Chapter I

Introduction and literature review

Vocherus Coiter in 1573 (reviewed by Harrison, 1948 b) was the first scientist who reported the presence of a solid structure in the ovary of a woman. It is now well established that this structure is transformed from the post ovulatory follicle and named "corpus luteum (CL)". It was processed by enlargement of granulosa cells and accompanied by capillary invasion from the thecal tissue (Basset, 1943), forming a rich network among the enlarging granulosa luteal cells. The extent and nature of the contribution from the theca interna varied from species to species (Corner, 1956; Mossman and Duke, 1973; Mori et al., 1983). The transformation of a membrana granulosa cell into a luteal cell was accompanied by hypertrophy in some species, hyperplasia in others or of both hypertrophy and hyperplasia in still others. The fully formed CL consitsted of large cells containing a yellow pigment, the luteal cells separated from one another by anastomosing frame work of connective tissue enriched with blood supply (Mossman and Duke, 1973).

Although, a uniform pattern of development and control of the ovarian follicle, has been established, the mechanisms of formation and maintenance of CL vary considerably from species to species. In rabbits, cats and ferrets, ovulation is induced by the copulation. The copulation triggered a neurohumoral mechanism which released pituitary gonadotrophins resulted in necessity for induction of ovulation and transformation of the thecal tissue into the corpus luteum

(Brambell, 1956; de Feo, 1963; Smith et al., 1975; de Greef and Zeilmaker, 1978). In cycling rats and mice, ovulation occurrs spontaneously, but the CL has relatively short life and starts to regress within a few days (Long and Evans, 1922; Everett, 1956; Hilliard, 1973). The copulation caused in the release of sufficient additional gonadotrophins to maintain CL to persist for 18 days if implantation has occurred, and to last for 12 days after an infertile mating (Long and Evans, 1922; Greenwald and Rothchild, 1968; de Greef et al., 1977). In most mammals including guinea pigs, monkeys and man, ovulation and formation of functional CL occurred spontaneously (Hilliard, 1973; Knobil, 1973). The CL of human females functioned for 12 to 14 days during normal cycle and extended up to 2 to 3 months in pregnancy (Bregman, 1949; Johnson and Evertte, 1980; Short, 1984). For the rhesus monkey and cynomolgus monkey, the functional life of the CL had been estimated to be about 13-17 days in the normal cycle and longer than 30 days when pregnancy intervened (Hisaw 1944; Smith 1954; Meyer, 1972, Knobil, 1973; Hodgen and Tullner, 1975; Varavudhi and Yodyingyuad, 1979; Varavudhi et al., 1982; Kasemsatayakorn, 1987).

Classical studies on the formation of the CL in the mouse and rat have shown that the majority of luteal cells was formed from hypertrophy of the membrana granulosa. In addition, some of the theca interna cells also persisted in the periphery of the developing luteal tissue and may differentiate to the nutritive cells, while the connective tissue was formed from both the theca interna and theca externa (Togari, 1923). In rabbits, the luteal cells arise chiefly form hypertrophy and hyperplasia of the membrana granulosa. The majority of the theca interna cells degenerated during CL development, but some of these cells contribute to the thecal luteal cells. In mice, no luteal cells

were found arising from the theca interna (Togari, 1926). In sheep, luteal cells arised exclusively from the membrana granulosa (Marshall, 1903). The luteal cells of pigs, mares and goats are formed by hypertrophy of granulosa cells. During this process the granulosa cell is invaded by blood capillaries from the theca interna which give rise to the vascular plexus of the corpus luteum. The large lipoid laden cells of the theca interna multiply mitotically, they give rise to two types of elements. The first type occupies their original position in the peripheral zone and along the vascular trabeculae by folds of the thecal wall of the collapsed follicle. The second type passes into luteal tissue, wandering among the luteal cells and becoming lodged throughout the whole structure. These thecal luteal cells are capable to be distinguished from the true luteal cells by having the smaller size. These cells persisted in the regressing CL after the true luteal cells have disappeared (Corner, 1942; Harrison, 1946; 1948a,b). In the rhesus monkey, luteal cells arised from granulosa and theca interna cells. Blood vessel invaded the granulosa cells and reached the inner part of the corpus luteum. Some of the theca interna invaded and differentiated to the nutritive cells and connective tissue network of CL (Corner, Bartelmez and Hartman, 1963). In baboons, the wall of the ruptured follicle folded extensively, resulting in tongues of theca interna and externa projecting inwards, both of theca interna and granulosa cells were transformed to luteal cells by hypertrophy which chiefly due to increasing of cytoplasm upon the central cavity (Zuckerman and Parkes, 1932). Human luteal cells derived from the membrana granulosa and some of the theca interna (Wartin 1924, 1926).

Corpus luteum is a transient endocrine gland which has the ability to secrete progesterone (P) and estradiol (E_2) (Greenwald and

Rothchild, 1968; Moudgal et al., 1972; Knobil, 1973; Speroff et al., 1983; Hutchison and Zeleznik, 1984). However, CL differed from other endocrine gland, since CL has no single trophic pituitary or other hormones for all species. Some species may have luteinizing hormone (LH), others may have prolactin (PRL), or combinations of PRL and LH (Rothchild, 1960; Everett, 1963; Rothchild and Schubert, 1963; Freeman and Sternman, 1978; Van der Schoot et al., 1978; Takahashi et al., 1980). The CL has a limited life span which can not be prolonged beyong gestation peroid in all mammals (Rothchild, 1981). The control of corpus luteum function and life span involved a complex interplay of luteotropic and luteolytic agents. However, the responsiveness of the CL may altered with time and physiological environments (Stouffer et al., 1976, 1977; Khan et al., 1979; Wu et al., 1976; Stouffer Bennett and Hodgen, 1980; Richardson and Masson, 1980; Ahren, Khan and Selstam, 1981; Hunter and Baker, 1981; Sridaran, Hunzicker-Dunn and Gibori, 1983; Patwardhan and Lanthier, 1984).

The endocrine mechanism on the maintenance of the CL during non-pregnant cycle and pregnancy differ according to species. In essence, the nonpregnant state there are two types of luteal phases. Those in which the luteal phase is short or incomplete, ranging from 1-2 days (most laboratory rodents inculding rats,mice and hamsters) and those in which the luteal phase is long or complete (guinea pigs, pigs, mares, cows, sheep, rhesus monkeys and women) (Rothchild, 1981). In animals with incomplete luteal phase, the CL of estrous cycle secreted a relatively low level of P during the first 24 hours of luteinizaiton, but the levels declined on the following day in rats (Eto et al., 1962), mice (Michael, 1976) and hamsters (Lukaszewska and Greenwald, 1970). Animals with incomplete luteal phase could obtained a complete luteal phase either by mating or stimulation of the major

external genitalia (cervix) or directly to the C.N.S. (posterior fornix)(Long and Evan, 1922; Shelesnyak, 1931; Harris, 1936; Carlson and De Feo, 1963; De Feo, 1963; Diamond and Yanagimachi, 1968). This stimulus evoked two daily surges of PRL secretion which rescued the CL and stimulated it to secrete P later during pregnancy and pseudopregnancy (de Greef and Zeilmaker, 1978, Day et al., 1980). Moreover, luteinizing hormone (LH) was also required for P secretion at this time (Rothchild, Pepe and Morishige, 1974; Ford and Yoshinaga, 1975). Prolactin action in the early life of the CL was necessary for acquisition of LH receptors (Holt et al., 1976) as well as luteal estrogen receptors (Gibori and Keyes, 1978; Gibori, Keyes and Richard, 1978). In animals with complete luteal phase, the length of luteal phase is sufficient to permit the developing egg to reach the stage at which implantation is possible and to maintain uterine sensitivity long enough for implantation (Johnson and Everette, 1980; Short, 1984). During implantation, syncytiotrophoblast developed and in some species secreted chorionic gonadotropin (CG) to mantain secretory activity of the CL (Hodgen et al., 1972, 1974; Reinius et al., 1973; Buso and Johnson, 1974; Goodman and Hodgen, 1979) until the conceptus provided sufficient P to sustain pregnancy (Goodman and Hodgen, 1979b).

Corpus luteum of rats is capable in secreting P and E_2, but the majority is P (Hilliard, 1973; Elbaun and Keyes, 1976; Gibori and Keyer, 1978; Taya and Greenwald, 1982a). In the cyclic stage, plasma P levels were low and lasted for no more than 2-3 days (Rothchild, 1965; Butcher, Collins and Fugo, 1974; Smith et al., 1975). However, serum LH and PRL levels appeared consistantly low throughout the cycle

(Amenomori et al., 1970; Bast and Melampy, 1972; Nequin, Alvary and Schwartz, 1979). The CL of cyclic rat is generally beleived to be refractory to hypophyseal tropic hormone (Uchida, Kadawaki and Miyake, 1969; Smith, Freeman and Neill, 1975; Boehm, Plas-Roser, and Aron, 1984b). On the other hand, there were some evidences to show that the CL of cyclic rats are capable to response to either LH or PRL by increasing the secretion of progesterone in vivo (Boehm et al., 1980; Rodway and Garris, 1982; Taya and Greenwald, 1982; Kim and Greenwald, 1984) and in vitro (Anderson et al., 1973; Hashimoto et al., 1975), although CL of hypophysectomized animals retained its capacity to secrete other inactive progestogens including 20α-hydroxyprogesterone (Van Straalen and Zeilmaker, 1982; Taya and Greenwald, 1982a: Kim and Greenwald, 1984).

During pseudopregnancy (PSP), the ovulatory surge gonadotropin was inhibited (Rothchild,1965) but basal levels of gonadotropins were increased (Van Rees and de Greef, 1965) and plasma P level was high (Hashimoto and Wiest, 1969; Bartosik and Szarowski, 1973; Van Straalen and Zeilmaker, 1982). The surge of PRL which occurred after induction by cervical stimulation (Smith et al., 1975; de Greef and Zeilmaker, 1978) was an indispensable luteotropic hormone to maintain CL function througout pseudopregnancy (Greenwald and Rothchild, 1968; de Greef Dullaart and Zeilmaker 1977; Murakami et al., 1979; Basury et al., 1983), while LH became essential only after day 9 (day 1 = ovulation) (Rothchild et al., 1974; Akaka et al., 1977; Lam and Rothchild, 1977; Garris and Rothchild, 1980).

In pregnancy, maintenance of CL function, prior to the establishment of placental luteotropic function, depended solely on luteotropic stimuli from pitutary gland (Biswas et al., 1975). The pituitary gland was necessary for luteal maintenance during the first half of pregnancy (Pencharz and Long, 1933; Madhwaraj and Moudgal,

1970; Morishige and Rothchild, 1974): being a source of PRL for the first 8 days of pregnancy (Morishige and Rothchild, 1974; Smith et al., 1976), which increased and maintained LH receptor (Gibori and Richard, 1978) and being a source of LH for day 8-12 (Modhawaraj and Moudgal, 1970; Smith et al., 1975) which might be dued to stimulatory effect on luteal estrogen production and played an important role in the maintenance of CL function during pregnancy (Gibori et al., 1978; Gibori et al., 1982). Serum P level in both pregnant and PSP conditions were very similar at least up to day 11 (Pepe and Richard, 1974), which indicated that the presence and the absence of implanted embryos were not essential determining factor for CL function during the preplacental phase of pregnancy. During day 6-11 of pregnancy and PSP, decidual tissue may produce a prolactin-like hormone (decidual luteotropin), which sustained luteal function in the absence of PRL, but required LH to act in concert to sustain P production between day 8-11 (Gibori et al., 1984; Janyatilak et al., 1984). In hypophysectomized pseudopregnant amimals, however, the presence of fully differentiated decidual tissue is unable to maintain sufficient endogenous progesterone secretion needed for further survival of the luteal tissue (Varavudhi et al., 1966; Janyatilak et al., 1984). When placentation was sufficiently advanced (by day11), placenta secreted luteotropic agents to regulate luteal cell LH receptors (Gibori adn Richard, 1978) and to maintain ovarian production of both estrogen and progesterone (Gibori, et al., 1982) by producing 2 hormones; one was a prolactin like hormone (placental lactogen, PL) (Gibori adn Richard, 1978; Blank and Dufau, 1983; Glaser, et al., 1984) which increased and maintained LH receptor content, the other was a LH-like hormone, chorionic gonadotropin (CG) which would be more likely to stimulate ovarian E_2 and P production (Gibori

and Richard, 1978). Furthermore, feto-placental unit actively synthesized P as well as androgens in the second half of pregnancy (Chan and Leather, 1975). While, the maternal CL maintained to be an active site of estradiol produciton in addition of progesterone (Gibori et al., 1981) and luteolysis is induced in rats during late pregnancy by fetoplacental removal (Smith and Kuhn, 1980).

In lactating stage, cyclic ovulaiton was interupted (Rothchild, 1960; Wang et al., 1980; Dorrington and Gore-Langton, 1981; Uilenbroek et al., 1982; Hansen et al., 1983), CL formed after the postpartum ovulation were functionally active (Tomogame, Oto and Yokoyama, 1969; Smith and Neill, 1977). This stage temporarily caused hyperprolactinaemia, inhibition of LH release, but not FSH secretion (Rothchild, 1960; Lu et al., 1976; Smith and Neill, 1977; McNeilly et al., 1978; Smith, 1978, 1982; Taya and Sasamoto, 1981; Hansen et al., 1983). The maintenance of CL activity seems to relate the suckling stimulus (Nicoll and Meites, 1959; Zeilmaker, 1964; Selmanoff and Selmanoff, 1983). The duration of lactating pseudopregnancy was increased with the increase of litter size (Rothchild, 1960; Maneckjee and Moudgal, 1975) and was decreased by litter removal (Takahashi and Suzuki, 1971; Ford et al., 1975; Maneckjee and Moudgal, 1975). Prolactin titre fell off in the late lactation in mother continuously kept with their pups and plasma PRL level decreased (Amenomori et al., 1970; Ford and Malampy, 1970; Lu et al., 1976).

Luteinizing hormone was an important luteotropic hormone in many species including rats, hamsters, rabbits, monkeys and human (Greenwald and Rotchild, 1968; Hillard, 1973; Hansel et al., 1973, diZerega and Hodgen, 1980; Speroff et al., 1983; Groff et al., 1984; Hutchison and Zeleznik, 1984). The action of LH on the CL was beleived to be

mediated by the adenyl cyclase system (Marsh, 1970; Lamprecht et al., 1973). The positive correlation between LH stimulating adenyl cyclase activity and P secretion was observed (Day, Kirchick and Birubaumer, 1980). Luteinizing hormone was capable to maintain the pregnancy in hypophysectomized rats (Yoshinaga, Macdonal and Greep, 1971) increase PRL receptor of CL (Holt et al., 1976), stimulate P production in dispersed luteal cells of pregnant rats and hamsters (Wada and Greenwald, 1984), and pseudopregnant rats (Menon, Peegal and Menon, 1985), rat granulosa cells (Jone, Valk and Useh, 1983) and of human luteal cells (Rice et al., 1964; Marsh and Lemaire, 1974; Stouffer et al., 1976; 1977; Richardson and Masson, 1980; Hunter and Baker, 1981; Laherty et al., 1985), stimulate estradiol production by rat luteal cells (Kalison, Warshaw, and Gibori, 1985), enhance the esterase activity and there by increasing cholesterol for P synthesis (Behrman and Armstrong, 1969). Furthermore, it increased 3β-hydroxysteroid dehydrogenase in granulosa cells (Jones et al., 1983), stimulated adenyl cyclase and increased cyclic-AMP, which contributed to P production (Channing and Tsafrini, 1977; Sala et al., 1979; Ahren et al., 1981). It is of interest that LH was capable to antagonize luteolytic of $PGF_{2\alpha}$ in rats _in vivo_ (Chatterjee, 1976) and _in vitro_ (Wright et al.,1980) and prevented luteolytic action of estradiol in monkeys (Schoonmaker et al., 1982). However, the ovarian specific binding of LH was high in proestrus of cyclic rat, during day 14-18 of pregnancy (Cheng, 1976; Van Straalen adn Zeilmaker, 1982), and was the highest on day 7 and decrease from day 7 to day 14 of pseudopregnancy (Hwang and Menon, 1986). As well as the responsiveness of luteal cells to hCG was paralleled to the hCG binding activity of luteal cells during the functional life of CL (Lee, Tatcishi, Ryan and Jiang, 1975).

Prolactin constituted part of the luteotropic complex necessary for the maintenance and secretory activity of the CL in rat (Aswood, 1941; Wang et al., 1979), mouse (Kavavic, 1969), rabbit (Spies, et al., 1968), hamsters (Greenwald and Rothchild, 1968) and sheep (Demamur, et al., 1973). Prolactin exerted stimulatory effect on P production in vitro and in vivo of rat ovary (Gibori and Richard, 1978; Jones, Valk and Hsueh, 1983; Menon, et al., 1985; Murphy and Rajkumar, 1985). Moreover, it was capable to maintain CL function in hypophysectomized rat (Aswood, 1941), maintain rat granulosa cells in culture (Cripp, 1977), retain luteal cells viability and functional capacity in response to LH-PRL stimulation, maintain transferase activity (Behrman et al., 1970), maintain and increase LH receptors (Grinwich, Hichens and Behrman, 1976; Holt et al., 1976; Van Straalen and Zeilmaker, 1982; Murphy and Rajkumar, 1985), inhibit the formation of 20α-hydroxyprogesterone (Wang et al., 1979) and enhance substrate incorporation for P synthesis (Murphy and Rajkumar, 1985). Furthermore, PRL could act synergistically with LH to stimulate P synthesis of CL in vitro (Armstrong et al., 1969; Uilenbroek and Van der Linder, 1984; Kalison et al., 1985), and could act synergistically with estrogen to stimulate P secretion in hypophysectomized rats' corpus luteum in vivo (Rodway and Garris, 1982).

corpus luteum of primate was capable to synthesize and secrete both P and E_2 (Mikhail, 1970; Baird et al., 1975; Stouffer Bennett and Hodgen, 1980). Nevertheless, it was the major source of P in nonpregnant primate, the cessation of luteal function (luteolysis) was manifested by declining plasma P level and the onset of menstruation (Neill et al., 1969, 1970; Kirton et al., 1970; Knobil, 1973; Goodman, Nixon, Johnson

and Hodgen, 1977) function in primate was reported to be influenced by circulationg levels of LH (Van de Weile et al., 1970; Moudgal et al., 1972; Knobil, 1973; Hutchison and Zeleznuk, 1984), LH and PRL (Schulz et al., 1976; Espinoza-Campos et al., 1978; Castracane and Shaikh, 1980; Jaffé, 1981). In vivo and in vitro studies demonstrated that exogenous LH of hCG were capable to stimulate and maintain luteal P production in monkeys (Macdonald and Greep, 1972; Stouffer et al., 1977; Wilk and Nobel, 1983) and human (Hunter and Baker, Goldsmith et al., 1981). However, hyperprolactinaemia and/or galactorrhea monkeys showed suppression of gonadal function associated with anovulatory cycle in most cases (Cholwanich, 1986). It is of interest that normal luteal function could be maintained in the monkey hypophysectomized during luteal phase of the cycle (Asch et al., 1982), in woman with transient hypoprolactinaemia (del Pozo et al., 1975; Sarris et al., 1978; Fleming et al., 1980), as well as either in cynomolgus monkeys (Castracane and Shaikh, 1980) and rhesus monkeys (Richardson et al., 1985).

During early pregnancy, a gonadotropin of placental origin, chorionic gonadotropin (CG), provided the stimulus for continuation of luteal P production (Hodgen et al., 1974). In Macaca fascicularis CG is detected in pregnancy urine during day 17-27 of gestation (Lamsa-ard, 1984). After the first three weeks of pregnancy the macaque CL underwent structrually regressive changes (Corner, 1956; Koering et al., 1973) and presumably relinquished the steroidogenic functions which were provided to maintain of normal pregnancy, to the developing placenta. In the rhesus monkeys, the ovaries can be removed as early as the 21st day of pregnancy without altering the normal pattern of plasma P concentration (Koering, Wolf and Meyer, 1973) or terminating

pregnancy (Hodgen and Tullner, 1975). Nevertheless, the CL of macaques established its functional capability near the time of parturition by being capable to secrete P and respond to hCG (Stouffer et al., 1976; Stouffer et al., 1979) and by structural analysis of the corpus luteum in Macaca fascicularis (Koerning et al., 1973; Srivara, 1973; Gulyas, 1974; Gulyas et al., 1976).

The primate luteal function during luteal phase of the menstrual cycle apparently requires LH support (Knobil, 1973; Ellinwood et al., 1984). Several investigators reported that continuously LH exposure is required for normal P production and menstrual cyclicity in human (Van de Weile et al., 1970; O'dell, 1979); Speroff, Glass and Kase, 1983), and in cynomolgus monkey (Moudgal et al., 1971; 1972; dizerga and Hodgen, 1980; Groff et al., 1984). Moreover, the role of LH on the corpus luteum function can be supported by the data demonstrating the presence of LH/hCG receptors in corpus luteum which changed in different stages of menstrual cycle (Cole et al., 1973; Rao et al., 1979; Cameron and Stouffer, 1982). Furthermore, these LH receptors increased to maximal level during mid luteal phase (Wardlav et al., 1975; McNeilly et al., 1980) which was concomitant with the increased LH responsiveness of CL (Butcher et al., 1974; Smith et al., 1975; Van Straalen and Zeilmaker, 1982).

The role of PRL in the function of the primate CL remain unclear. As for the pattern of PRL level during menstrual cycle of primate, there was no surge in serum PRL during any states of the menstrual cycle in rhesus monkeys (Quadri and Spies, 1976; Bulter et al., 1975; Milmore, 1978), in cynomolgus monkey (Varavudhi et al., 1982), in chimpanzee (Reyes et al., 1975) and in woman (Ehara, Siler, Vandenberg, Sinha and Yen, 1973; Tyson and Friesen, 1973). Moreover, PRL

was unable to alter P secretion of human granulosa and luteal cells (Erwards, Hyness and Wilson, 1982; Tan and Biggs, 1983), luteal cells of cynomolgus monkey and rhesus monkey (Stouffer, Coensgen and Hodgen, 1980; Laherty et al., 1985). However, a number of reports showed that PRL is capable to inhibit P secretion in human ovarian tissue fragments (Demura et al., 1982), human granulosa cells (McNatty, 1974), human luteal cells (Polan, Laufer and Dlugi, 1984; Hunter, 1984). These have been supported further by the presence of PRL receptors in human CL (McNeilly, Kevin, Swanton and Bramby, 1980).

Corpus luteum regression (luteolysis)

Structural regression

The first stage of structural regression of CL is marked by fatty degeneration, accompanied by shrinkage and disappearance of the luteal cells. The disappearance of the luteal cells take place gradually. The removal of the debris of luteal cells is assisted by reduction and ultimate atropy of the vascular supply of the corpus luteum apparently through collaspe of the vessel accompanied by connective tissue invasion. In larger animals, this appeared to be a real increase in the amount of connective tissue, which became collagenous and gave rise to the dense avascular corpus fibrosum. The corpus fibrosum gradually became merged with the surrounding tissue of the ovarian stroma but it might remain distinct for a very long time before finally disappear (Loeb, 1910; Corner, 1921, 1942; Long and Evan, 1922). In woman corpus albicantia persisted as old scars for months or years (Young and Corner, 1961), while corpora lutea of rats and mice persisted for 2-3 estrous cycles (Long and Evan, 1922).

Functional regression

Functional luteolysis of the CL was indicated by the decline in circulating P levels (Neill, et al., 1967; 1972; Knobil, 1973; Goodman et al., 1977a), accompanied by a marked increase activity of the 20α-hydroxyprogesterone and increase activity of the 20α-hydroxysteroid dehydrogenase (20αOHSDH) (Wiest et al., 1968; Bast and Melampy, 1972; Takahashi, 1984; Naito et al., 1986), desensitization of the adenyl cyclase system (Hunzicker-Dunn and Bisubanmer, 1976; Khan et al., 1979), decrease in the number of LH receptors (Greary and Rice, 1973; Rao, Estergreen and Carman, 1979a, Cameron and Stouffer, 1982), decrease in the binding of LH (or hCG) in CL (Wardlaw et al., 1975; McNeilly et al., 1980a) and followed by the onset of the menstruation in higher primates including human (Neill et al., 1967; Kirton et al., 1970; Knobil, 1973). Nevertheless, the precise physiological mechanism of luteal regression is not known. Prostaglandin of the F2α series originating from the uterus has been implicated as one of the possible factors involved in the initiation of luteolysis in several species including pigs, cows, guinea pigs, sheep and rats (Nalbandov, 1973; Behrman and Caldwell, 1974; Hammerstein, 1974; Horton and Poyser, 1976; Zor and Lamprecht, 1977; Behrman, 1979). Hysterectomy caused luteal function to prolong in sheep, rats, mice, guinea pigs, rabbits and hamsters (McCraker et al., 1970, 1973. McCraker et al., 1972; Golberg and Romwell, 1975; Hall and Robinson, 1978; Behrman, 1979), but failed to alter cyclic ovarian in woman (Beavis et al.,1969; Beling et al., 1970; Eraser et al., 1973) and rhesus monkey (Neill et al., 1969). These evidences supported the view that the uterus in the subprimate releases a "uterine luteolysin" responsible for maintenance of the CL.

In primates, $PGF_{2\alpha}$ was postulated as a major intraovarian luteolytic agent since luteal tissue of these animals were capable to synthesize $PGF_{2\alpha}$ (Challis et al., 1976; Balmaced et al., 1978; Valenznela et al., 1983) and the production of prostaglandins by CL during late luteal phase appeared to be higher than during earlier phases (Shutt et al., 1976; Balamaceda et al., 1978; Patwardhan and Lanthier, 1980; Vajaykumar and Walter, 1983). Elevated $PGF_{2\alpha}$ levels in ovarian plasma draining from the ovary bearing the CL has been reported (Walter et al., 1978; Aksel et al., 1979). Furthermore, a negative correlation existed between plasma P concentration and $PGF_{2\alpha}$ in luteal tissue throughout the cycle (Shutt et al., 1976; Vajayakumar and Walter, 1983).

The luteolytic activity of $PGF_{2\alpha}$ was first demonstrated in rats by Pharris and Wyngarden (1969) who showed that uterine infusion with $PGF_{2\alpha}$ in the pseudopregnant rat depressed ovarian P levels and since $PGF_{2\alpha}$ was venoconstrictor, they hypothesised that the luteolytic effect was due to restriction of ovarian blood flow resulting from utero-ovarian venoconstriction. The luteolytic action of $PGF_{2\alpha}$ has also been demonstrated in other animals, thus PSP was shortened in guinea pigs, mice, rats and hamsters (Blatchley and Donovan, 1969; Barth, Mereill and Baker, 1972; Lau, Saksena and Chang, 1974; 1979). Prostaglandin $F_{2\alpha}$ was capable to decrease the plasma P of rat _in vivo_ (Behrman and Hichen, 1972; Grinwich and Behrman 1974; Behrman et al., 1978; Pang and Behrman, 1981, and pig (Barb et al., 1984), induce a loss of LH action on luteal cells of rat _in vivo_ and _in vitro_ (Behrman et al., 1971; Behrman and Caldweel, 1974; Khan et al., 1979; Wright,et al., 1980; Jordon, 1981), reduce hCG binding capacity of luteal tissue (Hichen et al., 1974; Luborsky et al., 1984), prevent action of PRL on maintaining

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luteal function in hypophysectomized rat in vivo (Behrman et al., 1971), antagonize luteotropic action of PRL and LH in vitro (Behrman and Caldwell, 1974; Hichen, et al., 1974; Lahav, et al., 1976; Thomas, et al., 1978; Hall and Robinson, 1979; Ueda et al., 1980; Richardson and Peddie, 1982; Behr et al., 1983). However, the luteolytic action of $PGF_{2\alpha}$ was strongly dependent upon the age of the CL (Lamprecht, et al., 1975; Horton and Poyser, 1976; Henderson and McNatty, 1977; Khan et al., 1979). $PGF_{2\alpha}$ induce functional luteolysis in rhesus monkey by decreasing cycle length (Kirton, et al., 1970; Kirton and Koering, 1973; Spittman et al., 1977; Auletta et al., 1984), inhibit the stimulation of P formation in human CL by hCG in mid and late luteal phases (Patwardhan and Lathier, 1984). It is of interest that $PGF_{2\alpha}$ do not alter plasma LH level but induced loss of LH/hCG receptor (Sotrol et al., 1981), decrease the ability of CL to bind ^{125}I-labelled gonadotropin (Hickens, et al., 1974; Rajaniemi et al., 1977; Dickman et al., 1978; Stouffer, et al., 1979; Luborsky et al., 1984). These are evidences of the presence of $PGF_{2\alpha}$ receptors in luteal cells (Powell et al., 1974; Rao, et al., 1977). The inhibitory effect of $PGF_{2\alpha}$ on P secretion by human luteal cells and granulosa cell culture (O'Grady et al., 1972; McNatty, et al., 1975; Richardson and Mossman, 1980), rhesus granulosa and luteal cell culture (Channing, 1970; Stouffer, et al., 1979) indicated the involvement of $PGF_{2\alpha}$ in the luteal regression.

Primate corpus luteum was also capable to synthesize prostaglandins, it also synthesizes at least two polypeptide hormones, relaxin and oxytocin. The former was not detect in primate during the non fertile menstrual cycle, but became measurable during the interval when chorionic gonadotropin (CG) rose in early pregnancy (Austin and Short, 1984; Ottobre et al., 1984). Moreover, peak of

relaxin secretion in the rhesus monkey was associated with the declining steroidogenic function of CL (Ottobre et al., 1984). Relaxin induced softening of the inter-pubic ligament and reduced the contractility of the uterus (Austin and Short, 1984) which did not involve the luteolysis. Oxytocin has been found in relatively high concentrations in the CL of of sheep and human (Austin and Short, 1984) and in cynomolgus monkey. Peak of oxytocin found in mid luteal phase (Khan-Dawood et al., 1984). A dirrect chronic diffusion of oxytocin (10 min/µl/h) into the CL of normal cyclic rhesus monkeys in vivo began from 6 days after the preovulatory estradiol surge, caused a significant decrease of P and E_2, beginning 1 and 4 days after treatment respectively. The peripheral LH concentration remained unchanged but the duration of the luteal phase, menstrual cycle and the onset of menses from the initiation of oxytocin infusion were significatly shorter (Auletta, 1984). In vitro evidence oxytocin indicated that at low concentrations (4-40 miu/ml) significantly enhanced P produciton of dispersed bovine luteal cells from early pregnancy after incubation for 3 hour. On the contrary high oxytocin concentraiton (800 miu/ml) markedly inhibit the response of luteal cells to hCG.(Ottobre, 1984). These results show that oxytocin is also involved functional luteolysis of corpus luteum in primate.

Objectives

The regulation of corpus luteum function is very complicated by the fact that tropic hormones required for *in vivo* luteal function appeared to fluctuate and may under the influence of several factors. To avoid the *in vivo* factors, cultivation of isolated luteal cells *in vitro* system was employed to study the corpus luteum function of rats and *Macaca fascicularis* and the responsiveness of CL to potential luteotropin and luteolysin.

The main objectives of the present study are as follow:

1. Establish the luteal cell culture under various physiological stages of rats and monkeys.

2. Establish baseline data of P and E_2 secretion of luteal cells during short and long term incubations.

3. Study the effect of certain potential luteotropic and/or luteolytic agents on *in vitro* P and E_2 secretion of luteal cells.