during severe hypothyroidism (no.77 and 101) . The mechanism is differed from greater levels of TBG during pregnancy.

5. Baseline levels of thyroid hormones, TSH, PRL, TBG, E_2 and P in normal pregnancy and pregnancy previously treated with MMI (no 63)

Post-MMI treated monkey (no. 63) showed normal gestational period of 158 days (normal 162-171 days). This is in agree with previous record in the colony (158-174 days) (Varavudhi *et al.*, 1982). Indeed, the monkey previously treated with MMI exhibited the shortest gestational period ever recorded in this breeding colony and delivered smaller body weight at birth (280 gms) with no any symptoms of cretinism.

In human normal pregnancy, an elevated renal iodide clearance subsequently results an increase in thyroid ^{131}I uptake, thyroid iodide clearance rate and thyroid gland size (Burrow, 1980; Freink *et al.*, 1985; Gregerman, 1986). The observed lower T3, T4 and fT4 levels coincided with elevated TSH during D8 - D36 of gestation may directly relate with enhanced maternal pituitary-thyroidal axis during critical time of

implantation and placentation as well as transformation of corpus luteum P production to the syncytiotrophoblastic tissue. With regarding to the serum levels of thyroid hormones prior to the critical time of implantation, reduction of T4 : T3 ratio is evidenced. Indeed, T3 levels is abruptly increased during progestational period of pregnancy and remained high until critical time of implantation, presumably, directly converted from T4. Greater T3 levels are mainly retained in a large pool at the intracellular fluid (lngbar, 1985, Larsen, 1986). It is suggested that an elevated T3 levels may need for rise in BMR as well as initiation of blastocyst implantation. It is of interesting that an elevated TSH levels on D15 - D29 is compatible with the time which detected monkey chorionic gonadotrophin (mCG) in urine during D17 - D28 of gestation (Varavudhi *et al.*, 1982). Although early and term placenta of rhesus monkeys still contain biologically and immunologically active CG and having the fractionated components, CG L and CG B subsnits, similar with hCG (Hobson and Wide, 1981). TSH is a glycoprotein hormone showing immunologic cross reactivity with hCG and other glycopeptide hormones (Norman *et al.*, 1985; Ingbar, 1985; Carayon *et al.*, 1980; Pekonen *et al*, 1988). On the other hand, very low cross reaction (< 0.1%) between hCG and hSH is evidenced in this assay. Moreover, the levels of TSH do not rise throughout the latter part of normal pregnancy. But TSH levels of the monkey previously

treated with MMI (no 63) declined gradually. Although it is widely accepted that hCG has a stimulatory thyrotropic activity (Carayon *et al.*,1980;Norman *et al.*, 1985; Pekonen *et al.*, 1988), there still has no evidence of intrinsic thyrotropic activity of mCG in the monkey. It is emphasized that no suppression of TSH corresponding with lower T3, T4 and fT4 levels occurred during the active period of mCG secretion (D17 - D28) as reported previously in human (Kannern *et al.*, 1973; Kvetny and Poulsen, 1984). Similarly, an elevated TSH levels during pregnancy in women are earlier reported (Kannon *et al.*, 1973; Skjoldebrand *et al.*, 1982; Rasmussen *et al.*, 1989; Price *et al.*, 1989).

The ratio of T4 : T3 increased abruptly during late first trimester and lasted throughout the third trimester in which the serum T3 levels are still consistently lowered. These clearly indicate the fetal requirement of maternal T4. At this time, serum levels of TSH reduced immediately reaching the pretreatment values whereas the fT4 levels increased prominently with consistently slight increase in T4 levels.

Additionally, an increase in the carrier protein (TBG) coincides with elevation of E_2 levels with good correlation ($r = 0.60$) only during the early period of gestation points out the important role of TBG and thyroid hormones in this period (Suwanprasert *et al.*, 1989, 1990). E_2 secreted from the feto-placental unit is widely

accepted and believed to be the main factor responsible stimulation of TBG synthesis and secretion in monkeys (Glinoer *et al.*, 1978; 1979; 1982). However, time-response difference of individual results uncommitant increase in E_2 and TBG during mid and late pregnancy, hence, poor correlation are obtained. Similar supportive evidences of poor correlations within the individual two-week intervals of E_2 and TBG increment are also noticed in pregnant women (Skjoldebrand *et al.*, 1987). Primary alterations of elevated TBG concentrations subsequently result physiological increase in T4 concentrations as showing the positive correlation between T4 and TBG ($r = 0.55$) throughout the gestation. This finding indicates that predominent bound of T4 to TBG does occur in this pregnent monkeys. Consequently, slight increase but not reach significant in fT4 concentrations are observed throughout the gestation. With regard to the first report of the fT4 measurement in cynomolgus monkeys, comparative two-step RIA method which showed excellent correlation with direct equilibrium dialysis ($r = 0.99$) is considerably approached for the monkey sera (Wirquin *et al.*, 1987). Direct equilibrium dialysis is purposed to be reliable and given more closely to the clinical pictures such as pregnancy, nonthyroidal illness, analbuminemia, during heparin therapy, etc. (Ekins, 1978; 1979; 1982; 1984; 1990; Hashimoto and Matsubara, 1987; Witherspoon *et al.*, 1988). There are conflicting reports regarding the constancy of

the concentration of fT4 during pregnancy in women (Ekins, 1978, Boss and Kingstone, 1981). Some investigators reported constant fT4 concentrations throughout pregnancy, others showed a slight decline. Skjoldebrand and his colleagues (1982) have been reported the contemporary decline in serum fT4 concentration when the concentration of TBG increases. Their findings would be elucidated that the decrease in fT4 concentration might also reflect the true physiological state as, at a constant increase in the total concentration of T4, the increase in concentration of fT4 should gradually decrease with increasing TBG concentrations (Hawe and Francis, 1962; Ingbar, 1985; Woeber, 1986).

It is generally accepted that estrogen is the chronic modulators of PRL secretion at the pituitary level and an increase in E_2 reflects to enhance PRL secretion (McNeilly, 1986). Additionally, elevated PRL might be stimulated from the common regulatory TRH, one of stimulator, corresponding with slight increase in TSH levels during second and third trimesters. Exact mechanism of elevated TSH during pregnancy is not clear. However, the quantitation of TSH is lesser than those in severe hypothyroidism but showing much greater PRL levels. Significant decrease in T3 levels in supportive evidence for slight increase in TSH concentrations. Controversial reports in human claimed that T3 unchanged during the first and second trimester but increased in the

third trimester (Skjoldebrand *et al.*, 1982; Price *et al.*, 1989). Finally, pregnancy pattern of serum P levels during the entire period of gestation in this macaque is more like the pattern of the woman than the rhesus macaque (Lanman, 1977). But its pattern of serum E_2 levels are almost identical with the value previously found in rhesus monkey (Hodgen, 1972).

Thyroid functions during pregnancy are very complicated and complex mechanisms. Human feto-placental unit is capable of producing and secreting several steroid and peptide hormones (Johnson and Everitte, 1980; Casey *et al.*, 1985). It is interesting that human chorionic thyrotropin (hCT) has been isolated from extracts of placenta and hydatidiform molar tissue and does not appear to be identical to that of the TSH produced by the human anterior pituitary (Casey *et al.*, 1985; Ingbar, 1985). The role of hCT is unclear, indeed, the principal component of hCT is similar to bovine TSH (Casey *et al.*, 1985). Aparts from these, the TRH-like substance is present in extract of placenta and claimed to control hCG and hCT production by trophoblastic tissue (Gibbon *et al.*, 1975; Casey *et al.*, 1985). Recently, Ekins (1989) have been postulated that the feto-placental unit control the economy of feto-maternal thyroid hormones.

In monkey previously treated with MMI (no. 63), it is obviously observed that only TSH declined gradually and in contrast to the elevated pattern of TSH in normal

pregnancy (fig.51). In addition, fT4 levels fluctuated and trended toward low. In spite of only one case of monkey previously treated with MMI being pregnancy, a large number of variations cause very difficult to elucidate exactly in the case of TSH and fT4 concentrations. It is more sililar pattern of T4 levels and those from normal pregnancy whereas the serum levels of T3 increased during early second trimester and declined during late second and third trimester. Consequently, T4 : T3 ratio is diminished during the early second trimester and contrasted to greater T4 : T3 ratio in the normal pregnancy. This findings might be the secondary consequence of recovery from the state of mild hypothyroidism. Moreover, an increase in E_2, P, PRL and TBG concentrations throught pregnancy (no. 63) resembles to those in normal pregnancy indicating the same functional roles in pregnancy.

In conclusion, strinking increase in E_2 levels cause an elevation of TBG synthesis and secretion which subsequently reflects to increase in T4 and slight increase but not significant in fT4 concentration. These changes may enhance the metabolic state during pregnancy (Burrow, 1980; Freinkel, 1985).

6. Influences of MMI-induced hypothyroidism and MMI-withdrawal on serum E_2 and P profiles

Weekly measurement of serum E_2 levels during early

MMI treatment showed no definite E_2 preovulatory peak in the late follicular phase. However, serum P levels during the last two weeks prior to the onset menstrual bleeding may be able to confirm whether such cycle was accompanied by ovulation and with normal corpus luteum function. The pattern changes of both E_2 and P levels were further confirmed by measurements of major urinary metabolites, estrone-3-glucuronide (E_1-3-G) and pregnanedione-3 L -glucuronide (Pd-3L -G) (Chaiseha, 1988). both urinary metabolites have been used as useful indicators of ovulation and corpus luteum function during normal menstrual cycle in the rhesus monkeys (Breckwoldt *et al.*, 1972, Monfort *et al.*, 1987) as well as in woman (Scommegna and Chattorai, 1967, Samarajeewa *et al.*, 1979).

Three patterns of attenuated E_2 and P levels in response to MMI treatment were evidenced : firstly, rapid reduction of E_2 and P levels were compatibly with immediate decrease in fT4 levels; secondly, ovulation were always detected during the rapid decrease in fT4 levels and thirdly, insufficient E_2 and P secretions were evidenced in monkeys with delayed decrease in fT4 levels. Both lower serum E_2 and P pattern were concomitantly with decrease in urinary E_1-3-G and Pd-3 L -G concentrations (Chaiseha, 1988). The precise mechanisms of how thyroid hormones influence the serum concentrations of E_2 and P with three different characteristics are uncertainly. Indeed, menstrual bleeding of the first treatment cycles

ranging 24-34 days are considered to be within the normal range in this monkeys (Varavudhi *et al.*, 1982). However, a great deal of irregularity of the second treatment cycles ranging 20-248 days were evidenced. These corresponded with sharp decline in T4, T3 and fT4 levels (Suwanprasert *et al.*, 1987; Varavudhi *et al.*, 1988;1989). Menstrual irregularities are commonly accompanied by the hypofunction of thyroid gland, similar to situation in human (Hodges *et al.*, 1952, Goldsmith *et al.*, 1952, Scott and Mussey, 1964). Obviously, direct suppression effect of thyroid hormones on serum levels of E_2 and P were observed during severe hypothyroidism in all MMI treated monkeys. These clearly indicated that severe hypothyroid state would seriously affect normal steroidogenesis from the ovary and concomitantly with amenorrhea and galactorrhea. This findings are in agreement with clinical reports in the primary hypothyroidism patients (Toft *et al.*, 1973; Onishi *et al.*, 1977; Honbo *et al.*, 1978). During severe hypothyroidism, serum levels of PRL of treated monkeys increased significantly from the previous pretreatment (500-3700 mIU/L) and accompanied by galactorrhea. It is likely that the elevated serum PRL levels of these severe hypothyroid monkeys are mediated by feedback induced TRH secretion and may accord with antagonistic action of DA (Onishi *et al.*, 1977; Burrow *et al.*, 1977; Tkachenko *et al.*, 1989). In women, galactorrhea is not common syndrome following primary

hypothyroidism (Toft *et al.*, 1973, Honbo *et al.*, 1978). Conversely, galactorrhea is found in all treated monkeys during severe hypothyroidism and lasted until the end of treatment. It is possible that the elevated PRL levels should be one of the co-existence factors promotes amenorrhea in hypothyroidism. For the thyroid hormones only, four posibilities should be claimed and described the direct effect of thyroid hormones on E_2 and P production and/or secretion. They involve three levels of ovary-pituitary-hypothalamic axis and sex hormone binding globulin (SHBG).

At the ovary level, both T3 and T4 synergize with FSH to exert direct stimulatory effects on granulosa cell functions, including morphological differentiation, LH/hCG receptor formation and steroidogenic enzymes (3B-hydroxy steroid dehydrogenase and aromatase) induction (Maruo *et al.*, 1987). Therefore, decreased ovarian functions during the state of severe hypothyroidism may account for diminished responsiveness of the granulosa cell to FSH. Moreover, a high affinity of T3 receptors in highly purified nuclear preparations from human corpus luteal cells were also evidenced (Bhattacharya *et al.*, 1989). These investigators also claimed that thyroid hormones may exert a direct effect on corpus luteum cells steroidogenesis.

At the pituitary level, Distiller *et al.*, (1975) reported that the response of LH was blunted to LRH (100

ug) in hypothyroidism patients, due to limitation of pituitary LH reserve. Moreover, Larochelle and Freeman (1974) further pointed out in rats that thyroid hormones played a role in modulating the secretion of FSH and LH and this effect was not due to alteration of the metabolic clearance rate (MCR) of the gonadotrophins. The withdrawal of thyroid hormones leave the ovary hypersensitive to exogenous gonadotrophin secretion, but, in the absence of exogenous gonadotrophin, the ovaries of hypothyroid rats were diminished in ovarian weight and minimum function (Hagino, 1971). Clearly, T4 attenuates preovulatory surge of LH by inhibit the release of LHRH from Gn-RH neurons in the arcuate nucleus (Freeman *et al.*, 1976). At present, altered thyroid status may not influence the synthesis and metabolism of LH but does exert a profound effect on the secretion of this hormone, presumably acting on the hypothalamus-pituitary-gonadal axis.

At the hypothalamic level, it is well recognized that the arcuate region is the primary structure mediating the hypothalamic control of gonadotrophin secretion in the rhesus monkey (Plant *et al.*, 1978; Knobil, 1980; Knobil *et al.*, 1980) as well as in human (Johnson and Everitt, 1980). In addition, the dopaminergic neurons in the arcuate nucleus are closely related with the portal vesels in the external layer of the median eminence (Everitt and Hokfelt, 1986). These neurons are capable of accumulating

E_2 and have been speculated the possible participation of dopaminergic mechanisms in the feedback regulation of GnRH secretion (Greenstein, 1986). Indeed, E_2 in women is reported to increase dopamine turnover in tuberoinfundibular neurons, indicating that the amine acts to inhibit GnRH secretion (Fuxe *et al.*, 1969). Dopamine turnover decreases immediately preceding the preovulatory LH surge in rat (Fuxe and Hokfelt, 1969). Dopamine receptor agonists clearly inhibit LH release in ovariectomized rats whereas dopamine receptor antagonists may actually induced ovulation (Fuxe *et al.*, 1975). Besides the established negative feedback mechanism of thyroid hormones to pituitary TSH, thyroid hormone is capable of stimulating hypothalamic dopamine secretion as an intermediate step in inhibition of TSH secretion by pituitary thyrotropes (Feek *et al.*, 1980). Eventhough, the precise functional importance of dopaminergic mechanisms in the regulation of GnRH secretion remains unclear, additionally, clinical data are rather consistent in showing that dopamine agonists reduce effectively plasma LH concentrations unless given to hyperprolactinemic patients (McNeilly, 1986).

Finally, hypothyroidism is associated with an increased metabolic clearance rate (MCR) of testosterone and conversion ratio of testosterone to androstenedione (Gordon *et al.*, 1969). These results lead to expect that the sex hormone binding globulin increases binding

affinity for testosterone in hypothyroidism patient. Furthermore, the increased androgen-estrogen conversion is a direct effect of thyroid hormone manifested mainly by the increased conversion of androstenedione to estrogens (Southren *et al.*, 1974). Therefore, hypofunction of thyroid hormones may reflect to diminish conversion of androstenedione to the estrogens at the granulosa cell of the ovarian follicle which may lead to suppression of E_2 peak interrupted the positive feedback regulation of gonadotrophin surge required for stimulated ovulation and luteinization. Considerable resumption of menstrual bleeding cycles with detectable greater E_2 and P concentrations in the follicular and luteal phases of the rebound period were evidenced concomitantly with those during the state of mild hypothyroidism. These further indicate the close relationship between thyroid hormones and reproductive hormones production and/or secretion. 4 out of 7 monkeys (no. 25, no. 78, no. 77 and no. 63) showed regularity of menstrual bleeding cycle but the remaining monkeys (no. 33, no. 87 and no. 101) exhibited irregularity of cycle and only one monkey (no. 101) showed two period of severe hypothyroidism states. Many characteristic patterns are due mainly to the response to time-difference adaptation in thyroid hormone secretion following gradual release suppression by MMI withdrawal. Several lines of evidences indicated that the replacement of T4 can restore normal reproductive functions in primary

hypothyroidism patients (Boroditsky and Faiman, 1972; Snyder *et al.*, 1973; Refetoff *et al.*, 1974; Onishi *et al.*, 1977). Similarly, increase in thyroid hormones directly reflects fT4 increment and leads to restore the ovarian function in this experimental monkeys.

It is worth emphasizing the occurence of ovulation during the state of mild hypothyroidism from this study. These finding is in agreement with uncommonly successful outcome of pregnancy in hypothyroid mothers (Montoro *et al.*, 1981). A dramatic serum patterns of E_2, P and LH levels indicated normal ovulation of 34 day cycle during the state of mild hypothyroidsim in monkey no. 33. After complete withdrawal of MMI for 8 days, successful pregnancy of monkey no. 63 was evidenced even in the first post-treatment cycle whereas the fT4 levels fluctuated to approach the pre-treatment values. It is indicated that MMI is a suitable antithyroid drug used to induce harmless and reversible hypothyroidism in this macaqe (Varavudhi *et al.*, 1989). Although another six monkeys failed to induce successful pregnancy but ovulations and normal meustrual cycles could easily recognized in most animals. In monkey no. 33 and 87 showed slightly delay recovery of ovarian function for 46 and 108 day cycles respectively. Different response of ovarian function leads to further suggest that thyroid hormones may affect not only the target endocrine gland like the ovary, but also regulate other organ systems including the adenohypophysis and the

hypothalamus. For focus on the aims, it should be concluded that an ovulation could be induced in the state of mild hypothyroidism in the cynomolgus monkeys leading to induction of pregnant monkeys as a model for further study in differentiation of the fetal nervous system.

Finally, all evidences from this study are summarized that

(1). MMI can induce transient states of hypothyroidism in cynomolgus monkeys.

(2). During severe hypothyroidism, the levels of thyroid hormones are decreased while the TSH levels are increased. Slight increase in thyroid hormones is observed during mild hypothyroidism with a moderate decrease in TSH levels.

(3). Hyperprolactinemia with galactorrhea as well as complete cessation of E_2 and P production with amenorrhea are common complications found during severe hypothyroidism. In some case, the TBG levels are slightly increased which contrasted to lower levels of thyroid hormones.

(4). Ovulation can be detected during mild hypothyroidism.

(5). The profile patterns of thyroid hormones, PRL, TBG, E_2, and P in the pregnant monkey recovered from MMI treatment and in normal pregnancy group are similar.