Chapter IV

Discussion

Luteal cell viability

The viability of luteal cells obtained from different reproductive stages of rats during 11 days of incubation were not significantly different. Rat's as well as monkey's luteal cells obtained from different reproductive stages showed no significantly different viability during various incubation peroids up to 11 days. During the first five days of incubation viable luteal cells of rats were found in more than 80% and more than 65% on day 11 of incubation. Similar proportions were also observed in monkey luteal cells incubated for 11 days irrespective of the stage of the CL obtained. It is conceivable that the cell dispersion technique and incubation process done in this study are suitable for long term study at least up to 11 days of incubation.

In the presence of hCG, PRL and $PGF_{2\alpha}$, it was found that PRL maintained viability of luteal cells in culture from any stage of rats and monkeys by detection of high percentage of viable luteal cells on day 11 of incubation, and these present data showed significant diference from the control, hCG and $PGF_{2\alpha}$. Administration of hCG, PRL and $PGF_{2\alpha}$ into the medium did not show any influence on the lowering of luteal cells' viability. However, PRL administration showed a significant increment of cell survival compared with untreated groups of the same peroid of incubation. These results seemed to concord with the work of Wu et al. (1976), who reported that dispersed rat luteal cells obtained from D_2 (L_1) of pregnancy which preincubated at $36^{o}C$ for 24 hours in medium containing PRL or LH and PRL, but not LH alone,

were capable of retaining luteal cell viability and functional capacity in response to LH+PRL stimulation for a period of 48 hours during perfusion method with a flow rate of 5 ml/10 min.

P secretion in medium of luteal cells from various reproductive stages of rats presented similar patterns. These hormone contents in medium were high on day 3, declined gradually on the following day, but still remained detectable until day 11 of incubation. These present results coincided with a study of bovine luteal cells culture during 11 days incubation (Pate and Condon, 1982; O'Shaughnessy and Wathes, 1985), in rhesus monkey luteal cell culture during 8-10 days incubation (Gulyas et al., 1979, 1980; Stouffer et al., 1980b), in mouse granulosa cell culture during 13 days incubation (Corredor and Flinkinger, 1983) and in rabbit granulosa cell culture during 10 days incubation (Yuh et al., 1986). In the present study, the sharp decline in P secretion of luteal cells in culture occurred after 7 days of incubation, and may be caused of a depletion of cholesterol in the luteal tissue (Behrman and Armstrong, 1969; Christie et al., 1979). Gulyas et al. (1980) suggested that luteal cells "age" in culture, since the increasing time in culture caused the increased E_2 : P ratio in medium which indicated that steroidogenesis of P reduced more rapidly than that of E_2 and was related to ovarian steroidogenic changes during the late luteal phase. However, Veldhius et al. (1983) reported that the decline in P secretion in culture may be caused by a loss of cholesterol side chain cleavage activity which was increased by stimulation of c-AMP (Funkenstein et al. 1983).

Corpus luteum of rats in vitro

Corpus luteum of estrous cycle

The P secreting ability of luteal cells from estrus and diestrus-1 were high and markedly low in luteal cell culture from early and late diestrus-2 which directly related to the change of plasma P level, indicating that the CL of estrous cycle secreted P very soon after 48 hours of its formation. Anderson et al. (1973) reported that P and 20α hydroxyprogesterone (20α OHP) could still be detected from sliced ovarian tissue of diestrus-2 perfused continuously with buffer-saline. The evidence was in agreement with the present study since it is well established that luteal 20α-OHP production is an evidence of CL regression which will be followed by the refractoriness of the CL to all luteotropic agents, presumably be due to the reduction of hormone binding sites (Cheng, 1976). In the present sutdy, only hCG and PRL were capable of stimulating basal P production of luteal cells from estrus and diestrus-1 significantly. Moreover hCG and PRL also showed synergistic effects on stimulation of P produciton. The result was in agreement with Murakami et al. (1982), who perfused 2 μg/ml LH and 20 μg/ml PRL into the isolated luteal cells and found that luteal cells obtained between the evening of the estrus and morning of diestrus-2 were capable of responding to LH and PRL stimulation with a significant increment in P output into the perfusate, while those obtained on the evening of diestrus-2 were refractory. Unlike PRL and hCG, the presence of $PGF_{2\alpha}$ did not affect the basal P secretion of CL cells in cycling rats during short term incubation but showed a significant stimulatory effect on P production of luteal cells from estrus and diestrus-1 in long therm incubation

only. This finding is of great interest since most of previous studies stressed effects of prostaglandins on luteolysis in the late luteal phase (Rothchild, 1981) but did not pay much attention to the effect during earlier phase of the CL life. Indeed a few previous in vitro studies support this finding : $PGF_{2\alpha}$ stimulated P production from bovine CL (Hixon and Hansel, 1979), and from human CL (Channing, 1972; Henderson, 1970; Gould et al., 1977).

Luteal E_2 secreting ability of cyclic rats were quite low during 3 hours and 11 days incubation; highest level was observed in diestrus-1 luteal cell culture and declined to minimum in diestrus-2. These luteal E_2 secretions were related to the luteal P production since plasma E_2 levels were quite low throughout the luteal phase of cycle. A small rise of E_2 during the late diestrous phase may probably be secreted from ovarian tissue of extra CL origin (Johnson, and Everett, 1980; Rothchild, 1981). Only the presence of hCG (0.5 iu/ml) was capable to stimulate basal luteal E_2 secretion from the CL cells obtained from estrus and diestrus-1 significantly. These results agreed with the in vivo study of Taya and Greenwald (1982a), who found a single injection of LH is capable to stimulate E_2 production from the ovary of immature rats treated with PMSG. The mechanism by which LH or hCG stimulated luteal E_2 production is presumed to be intraluteal stimulation of aromatase activity (Tsai-Morris et al., 1983; Kalison et al., 1985) and/or action on the thecal lutein cells to stimulate androgen production which in turn aromatized to E_2 by the granulosa luteal cells (Keyes et al., 1979).

From these present results, it may be concluded that CL of cycling rats could function autonomously and secrete P and E_2 at least during the first 48 hours after their formation. They were capable to respond to hCG, o-PRL as well as $PGF_{2\alpha}$ stimulations until the morning of diestrus-1.

Corpus luteum of pregnancy

Luteal P secretion of pregnancy stages L_6 and L_{12} were higher than that of pregnancy stages L_2 and L_{20} during the short term (3 houus) as well as the long term (11 days) incubation. These results seemed to concord with the works of Wada and Greenwald (1984) who reported that P secretion of luteal cells from pregnancy stage day 13 (L_{12}) was almost as high as those obtained from stage day 8 (L_7) which were higher than those found during stage day 2 (L_1) during 3 hours incubation. The present results of luteal P secretion also showed a positive correlation with plasma P levels during the first half of pregnancy, although a sharp increment of serum P levels was seen during the second half of gestation and lasted until 1-2 days prior to delivery (Siriprasomsub, 1984). It is conceivable that rat corpora lutea are not the main source of peripheral serum levels P during the second half of pregnancy since hypophysectomy during the second half of gestation failed to terminate pregnancy in rats and mice (Pancherz and Long 1931, 1933; Selye et al., 1933) and failed to lower plasma P levels (Takahashi et al., 1979). Although rat corpus luteum function is the minor source of P production during the second half of pregnancy, *in vitro* evidence in this study clearly indicated that corpus luteum of pregnancy has the capacity to secrete P at least up to day 21 (L_{20}) of gestation.

That luteal cells from all stages of pregnancy either during short term (3 hours) or long term (7 days) incubations were capable to respond to hCG as well as to increase P production were in agreement with the work of Wu et al. (1976) on in vitro incubation of isolated luteal cells and Lahav et al. (1976) on incubation of the whole CL. Maximal capacity of luteal cells to respond to hCG and hCG in combination with PRL was found during stage L_6 and L_{12}, and may probably be related to the high levels of specific receptors for LH within the corpora lutea of pregnancy (Cheng, 1976; Van Straalen and Zeilmaker, 1982).

The in vitro responsiveness of luteal cells to PRL was found in almost all stages of pregnancy except stage L_{20}. These results were partially compatible to the work of Wu et al. (1976), who reported that PRL (200 ng/ml) failed to stimulate increment of P production from the isolated luteal cells on stage day 2 (L_1) of pregnancy by perfusion method, although these cells were capable to respond to LH stimulation for P production as well as for maintaining viability of luteal cells. These partial contradictory results may possible be dued to the different ages of luteal cell preparation; presumably luteal cells obtained during the first 24 hours of post coitus still did not have sufficient LH support needed to function autonomously (Rothchild, 1981). Indeed, the response to PRL in L_2 of luteal cells preparation showed only a border line significant increment in comparison with the untreated control group while luteal cells prepared from L_6 and L_{12} of pregnancy were be able to respond to PRL to a much greater extent.

Contrary to PRL, $PGF_{2\alpha}$ was incapable to alter P production of rat luteal cells from all stages of pregnancy during short term incubation. However, $PGF_{2\alpha}$ showed a long term stimulatory effect on basal P secretion of luteal cells from stage L_2, L_6 and L_{12} but exhibited an inhibitory effect on hCG-stimulated P secretion from stage L_{20}. Although the stimulatory effect of $PGF_{2\alpha}$ on P secretion from the luteal cells of pregnant rats were not reported by previous works, these stimulatory effects were found in other pregnant animals such as incubation of bovine CL slice with $PGF_{2\alpha}$ 100 µg/ml (Speroff and Ramwell, 1970) and slice of luteal tissue from woman in early pregnancy (Siginami et al., 1976). Like other protein hormone, the action of hCG or LH on the CL is mediated via adenyl cyclase system and the binding of hCG is antagonized by $PGF_{2\alpha}$ (Hickens et al., 1974). The inhibitory effect of $PGF_{2\alpha}$ on LH stimulation of P secretion of luteal cells of pregnant rat stage day 4 (L_3) and day 8 (L_7) _in vitro_ was prevented in the presence of high concentration of LH (100 ng/ml) (Wright et al., 1980). However, intraluteal production of $PGF_{2\alpha}$ and its effect on luteolysis was also prevented by PRL (Ueda et al., 1985). This evidence favors the possibility that a stage of high luteal P secretion has the tendency to protect the luteolytic effect of $PGF_{2\alpha}$ and the present results may further indicate that availability of a physiological level of $PGF_{2\alpha}$ during early luteal phase is capable to exert dirrect luteotrophic action on rat's CL cells in addition to its known antigonadotrophic effect during late pregnancy.

In addition to the P production, luteal E_2 secretion during pregnancy increased correspondingly with an increased stage of pregnancy during short term and long term incubation, indicating that CL of pregnant rats are capable to secrete E_2 autonomously. It is of interest

that hCG, but not PRL and $PGF_{2\alpha}$, is capable to stimulate a significant increase of E_2 production from rat CL cells obtained during L_2-L_{12} of pregnancy. However luteal cells from pregnancy stage L_{20} seemed to loose this responsiveness. These results were in agreement with an in vivo study of Gibori and Keyes (1980) who found a high luteal E_2 content during pregnancy in rats. The action of hCG on luteal E_2 production was known to be mediated by increment of aromatizing enzyme (Tsai-Morris et al., 1983). Futhermore, LH-antiserum administration on day 8 (L_7) of pregnancy caused a decrease in in vitro production of rat luteal estradiol (Teranova and Greenwald 1981).

Corpus luteum of pseudopregnancy

P secreting ability of luteal cells during incubation for 3 hours and 7 days showed highest in PSP stage L_6 and sharply declined in the luteal cell culture from stage L_{12}, indicating that the luteal cells during PSP are capable to secrete P autonomously at least up to the peroid between L_2 and L_{12} of PSP. These present results coincide with the work of Menon et al. (1985) who prepared collagenase dispersed cell suspension from superluteinized rat ovaries and found that basal P production was highest in luteal cell culture obtained from day 7 (L_6) of PSP, declined after day 7 (L_6) until reaching a nadir level in luteal cells from day 14 (L_{13}) of PSP.

The luteal cells of PSP stage L_2 and L_6 were capable to respond to hCG and PRL to stimulate significant elevation of P secretion during 3 hours as well as 7 day incubations. These responsivenesses of luteal cells obtained from PSP L_6 was higher than of luteal cells from PSP L_2 significantly, whereas low basal P secretion and low responsiveness to hCG were observed in the luteal cells from PSP L_{12}. Moreover, the

synergistic effect of hCG and PRL treated group were also evidenced in short term as well as long term incubation, further indicating that hCG (or LH) and PRL are the major components of the luteotrophic complex of the rat and possibly in the tree shrew (Varavudhi, 1987). These present results coincide with the work of Menon et al. (1985), who reported that responsiveness to hCG (10-100 ng/ml) and PRL (5-10 μg/ml) of collagenase dispersed cell suspension prepared from super-luteinized rat ovaries during 3 hours incubation was maximum in the luteal cells from PSP day 7 (L_6) and was lowest in luteal cells from PSP day 12-day 14 (L_{11}-L_{13}) which were refractory to either hCG or PRL. However, this present study contrasts to the previous work of Murakami et al. (1982) who used the perfusion rat luteal cells preparation from PSP L_4 and L_8 with flow rate 1 ml/5 min and failed to stimulate P secretion with PRL (20 μg/ml). This contradictory result may possibly be due to the different method for luteal cells preparation. However, the P secreting ability of luteal cells from PSP rats from this study seemed to relate dirrectly to the change of LH receptors on the membrane of luteal cells which showed a significant decrease after L_6 of PSP (Hwang and Menon, 1986).

In the presence of $PGF_{2\alpha}$ (250 ng/ml) it was incapable to alter basal P production during 3 hour incubation but it was capable to mimic luteotrophic effect to stimulate P secretion of luteal cells PSP stage L_2 and L_6 during long term incubation, whereas luteal cells from PSP L_{12} were refractory in all cases. The stimulatory effect of $PGF_{2\alpha}$ on P secretion from the luteal cells of PSP L_2 and L_6 seemed to coincide with the previous works of Hall and Robinson (1979) who found luteotrophic effect of $PGF_{2\alpha}$ (250-500 ng/ml) in dispersed rat luteal cells from PSP

day 6. These stimulatory effect were also reported in other animals such as pregnant bovine sliced corpora lutea with $PGF_{2\alpha}$ 10 μg/ml (Speroff and Ramwell, 1970), slice of luteal tissue from woman in early pregnancy with $PGF_{2\alpha}$ 1 μmol (Suginami et al., 1976), dispersed luteal cells from luteal phase of human with $PGF_{2\alpha}$ 1 μmol (Richardson and Mossman, 1980), and dispersed luteal cells from mid luteal phase of rhesus monkey with $PGF_{2\alpha}$ 500-5,000 ng/ml (Stouffer et al., 1979). However, some in vivo evidence was not in agreement with this study since $PGF_{2\alpha}$ was well known to be a luteolytic agent in PSP rats, when infused into the uterine lumen during day 5 and day 6 of PSP (Pharris and Wyngarden, 1969), and a silastic-PVP tube containing 600 μg of $PGF_{2\alpha}$ inserted under the neck skin on day 5 of PSP was capbale to terminate PSP within 2 days (Lua et al., 1979). These contradict results of the present in vitro study and the provious in vivo reports may show that luteolytic effect of $FGF_{2\alpha}$ in vivo is unable to effect the luteal cells directly. Previous results in CL of pregnancy were in agreement with the CL of PSP except the functional capacity of the luteal cells of pregnancy could be extended until near term.

Similarly to P production, luteal E_2 production during short and long term incubation were high in PSP L_2, highest in PSP L_6 and sharply declined in PSP L_{12} which related to the serum level of P. As for the luteal cells of pregnancy, only hCG was capable to stimulate a significant increase in E_2 production from the luteal cells of PSP L_2 and L_6 whereas luteal cells from PSP L_{12} were incapable to respond. These results were related to the in vivo study of Tsai-Morris et al. (1983) who found that LH is capable to stimulate luteal aromatizing enzymes of superluteinized CL obtained from immature rats injected with LH (20 μg) after 48 hours of PMSG administration and killed 4

hours later. Gibori et al., (1978) and Gibori and Keyes (1978,1980) found that administration of LH-antiserum resulted in decreasing plasma P and estradiol concentration.

Finally, the incapability of luteal cells of PSP L_{12} to secrete P + E_2 autonomously and their inability to respond to all agents, including PRL, were in agreement with in vivo study of Murakami et al. (1979) who failed to stimulate P production with PRL in rats. It seemed that the intrinsic nature of luteal cells have completly lost their responsiveness to hormonal stimulation since luteal content of 20α hydroxysteroid-dehydrogenase (20α-HSD), an enzyme converted P to 20α-OHP, is increasing toward the end of PSP (Wiest et al., 1968; Bast and Melampy, 1972; Takahashi, 1984; Naito et al., 1986) whereas adenyl cyclase activity declined greatly (Hunzicker-Dunn and Birrbanmer, 1976; Khan et al., 1979). Furthermore, LH receptors decreased by 20 and 45% on day 11 and day 14 of PSP respectively (Hwang and Menon, 1986) which were related to the phase around the time of luteolysis occurred.

Corpus luteum of lactating pseudopregnancy.

Luteal P secretion from the CL of lactating PSP was highest in luteal cells from stage L_{12}. The level was higher than luteal cells from stage L_2 and L_{20} significantly. The low P secreting ability of luteal cells from L_2 and L_{20} was directly related to the lower levels of plasma P. These results seemed to relate to the serum PRL levels which were high during early and mid lactation, and markedly declined in late lactation (Amenomori et al., 1970; Ford and Melampy, 1973; Lu et al., 1976; Grovenssor and Whiteworth, 1979). Administration of hCG (0.5 iu/ml) or o-PRL (5 µg/ml) was capable to stimulate P secretion from rat luteal cells in all stages of lactation (L_2-L_{20}) during either

short term or long term incubation. This responsiveness of luteal cells to hCG or PRL from lactating stage L_{12} was also significantly higher than stage L_2 as well as stage L_{20}. Moreover, the synergistic effect of hCG and PRL were also found in all treated groups, indicating that continued suckling could be able to prolong functional life of CL to secrete P autonomously and the capability of responding to major luteotrophic hormones, hCG and PRL, to increase luteal P production at least up to 21 days of lactation. It is generally accepted that the major luteotrophic support during lactation is PRL (Nicoll and Meites, 1959; Selmanoff and Selmanoff, 1983) since administration of a dopamine agonist, ergocornine maleate, on day 6 and day 7 of lactation could induce a fall in P concentration to an undetectable level (Tomogame et al. 1975) while a high serum concentration of P was observed in rat bearing pituitary grafts which secreted PRL continuously (Eto et al., 1962). Moreover, ergocornine given into hypophysectomized pregnant rats autotransplanted into the kidney capsule caused the CL to be unable to secrete sufficient P needed for stimulation of blastocyst implantation in the presence of estradiol (Varavudhi et al., 1966). PRL was known to inhibit 20α hydroxysteroid dehydrogenase activity (Wiest et al., 1968; Richard and William, 1976; Grinwich et al., 1976; Casper and Frickson, 1981; Jones et al., 1983; Rajkumar et al., 1985), to increase LH receptor (Holt et al., 1976), and to maintain and induce LH receptor turnover (Casper and Frickson, 1981). The increase LH receptor concentration was considered to be requistite for increasing P produciton. It is conceivable that either hCG or PRL played a significant role for regulation rat CL function druing lactation.

$PGF_{2\alpha}$ was capable to stimulate P secretion of rat luteal cells from lactation stage L_2 and L_{12}, but exhibited an inhibitory effect on hCG-stimulated P secretion from stage L_{20} during long term incubation. These stimulatory effects of $PGF_{2\alpha}$ on luteal cells of lactating rats were not reported by previous workers but the result was in agreement with long term in vitro incubation with all types of rat CL especially during the active phase of progesterone production.

Results of E_2 secreting ability from the CL of lactating PSP increasing accordantly with an increased stage of lactation may be related to the plasma LH levels which were low during early and mid lactation but slightly elevated during late lactation (Lu et al., 1976). Like CL of pregnancy and pseudopregnancy, the presence of hCG, but not o-PRL or $PGF_{2\alpha}$ was capable of stimulating an increase in E_2 secretion of luteal cells from all stages of lactation during short term and long term incubation, further indicating a unique role of hCG and related glycoprotein on stimulation of E_2 production from the corpus luteum.

Monkey corpus luteum

The CL of cycling cynomolgus monkeys (Macaca fascicularis) were obtained by partially lutectomization during early, mid, late luteal phase and luteolytic phase of the cycle. With the aid of cystoscope, evidence indicated that the ovulation pattern of cynomolgus menkey alternates from one side of the ovary to the other side, cycle by cycle, which is in agreement with the rhesus monkey (Goodman et al., 1977 b; Goodman and Hodgen, 1979 a & b).

The plasma P levels were 4.61-5.76, 6.80-13.60 and 4.59-7.61 nmol/L during early, mid and late luteal phase of the cycle respectively. These results generally agreed with the work of Stabenfeldt and Hendricks (1973) and Varavudhi et al. (1982). Partial lutectomy caused abruptly decreasing peripehral plasma P levels within one day, whereas plasma E_2 level was not affected. These results were in coincidence with previous works which studied in the rhesus monkey (Goodman and Hodgen, 1977, 1979 b; Goodman et al., 1977 b, 1982), and in the cynomolgus monkey (Sopelak and Hodgen, 1984). Furthermore, the regularity of the menstrual cycle still persisted.

From the present study, the ability of luteal cells of cynomolgus monkey to secrete P in vitro varied markedly and was related to the life-span of the CL of menstrual cycle. The P secreting ability was greatest in luteal cells isolated from the mid luteal phase and significantly higher than luteal cells obtained from the late and early luteal phases, except luteal cells which were obtianed from old and in fertile monkey #24 had a relatively short menstrual cycle (21 days). Progesterone secretion from the isolated luteal cells of monkey # 24 was lowest in comparison with other animals. P secreting ability of the luteal cells in vitro from all animals correlated very well with the peripheral plasma P levels, indicating that CL of the cynomolgus monkeys is the principle source of peripheral plasma P during the luteal phase of the menstrual cycle.

Generally, the P secreting pattern of luteal cells from menstrual cycle during the 11 days of incubation was high on day 3 and declined slightly after day 3 of incubation but remained detectable until day 11 in all cases, indicating that luteal cells obtained from the cycling

monkeys were capable to secrete P autonomously throughout 11 days of incubation. These P secreting patterns were similar to the previous results studied in rhesus monkey luteal cell culture (Gulyas et al., 1979, 1980), bovine luteal cell culture (Pate and Condon, 1982), human granulosa cell culture (Polan et al., 1984) and in rabbit granulosa cell culture (Yuh et al., 1986). The decline of P secretion throughout culture period may be related to a loss of cholesterol side chain cleavage activity (Veldhius et al., 1983). However, the present study showed that monkey luteal P secreting ability during the menstrual cycle was greater than rat luteal cells from various reproductive stages of cultures in the same condition. It is conceivable that gonadotrophin from the pituitary gland was not the essential requirement for maintaining CL function during menstrual cycle since serum LH levels were quite low throughout the luteal phase (Yoshida et al., 1984 a & b). Moreover, rhesus monkeys hypophysectomized on the day of ovulation still had normal P levels in the serum (Asch et al., 1982). Futhermore, exogenous administration of GnRH antagonist to the rhesus monkey failed to affect normal luteal function (Balmaceda et al., 1983). However, there was some contradictory evidence that LH-antiserum administration to cycling cynomolgus monkey during mid luteal phase resulted in shortening of the luteal phase and premature menstruation (Groff et al., 1984). From this minor contradictory evidence the conclusion may be drawn that the need for luteotrophic support after ovulation and luteinization in higher primates is minimum and the CL may have capacity to produce P autonomously for several days. In the presence of hCG (0.5 iu/ml), P secretion of mid luteal phase cells was significantly higher than in the early and late luteal phases which had either lower basal P secretion or lower responsiveness. Furthermore, monkey # 24

which had low basal P secretion also showed positive relation of responsiveness to hCG. These present results studied in cynomolgus monkeys seemed to relate to the previous work in rhesus monkeys by Stouffer et al. (1977) who reported that P synthetic ability of the CL obtained during day 15-19 of the cycle was significantly greater than of cells from late luteal phase (day 22-28 of the cycle). Furthermore, P production by mid luteal phase was consistently stimulated ($P < 0.01$) by the presence of 100 ng hCG/ml, whereas late luteal phase cells were less sensitive or unresponsive to exogenous gonadotrophin. Stouffer et al. (1980 a) also found that in the presence of hCG (0.1-100 ng/ml), luteal E_2 and P production by day 21-22 cells of cycle was significantly less than that by day 16-19 cells during 6 hours incubation. Yoshida et al. (1986) reported that the binding of LH receptors decreased during the regression of the CL, but the number of LH receptors were not altered markedly throughout the menstrual cycle of cynomolgus monkeys. It is conceivable that the increase in responsiveness of mid luteal phase cells or the reduced responsiveness of late luteal phase cells to hCG is caused by altering the affinity of hCG to the receptors which developed a state of sensitivity or insensitivity to luteotrophic stimuli. This concept was in agreement with previous *in vivo* evidence since LH-antiserum administration during mid luteal phase resulted in a precipitous fall in serum progestin within 24 hours and followed by premature menstrual bleeding in cynomolgus monkeys (Groff et al., 1984). Similarly in rhesus monkey, LH administration during luteal phase of the menstrual cycle is followed by a dramatic rise in plasma P concentration and extended luteal function (Neill and Knobil, 1972). Moreover, GnRH antagonist administration on day 20 of the cycle (Collin et al., 1984) and radiofrequency lesions

in the arcuate region of the medial basal hypothalamus (Hutchison and Zeleznik, 1984) to prevent LH pulse caused a decline in plasma progesterone and premature menses occurred 2-5 days after.

PRL failed to alter basal P secreting ability of luteal cells in all cases. These present results agreed with the study of Stouffer et al. (1980 a) who reported that h-PRL (2.5-5000 ng/ml) failed to affect P production by luteal cells of cynomolgus monkeys in short term incubation. It indicated that PRL is not a luteotrophin of cynomolgus monkeys during menstrual cycle in vitro. This concept was supported by studies in other primates such as rhesus monkey; PRL (1000 ng/ml) had neither a stimulatory nor an inhibitory effect upon basal P and LH-stimulated P secretion of dispersed luteal cells obtained from 5-7 days of post ovulation, as well as prolonged bromocriptine administration (30 mg, im/day) for 7 days still unable to affect CL function (Richardson et al., 1985). Similarly, PRL also failed to affect P production of human granulosa cells (Edward et al., 1982). On the otherhand, earlier work of McNatty et al. (1974) showed that h-PRL (1-5 μg/ml) had a stimulatory effect on P secretion of human granulosa cells in culture.

In the presence of $PGF_{2\alpha}$, it was possible to stimulate increment of basal P secretion of luteal cells from early and mid luteal phase, whereas late luteal phase cells were unaltered. However, $PGF_{2\alpha}$ showed a significant inhibitory effect on hCG-stimulated P secretion of luteal cells obtained from the late luteal phase. These results were concorded with the studies of the rhesus monkey by Stouffer et al. (1979) who reported that P production by luteal cells obtained on day 16-18 of cycle or 4-7 days following preovulatory LH surge was

significantly increased in the presence of either PGE_2 50-500 ng/ml or $PGF_{2\alpha}$ 500-5,000 ng/ml but was less ($P < 0.01$) than that elicited by the presence of 1000 ng hCG/ml and the combination of hCG and $PGF_{2\alpha}$ produced a response no different from that of hCG alone. In contrast, P synthetic activity of luteal cells from the late luteal phase of the cycle (8-10 day after LH surge) was unaltered by the presence of 5-5000 ng/ml of $PGF_{2\alpha}$ but still capable of responding to 100 ng of hCG/ml by enhancing P production ($P < 0.01$). The combination of hCG and $PGF_{2\alpha}$ (5000 ng/ml) failed to increase P secretion. Other investigators had also reported that $PGF_{2\alpha}$ was capable to mimick LH-hCG action on P synthesis *in vitro* by luteal tissue from pregnant bovine with $PGF_{2\alpha}$ 10 μg/ml (Speroff and Ramwell, 1970), in the human CL from early pregnancy and luteal cell culture from mid luteal phase with $PGF_{2\alpha}$ 1 μmol (Suginami et al., 1976; Richardson and Masson, 1980). However, $PGF_{2\alpha}$ (5000 ng/ml) was capable of preventing *in vitro* stimulation P production from late luteal phase cells (day 8-12 after LH surge) by 1 mM dibutyryl cyclic AMP or hCG 100 ng/ml in rhesus monkey (Stouffer et al., 1979). There were *in vivo* evidences of rhesus monkeys (Kirton, 1970), which showed luteolytic effect of $PGF_{2\alpha}$ when injected 30 mg/day on day 11, 12 or 13 postovulation and such an effect was not obtained by injection earlier. Similarly, administration of $PGF_{2\alpha}$ (Kirton and Koering, 1973) or 15 Keto-$PGF_{2\alpha}$ (Wilk, 1977) to rhesus monkeys on day 22 of the cycle elicited premature luteolysis. This evidence suggested that the CL of the monkeys was more vulnerable to luteolysis in the later stages of the reproductive cycle. In the otherhand, the newly formed CL of several species exhibited resistance of $PGF_{2\alpha}$ in inducing luteolysis *in vivo* (Henderson and McNatty, 1977). $PGF_{2\alpha}$ was known to induce functional luteolysis by inhibiting of LH-stimulated c-AMP

accumulation (Thomas et al., 1978; Rothchild, 1981) and P secretion (Richardson and Masson, 1980). Furthermore, a high concentration of LH was capable of overcoming the inhibitory effect of $PGF_{2\alpha}$ *in vitro* (Wright et al., 1980). Therefore, it is conceivable that $PGF_{2\alpha}$ mimicked gonadotrophic effect on P secretion of early formed CL while in later phase acted on LH receptor to block the mechanism of hCG stimulation of P production which was switched from a steroidogenic to an antigonadotrophic effect as the luteal phase progressed and approached to the onset of regression which the LH binding of luteal cells was low (Hickens et al., 1974; Luborsky et al., 1984; Yoshida et al., 1986).

Plasma E_2 was 0.45-0.80, 0.41-0.88 and 0.47-0.75 nmol/L during early, mid and late luteal phase, respectively. These results were similar to previous works which showed no plasma E_2 peak during luteal phase in cynomolgus monkeys (Varavudhi and Yodyingyuard, 1980; Cholvanich, 1986) and in rhesus monkeys (Hotchkiss et al., 1971). In the present sutdy, luteal E_2 secreting ability was greatest in luteal cells from mid luteal phase and diminished in luteal cells from late luteal phase. The reduction in luteal E_2 secretion during late luteal phase did not relate to plasma E_2 levels and partial removal of CL was not interfere with plasma E_2 concentration, indicating that luteal cells of cynomolgus monkeys obtained during menstrual cycle were capable of secreting a small amount of E_2 autonomously and CL is not the major source of peripheral plasma E_2 levels. These results seemed to concord with the study in rhesus monkeys by Stouffer et al. (1980 a) who found that luteal cells produced in picogram quantities of E_2 and nanogram amount of P/5 x 10^4 cells during 6 hour incubation and luteal cells obtained from day 23-25 of cycles produced significantly ($P < 0.01$) less P and

E_2 than from day 16-19. Similarly to the results obtained in rats, only hCG was capable of stimulating E_2 production from luteal cells of the cynomolgus monkey. The responsiveness of luteal cells from early and late luteal phase to hCG was less than luteal cells from mid luteal phase. These results were in agreement with the study in rhesus monkeys (Stouffer et al., 1980 a) who reported that estrogen production from luteal cells during day 16-19 was significantly enhanced by hCG (0.1-100 ng/ml) which was significantly higher than that obtained during day 21-22. Whereas, luteal cells from day 23-25 of the cycle produced significantly less P and E_2 than day 16-19 of the cycle and failed to respond to hCG stimulation. Similarly, hCG (10 iu/ml) was also capable of sitmulating _in vitro_ P and E_2 production from human CL during mid and late luteal phase of the cycle (Hunter and Baker, 1981). Estrogen was known to be luteolysin in primates by inducing premature regression of the CL in cynomolgus monkeys (Westfahl and Resko, 1983), reduced functional life span of the CL in the rhesus monkey (Karsch et al., 1973; Stouffer et al., 1977; Laherty et al., 1985), in the baboon (Westfahl and Kling, 1979), and in human (William et al., 1979). However, it seemed from this study that luteal E_2 production during various ages of CL did not correlate to the luteolysis. There were _in vivo_ studies to support this concept since oral administration of an antiestrogen (clomiphinn citrate 10 mg/day) to cynomolgus monkeys failed to prolong luteal function whereas it was capable of inhibiting E_2-induced luteolysis (Westfahl and Resko, 1983). Similarly, twice daily and administration of aromatase inhibitor 1,4,6-androstratriene-3,17 dione (ATD 250 mg) to suppress systemic and intraluteal estrogen levels did not affect cycle length and length of the luteal phase in rhesus monkeys (Ellinwood and Resko, 1983). Since the amount of E_2 produced from the

cynomolgus monkey's CL is much less than the CL of human, great ape and baboon, it is conceivable to assume that under physiological conditions endogenous estrogen production may not be the major luteolysin in the cynomolgus monkeys.

Conclusion

Comparative studies on the in vitro capability of isolated luteal cells in response to various potential luteotrophins and luteolysins between rats and cynomolgus monkeys were carried out. Significant findings could be condensed as follows: 1) Active viable luteal cells obtained from the same species has the capacity to respond to various agents in the same manner irrespective of the physiological state of the CL. 2) hCG or related glycoprotein hormone, presumably LH, is universal luteotrophin in all Eutherial mammals. 3). hCG (or LH) has a unique role in the stimulation of luteal cells to secrete P and E_2 in several Eutherial mammals studied whereas other known luteotrophins are limited to stimulating P production only. 4). $PGF_{2\alpha}$ has a common role on regulation of intracellular P production, presumably stimulating during early and mid luteal pahse and inhibiting in late luteal phase. 5). PRL has lost its luteotrophic property completely in higher primates but is still one of the major luteotrophic complexes in small mammals such as rodents.