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EFFECTS OF ESTRADIOL-17β ON HORMONE LEVELS AND LUTEAL FUNCTION IN CYCLING GILTS

by

LAURIE CONNOR

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DEDICATION

To Robert J. Parker

whose commitment to knowledge

and sensitivity to graduate students

are rare and treasured qualities

ABSTRACT

Two experiments were conducted to investigate the luteotrophic action of estradiol-17β (E_2-17β) in gilts. For experiment I, 8 cycling crossbred gilts 6-8 months of age were divided into two groups. In treatment 1 (T1), 4 gilts were injected intramuscularly (im) with 10 mg E_2-17β at 0800/h on day 10 of the estrous cycle. The 4 gilts in treatment 2 (T2) were injected im with 10 mg E_2-17β on each of days 10 through 14 of the cycle. Each animal, serving as its own control, was injected with peanut oil vehicle on corresponding days of the estrous cycle preceding the treatment cycle. Blood samples were taken daily at 0900 h from day 0 (first day of estrus) to day 9; immediately pre-injection (0800 h) and subsequently every hour for 14 h; then daily until the next estrus. Following E_2-17β injection, mean serum estrogens peaked at levels > 900 pg/ml within 1 to 4 h post-injection, returning to near pre-treatment means by day 18 (T1) to 22 (T2). During the 14 h period following each E_2-17β injection, serum progesterone (P) and prolactin (PRL) concentrations did not change significantly; the pulsatile pattern of LH secretion was suppressed but serum LH levels were significantly different ($P < .05$) from the pre-injection mean only on day 10 in T2 gilts. E_2-17β treatment prolonged luteal P production and significantly extended ($P < .01$) the interestrus interval by an average 6.25 (T1) and 7.71 (T2) days.

In experiment II, 13 cycling Managra and York x Managra gilts, 7 months of age were divided into 4 groups. Group I (G I) and Group II (G II) were controls, injected im with vehicle at 0830 h on days 10 through

14 of the estrous cycle. Groups III and IV (G III and G IV) were injected on the same days of the cycle with 10 mg E_2-17β. Ovaries were removed on day 15 (G I and G III) or day 20 (G II and G IV), and evaluated for several ovarian characteristics and luteal membrane receptor binding of oLH and oPRL. Blood samples were collected from G II and G IV immediately pre-injection; at 6 h and 12 h post-injection; then at 0830 h and 2030 h from day 15 to 20. Blood was collected from all gilts at ovariectomy (ovx). The effect of E_2-17β on serum hormone profiles and luteal function was consistent with observations in experiment I. Luteal P production was maintained until ovx on day 20, serum LH remained low, and no immediate effect on PRL secretion was apparent. Hormone concentrations at ovx were similar in G I and G III. Only P levels were significantly different ($P < .01$) between G II and G IV; being maintained in G IV at levels comparable to G I and G III. E_2-17β treatment inhibited ovarian follicular growth beyond the 3 mm stage; maintained corpora lutea weight; increased ($P < .01$) % specific binding (%SB) of oLH to luteal tissue receptor preparations by more than 2 fold above G I values; but did not affect %SB of oPRL to luteal receptor preparations.

Results suggest that part of the E_2-17β luteotrophic effect involves an increase in available luteal binding sites for LH and possibly maintenance of luteal receptor sites for PRL.

The final experiment was concerned with the effect of inhibiting PRL secretion on cyclic luteal activity. Ten cycling gilts of mixed breeds, 7-9 months old were divided into two equal groups. At 0830 h on days 4 through 11 of the estrous cycle, each gilt was injected im with either 1

ml 60% ethanol saline (controls) or 10 mg bromocriptine (Br) (treated). Ovaries were removed on day 11 and evaluated as in experiment II. Daily blood samples were taken immediately pre- and 12 h post-injection from 2/5 control and 3/5 Br-treated gilts. Samples were collected from all gilts at ovx. Mean levels of PRL decreased from the day 4 pre-injection mean of 13 ng/ml but remained above 4 ng/ml at all sampling periods until day 11. Br did effectively block the PRL response to stress at ovx. Serum P concentrations, corpora lutea numbers, weights, and %SB of oLH and oPRL to luteal tissue receptor preparations were not significantly affected by Br treatment.

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INTRODUCTION

The luteotrophic response evoked by injections of estrogen into the gilt at midcycle has been acknowledged for over two decades, yet there is a paucity of information regarding the mechanism(s) of this action. Prior to the beginning of the present studies no reports were available of the hormone profiles associated with estrogen treatment. Consequently, theories advocating estrogen-induced alteration of pituitary luteotrophin secretion lacked the supporting evidence that might have been gleaned from such measurements.

In general, hormonal regulation of porcine luteal function has not been clearly defined. Although, it is commonly accepted that porcine corpora lutea of the estrous cycle function autonomously, there is evidence to the contrary. As well, it is recognized that maintenance of luteal activity beyond the cyclic lifespan requires hypophyseal support, but again the nature of this requisite has not been fully elucidated. A luteotrophic role for luteinizing hormone (LH) has strong support. Evidence implicating prolactin (PRL) involvement in maintenance of porcine corpora lutea is more tenuous. At the time these studies were undertaken there was no information concerning the daily serum PRL concentrations during the porcine estrous cycle.

The first experiment was an investigation of the effects of estradiol-17β (E_2-17β) injection on the interestrus interval and serum concentrations of estrogens, progesterone, LH and PRL in cycling gilts. These observations were extended in the second experiment to include

evaluation of ovarian characteristics and luteal membrane receptors for LH and PRL subsequent to E_2-17β treatment.

The final experiment was concerned with inhibiting PRL secretion with bromocriptine treatment during the luteal phase of the estrous cycle, in order to examine a possible role for PRL in cyclic luteal function. Serum hormone levels were measured as an indices of ovarian and pituitary function; ovarian characteristics were evaluated and specific binding of LH and PRL to luteal membrane receptors was assessed.

LITERATURE REVIEW

Attempts to form unifying concepts and mechanisms concerning hormonal regulation of corpus luteum (CL) function have been hindered by reports of marked species differences in requirements for luteotrophic hormones. The hormonal mechanisms involved in control of formation and function of the CL have received the earliest and most extensive attention in rats; a species in which full CL development and activity is attained only during pregnancy or pseudopregnancy. It is now recognized that maintenance of morphological and functional integrity of the rat CL depends upon pituitary secretion of luteinizing hormone (LH) and prolactin (PRL). Estrogen is intimately involved with this complex but the respective roles of each of these hormones and their cellular mechanisms for regulation of CL function are not well defined.

The requirements and role of the above mentioned hormones in control of CL function in the large domestic animals are not so well established. This review will present a brief overview of the literature concerning the involvement of these hormonal factors in maintenance of normal CL function in cattle, sheep and swine. The first two sections will compare the requirements for pituitary support of cyclic CL maintenance and the hormone(s) involved. Next, luteolytic and especially luteotrophic actions of estrogen will be discussed. The final section will deal with some of the theories regarding estrogen involvement in the transformation of corpora lutea of the estrous cycle into corpora lutea of pregnancy in swine.

Role of the Pituitary in CL Maintenance

The concept that the porcine CL of the cycle is autonomous, not requiring pituitary support for development or function, has been perpetuated as fact for several years (Nalbandov, 1973, 1976). In part, the original hypothesis stemmed from results demonstrating that functional corpora lutea developed, produced progesterone (P) and lasted the normal duration of the cycle in pigs hypophysectomized immediately after ovulation (du Mesnil du Buisson and Leglise, 1963). Although hypophysectomy performed at estrus did not prevent the formation of apparently normal corpora lutea up to days 6 and 9, by day 13 of the cycle P content and weights of corpora lutea were lower than controls (du Mesnil du Buisson and Leglise, 1963, et al. 1964 as reviewed by Denamur, 1968). Removal of the pituitary after estrus did not affect CL P content on day 10 or 11, but did result in corpora lutea of lighter weight than in day 13 controls (Anderson et al., 1967). These observations suggest that the preovulatory LH surge is indeed sufficient stimulus to initiate luteinization, subsequent normal CL development and P production until at least the mid-luteal phase. Maximum morphological and functional integrity of luteal tissue beyond this point may depend on secretion of pituitary gonadotrophin(s).

In the ewe, hypophyseal support is required for normal development of luteal structures and maintenance of cyclic luteal activity. However, the two research groups who conducted most of the hypophysectomy studies were opposed in their interpretations. Kaltenbach et al. (1966) concluded from their research that the ovine CL required pituitary support for at least the first 5 days of the cycle. Denamur's group, in

France, (Denamur, 1968) found hypophyseal support was necessary only after day 5. The controversy may have evolved because of different criteria for development and functional activity as well as possible variations depending on the day of the cycle hypophysectomy was performed. Nonetheless, it is apparent that a luteotrophic contribution from the pituitary is required for the ovine CL of the estrous cycle to attain normal size and steroidogenic competency.

The effects of hypophysectomy in the cow have not received attention. However, pituitary stalk section on the day of ovulation (Anderson et al., 1966) resulted in corpora lutea on day 12 that were no heavier than would be expected on day 5 or 6 of the cycle, but did produce an apparently normal plasma P concentration (Gomes et al., 1963).

Thus, each of these species may have the potential for some autonomous CL growth and activity; the necessity of hypophyseal support being one of degree.

Nature of the Pituitary Luteotrophin(s)

Luteinizing Hormone. A luteotrophic role of LH for normal cyclic luteal function in cattle and sheep seems well established. In the bovine, it has been demonstrated that exogenous LH can increase P output from corpora lutea of normal cyclic (Carlson et al., 1971) or hysterectomized animals (Brunner et al.; 1969, Carlson et al., 1971) and prolong the interestrus interval by approximately 16 days (Donaldson and Hansel, 1965). Administration of antiserum to LH on days 2 through 6 of the cycle to intact heifers, or for 5 days to hysterectomized heifers, resulted in a significant reduction in corpora lutea weights and P content

(Snook et al., 1969). CL functions ceased, or was quantitatively inhibited, when LH antisera was injected on days 11 and 12 (Hoffman et al., 1974). These findings have been supported by several *in vitro* trials which demonstrated that LH was required to maintain morphological appearance and P secretion of bovine luteal tissue (Hansel et al., 1973; Gospodarowicz and Gospodarowicz, 1975). According to Armstrong and Black (1966), CL from day 14 of the cycle respond less to LH than do older ones.

LH also exerts important trophic actions on the cyclical CL of the ewe. Infusion of LH into the ovarian artery stimulated P secretion from ovaries autotransplanted to the neck (McCracken et al., 1971) or remaining *in situ* (Domanski et al., 1967). In sheep hypophysectomized on the day after estrus, LH increased both CL weight and P production (Denamur, 1974). As well, functional luteal tissue lifespan was approximately doubled by daily infusions of LH beginning on day 10 or 11. Repeated administration of anti-bovine LH serum decreased P secretion in ovary autotransplant ewes (McCracken et al., 1971) and resulted in partial regression of corpora lutea in intact ewes (Fuller and Hansel, 1970; Denamur, 1974).

A requisite for LH by the porcine CL of the cycle appears less obvious. As previously indicated, the results of hypophysectomy at estrus suggest that the preovulatory LH surge, or other events occurring by that time, are sufficient to initiate CL development. Subsequent differentiation and activity may proceed normally without pituitary support; at least, until the mid-luteal phase. Other investigatory approaches lend credence to this hypothesis. Cook et al. (1967) observed that porcine luteal tissue from mid-cycle (days 8-10), or early gesta-

tion, responded to incubation with LH by a significant increase in P synthesis. However, the response was highly variable and small (15%) compared to the response noted with bovine (40%) and ovine (30%) luteal tissue. More recently, Watson and co-workers noted a rapid transient LH stimulated increase in luteal P secretion followed by a prolonged stimulation in luteal tissue from non-pregnant (days 11-14) (Watson and Leask, 1975; Watson and Wrigglesworth, 1975) and early pregnant (Watson and Maule Walker, 1978) pigs. Although these studies demonstrate an ability of porcine corpora lutea to respond to LH, they do not define a requirement in vivo.

Spies et al. (1967) were unable to cause any significant reduction in corpora lutea weights or P concentration in non-pregnant gilts by the administration of anti-ovine LH on days 7 through 11 of the cycle. The same antiserum given to pregnant gilts resulted in CL regression and loss of embryos; thus demonstrating the necessity of LH for maintenance of corpora lutea beyond the normal cycle length. This observation gains support from studies using hypophysectomized animals. As in many other non-primate species in which the uterus exerts a luteolytic effect, hysterectomy during the porcine luteal phase results in prolongation of the CL lifespan (du Mesnil du Buisson and Dauzier, 1959). Anderson (1966) summarized the available information at that time and concluded that in sows hysterectomized and then hypophysectomized, corpora lutea will regress within 10 days. Corpora lutea could be maintained beyond 10 days by a variety of LH containing gonadotrophin preparations. These same compounds were without effect when the uterus remained in situ. Of interest in this context is the report of Denamur (1968) that a combined

LH and estradiol treatment, beginning on day 12, resulted in fully active corpora lutea on day 20 in sows hypophysectomized on day 2 of the cycle. Such results are of interest in view of the necessity for CL maintenance during early pregnancy and will be discussed later.

Prolactin: The role of PRL in maintenance of structure and function of the CL remains controversial. As previously mentioned, PRL is considered part of a luteotrophic complex in the rat. PRL action in the rat CL appears to include increasing LH receptor protein, maintaining enzymes essential for steroidogenesis and, at the same time, inhibiting enzymes which catabolize P (Ensor, 1978). Evidence implicating PRL as an essential luteotrophin during the estrous cycle in cows, ewes and sows is conflicting.

In cattle, PRL failed to overcome the inhibitory effect of oxytocin on CL function, whereas, LH did (Donaldson et al., 1965). Similarly, PRL treatment could not prolong CL function in intact heifers (Smith et al., 1957). In vitro studies were contradictory. While Hansel (1967) did not observe any effect of PRL on P synthesis by bovine luteal tissue, both Romanoff (1966) and Bartosik (1967) reported that PRL increased P synthesis in perfused luteal phase ovaries. However, the quantity and purity of the PRL hormone preparations used in the latter studies have been questioned (Hansel et al., 1973). Depression of plasma PRL concentrations with PRL antiserum or bromocriptine (CB-154), administered on days 11 and 12 of the cycle, did not affect circulating P levels (Hoffman et al., 1974). Plasma PRL concentrations could not be determined in the antiserum-treated group. But, in the two cattle treated

with CB-154, PRL levels declined by 83% and 88%. These reports, combined with those in the previous section, suggest that LH is the dominant luteotrophin in cycling cattle, whereas PRL appears to have little if any effect. But, a possible facilitating role in preservation of luteal tissue integrity cannot be excluded.

More evidence is available that PRL is required for normal cyclical CL function in the ewe, but, some controversy exists. Hixon and Clegg (1969), using an impure PRL preparation, were able to increase P secretion in hypophysectomized ewes. McCracken et al. (1971) were unable to duplicate this stimulatory effect in vitro or in intact luteal phase ewes. Similarly, in contrast to their results using LH preparations, Karsch et al. (1971) were unable to prevent CL regression by ovarian infusion of PRL. However, failure to detect a luteotrophic action in cycling animals during the luteal phase, does not rule out a requirement for this hormone for normal development and preservation of luteal structures and cyclic activity.

The luteotrophic properties of PRL have been demonstrated in hypophysectomized and hysterectomized-hypophysectomized ewes. PRL supplementation, for 12 days after hypophysectomy on day 2 of the cycle, produced an increase in corpora lutea weight of ca. 60% compared to controls (Kann and Denamur, 1974). Hypophysectomy following hysterectomy (days 9-12) resulted in near complete luteal regression within 4 days (Denamur et al., 1973). Daily PRL treatment, but not LH alone, maintained CL activity for at least 12 days; but, at a reduced level. Simultaneous administration of PRL and LH preserved CL function comparable to that of hysterectomized controls. Finally, consequences of pituitary

stalk-section on day 3 of the cycle also support a trophic role for PRL during the cycle. This surgical intervention resulted in a diminuition of circulating LH to undetectable levels (Kann and Denamur, 1973), persistance of appreciable amounts of plasma PRL (Bryant et al., 1971) and relatively normal CL development, up to the 12th day of the cycle (Denamur et al., 1966, 1970). Removal of residual LH, by injections of LH antiserum, still allowed greater luteal structure development than noted 7 days following hypophysectomy on day 2 of the cycle.

Although a luteotrophic requirement for PRL seems to be illustrated by the experiments cited above, suppression of PRL secretion by CB-154 injections from day 2 of the cycle did not affect the time of CL regression (Niswender, 1972; Kann and Denamur, 1974). This does not, necessarily, contradict a requisite for PRL, since blood PRL was still detectable (Kann and Denamur, 1974).

Comparatively little research has been reported regarding PRL in the pig. Measurements of pituitary PRL content during the estrous cycle of sows showed that the amount of hormone increased until estrus then declined (Day et al., 1959); the lowest values being immediately after ovulation and the highest in the luteal phase (Threlfall et al., 1972). If pituitary content is inversely related to plasma concentrations, these results agree with the recent report of Dusza and Krzymowska (1979). Plasma PRL levels in sows were greatest just before estrus, with a minor surge in some animals at estrus. The lowest plasma concentrations were observed during the luteal phase. This pattern is similar to that seen during the estrous cycle of the ewe (Kann and Denamur, 1974).

Incubation of porcine corpora lutea with PRL did not influence P

synthesis (Cook et al., 1967). The most definitive indication that PRL may have a part in porcine luteal function comes from the demonstration of specific receptors for PRL in the corpora lutea of non-pregnant and pregnant pigs (Rolland et al., 1976). Corpora hemorrhagica and albicantia demonstrated little specific binding, whereas corpora lutea from early to mid-pregnancy demonstrated a near 5 fold increase in specific binding above corpora lutea of the cycle. Binding site concentration increased with gestational age. Combined with the earlier observations of Rolland and Hammond (1975), it appears that around the time of ovulation, when luteinization of granulosa cells commences, PRL binding is minimal. As corpora lutea develop, PRL receptor sites become more numerous. If pregnancy occurs, a substantial increase in binding capacity ensues which increases during gestation, at least up to about day 46 of pregnancy.

The significance of these observations remains speculative, as yet. As results of hypophysectomy suggested, some pituitary support may well be required for optimum luteal integrity beyond mid-cycle.

Estrogens: Luteolytic or Luteotrophic?

Exogenous estrogens are generally considered to be luteolytic in sheep and cattle; an effect which requires the presence of the uterus (Brunner et al., 1969; Bolt and Hawk, 1972, 1975). Plasma estradiol appears to rise, in both species, just before luteal regression and has been implicated in the uterine synthesis and release of the presumptive luteolysin, prostaglandin F_2a (PGF_{2o}) (Hansel et al., 1973; Ford et al., 1973, 1975; Barcikowski et al., 1974; Cox et al., 1974). Although

the major luteolytic effect of estradiol requires the uterus, a direct action on the CL cannot be excluded. In hysterectomized heifers, exogenous estradiol resulted in a small decrease in CL weight and P content (Brunner et al., 1969; Gengenbach et al., 1977); when combined with PGF_{2o} treatment, luteolysis was more complete than with either estradiol or PGF_{2o} alone (Gengenbach et al., 1977). Bovine luteal tissue contain specific estrogen receptors and in vivo treatment with small amounts of estradiol-17β(E_2-17β) eliminated the stimulatory effects of LH on P secretion in vitro (Hansel et al., 1973) without significantly affecting plasma P concentration. Similarly, Williams et al. (1977) demonstrated E_2-17β inhibition of LH stimulated P synthesis by dispersed bovine luteal cells. The mechanism(s) of this estrogen-induced luteolysis remains elusive.

In the ewe, estradiol may exert a luteotrophic effect when administered during the early luteal phase (Stormshak et al., 1969; Hawk and Bolt, 1970). Presumably, this relates to the ability of estrogen to induce LH release when plasma P levels are low. Later, in mid-cycle, when P concentrations are elevated, estradiol does not usually evoke LH release (Howland et al., 1971) and estrogen's retrogressive influence predominates (Hansel et al., 1973; Cummings, 1975).

In apparent contrast to sheep and cattle, exogenous estrogens elicit a luteotrophic response in pigs. This was first demonstrated by Kidder and coworkers (1955) who observed a significant lengthening of the interestrus interval, by 6 days, when gilts were injected with diethylstilbestrol (DES) on day 11. The same treatment to gilts on day 6 was without effect. Nishikawa and Waide (1958; as reviewed by Denamur, 1968)

reported that corpora lutea persisted for 53 to 111 days from ovulation. Daily injections of stilbestrol were begun during the luteal phase (days 5 - 9) and continued for 7 to 10 days. The requirements for CL maintenance by exogenous estrogen treatment in the pig were summarized by Denamur (1968) as follows: 1) injection of an effective minimum dose: 5 mg for estradiol (Gardner et al., 1963; du Mesnil du Buisson, 1966), 10 mg for estrone, 1 mg for stilbestrol (Gardner et al., 1963), 8 mg for ethynylestradiol (Wagner and Veenhuisen, 1965); 2) estrogen administration during a precise period of the cycle, starting no later than day 11 (Kidder et al., 1955; Gardner et al., 1963; Wagner and Veenhuisen, 1965; du Mesnil du Buisson, 1966) and lasting at least 5 to 7 days (du Mesnil du Buisson, 1966).

The mechanism(s) by which estrogens exert their luteotrophic effect is unknown. Based upon examination of ovaries from gilts sacrificed 4 days after DES treatment on day 11, Kidder et al. (1955), assumed the extended cycle length was due to estrogen-effected LH release resulting in ovulation or luteinization of follicles. However, as in sheep and cattle, Foote et al. (1958) could find no evidence that estradiol caused LH release in pigs when plasma P levels were high; as in the mid- to late luteal phase or during exogenous P treatment. These observations were extended by several investigators. Multiple injections of E_2-17β maintained CL P concentration and content, inhibited ovarian follicular growth (Garbers and First, 1969; Chakraborty et al., 1972) and increased pituitary FSH and LH levels (Garbers and First, 1969). No difference was noted in pituitary PRL content, but results were inconclusive (Garbers and First, 1969).

Estradiol was not luteotrophic in hypophysectomized sows (Denamur, 1968) nor in pituitary stalk-sectioned gilts (Anderson et al., 1967). Combined LH and estradiol treatment, beginning on day 12 to hypophysectomized sows, maintained fully active corpora lutea as assessed on day 20 (Denamur, 1968). LH, alone, was ineffective.

Theories of Estrogen Involvement in Maternal Recognition of Pregnancy

Cow, sheep and pig embryos undergo a prolonged free-living stage in the uterine lumen before attachment. The earliest observed signs of morphological interaction between trophoblast and endometrium varies from about 13 to 15 days in the sow and ewe (Crombie, 1970; Boshier, 1969) up to 5 weeks after fertilization in the cow (Wimsatt, 1975). Consequently, the signal for initiating luteal maintenance and establishment of the CL of pregnancy likely occurs while the embryo is essentially free in the uterus. In these domestic species the production of an anti-luteolysin and/or luteotrophin by the embryo is essential to neutralize the uterine luteolysin, and 'rescue' the CL from regression.

Trophoblast cells from many species have the capacity to synthesize a variety of compounds, including steroids and glycoproteins (Cook and Hunter, 1978; Heap et al., 1979). Although, the influence of the embryo on CL maintenance has received considerable attention, the exact nature of its affect has yet to be clarified. Regarding cattle and sheep, evidence to date does not tend to favour embryonic estrogens as luteotrophic or antiluteolytic factors in early pregnancy (Gadsby et al., 1976; Carnegie and Robertson, 1978; Cook and Hunter, 1978).

The remainder of this section will deal primarily with swine and

evidence implicating estrogen involvement in maternal recognition of pregnancy, thereby establishing the corpora lutea of pregnancy.

Preimplantation pig embryos have been shown to be particularly active in the biosynthesis of estrone and E_2-17β by day 12 (Perry et al., 1973, 1976) - early enough to be involved in arresting luteal regression. Embryonic estrogens may be sulphated in the endometrium (Perry et al., 1976) and appear in the peripheral plasma of the pregnant sow, primarily as estrone sulphate, which can be detected there by about day 16 (Robertson and King, 1974; Robertson et al., 1978); reaching a peak between days 23 and 30. Probable target tissues for this estrogen, ie. hypothalamus, pituitary and CL, all have active sulphatases which could regenerate free steroid (Perry et al., 1976; Cook and Hunter, 1978).

Luteotrophic Effect. Perry and co-workers (1976) have suggested that rescue of luteal function in the pregnant pig is mediated by embryonic estrogens transported in the sulphated form to the CL. There, they are hydrolyzed to exert a luteotrophic effect which could augment that of LH secreted by the pituitary. In a previous section, evidence was presented that exogenous estrogens are luteotrophic when administered during this time to the non-pregnant gilt; an effect which required the pituitary intact.

Goldenberg et al. (1972) did observe a synergistic action of estrogens and HCG in stimulating P synthesis by cultured porcine granulosa cells. As well, in one experiment reported by Cook et al. (1968), estradiol gave a dose-related increase in P synthesis by luteal slices

obtained on day 10 of the cycle. In general, however, these latter investigators did not observe estradiol enhancement of P biosynthesis in incubation systems with corpora lutea from earlier in the cycle. Pig granulosa cells in culture specifically concentrated labelled estrogens and in particular, accumulated unconjugated estrone (Norris and Kohler, 1974). Two points may be noteworthy here. Firstly, Gardner et al. (1963) noted that daily injections of estrone (days 11-33) to the cycling gilt, maintained significantly larger corpora lutea than did E_2-17β, although no difference in P concentration was observed. Secondly, Pack and Brooks (1974) described a variation in uterine conversion of E_2-17β to estrone sulphate during the pig estrous cycle; a maximum (ca. 80%) was reached in the mid-luteal phase, then declined steadily. These observations were extended by Perry and co-workers (1976), who reported that the level of conversion attained in the mid-luteal phase did not decline in early pregnancy. As suggested by Perry et al. (1976), these last observations may relate to an anti-luteolytic effect of estrogens.

Anti-luteolytic Effect. The luteolytic action of $PGF_{2\alpha}$, administered during the late luteal phase, has been demonstrated in the cycling pig (Connor, 1976; Halford et al., 1974) and increasing amounts have been detected in the uterine veins just prior to luteal regression in the non-pregnant sow (Gleeson et al., 1974). Lesser quantities may be secreted from the pregnant uterus on days 13 to 17 (Moeljono et al., 1976), but are either insufficient to cause luteolysis or its effects are neutralized by an embryonic signal. In this regard, Bazer and Thatcher (1977) have proposed an anti-luteolytic action of estrogens via redirection of

uterine PGF in the pregnant sow. Inhibition or redirection of uterine PG production was suggested from observations of reduced concentrations of PGF in the utero-ovarian vein of pregnant, compared to nonpregnant, animals after day 12 post coitus, and the suppression of uterine PGF release into the utero-ovarian vein by estradiol valerate (EV) administration (Frank et al., 1977). In a subsequent study, they found that EV treated gilts had significantly elevated PGF concentrations in the uterine lumen. As a result, it was proposed that in pregnant, and non-pregnant estrogen-treated pigs, estrogen effects a redirection of $PGF_{2\alpha}$ secretion away from the utero-ovarian vein, and thus away from the ovary, into the uterine lumen.

As well, these latter researchers noted that EV treatment, days 11 through 15, resulted in a significant increase in peripheral estrone concentrations. This coincides with the observations that the conversion of estradiol to estrone by the pig uterus does not decline in the early pregnant animal (Perry et al., 1976) as it does in the cycling animal (Pack and Brooks, 1974). Therefore, estrogens, either exogenous or of embryonic origin, may exert an anti-luteolytic effect by redirection of uterine PGF flow. Suppression of the luteolytic mechanism may also be accomplished by maintenance of low uterine tissue concentrations of E_2-17β. This last possibility assumes that in the non-pregnant animal, increased secretion of estradiol from rapidly growing follicles normally enhances synthesis and release of uterine PGF; these events being preceded in the pregnant animal by trophoblastic steroidogenesis and maintained uterine enzymatic activities (Perry et al., 1976).

Altered Sensitivity of the CL. Evidence presented earlier in this section suggested that estrogens may also have a direct action on the CL. Cultured porcine granulosa cells concentrated estrogens (Norris and Kohler, 1974) and synthesized increased quantities of P under the influence of estrogens and HCG (Goldenberg et al., 1972). Estrogen receptors have been demonstrated in porcine corpora lutea (Cook and Hunter, 1978) and luteal slices from day 10 cycling gilts responded to estradiol by increased P secretion (Cook et al., 1968). Whereas, these observations may imply a direct luteotrophic effect, a slightly different possibility may be inferred from the work of Kraeling et al. (1975). They were investigating the susceptibility of porcine corpora lutea to the luteolytic effects of $PGF_{2\alpha}$. In 3 of 4 hysterectomized gilts, daily injections of 10 mg estradiol benzoate (EB) (days 20 to 25) prevented 5 mg $PGF_{2\alpha}$ (day 24) from initiating luteolysis. Corn oil or 5 mg EB were ineffective. This may suggest that the EB, either directly or indirectly, reduced the sensitivity of the corpora lutea to PGF.

This idea is not supported by the recent report that, in vitro, estradiol did not prevent a $PGF_{2\alpha}$-induced decline in P production by superfused porcine luteal tissue of early pregnancy (days 18-22) (Watson and Maule Walker, 1978); nor did it enhance P synthesis. On the other hand, LH enhanced P production and was antagonistic to luteolytic effects of $PGF_{2\alpha}$. It is not known whether a higher concentration of estradiol in the superfusion medium, or a longer exposure period could have resulted in observations more compatible with the in vivo findings of Kraeling et al. (1975). The in vitro observations do not, necessarily, preclude an effect of estrogens on luteal tissue in vivo,

nor an effect which normally would occur earlier than day 18-22.

Altered Gonadotrophin Secretion. The luteotrophic potential of LH is well accepted. It has the demonstrated ability to stimulate P synthesis in vitro (Cook et al., 1967; Watson and Maule Walker, 1978); to act synergistically with estrogens to increase P production by granulosa cells (Goldenberg et al., 1972) and to maintain corpora lutea in hypophysectomized sows (Denamur, 1968). Therefore, several investigators have ascribed part of the estrogen luteotrophic effect to elicitation of LH release by a positive feedback of estrogen on the hypothalamo-pituitary system (Perry et al., 1976; Cook and Hunter, 1978). A report of raised plasma LH levels during early pregnancy in the sow (Guthrie et al., 1972) is generally cited to support this theory. However, mean LH levels were higher than the non-pregnant controls from day 0 onward. No increase was obvious from day 12 until collections stopped on day 24. This would suggest that if pregnancy affected LH levels it was at the time of conception, not around days 10-12, when blastocyst estrogen synthesis begins (Perry et al., 1976). Also, Tillson et al. (1970) found the complete reverse; lower LH levels in pregnant sows. Consequently, it remains uncertain what effect, if any, embryonic estrogens may have on maternal plasma LH concentrations.

Generally, elevated P concentrations, as would be present around days 10 to 12, are considered antagonistic to estrogen induced LH release (Foote et al., 1958; Howland et al., 1971). The increased pituitary LH content noted concommitant with estrogen induced luteal maintenance in gilts (Garbers and First, 1969) may suggest suppression

rather than stimulation of pituitary LH release. A requirement for LH during early pregnancy was indicated by CL regression following LH antiserum administration to pregnant gilts (Spies et al., 1967). Whether estrogen stimulates LH release, acts synergistically or facilitatively with LH, remains to be elucidated.

In other species, high circulating estrogens are often associated with stimulation of pituitary PRL release (Neill, 1974; Ensor, 1978). At present, no evidence is available to show a similar effect of estrogens in the pig.

Effect on Luteal Tissue Hormone Receptors. One mechanism of estrogen action in the pregnant pig, which has received virtually no attention, is a possible interaction between estrogen, PRL and/or LH. The significance of specific PRL binding sites in porcine corpora lutea and the increase with gestational age (Rolland et al., 1976) can only be postulated.

In the rat, PRL is considered the hormone that transforms the CL of the cycle into the CL of pregnancy (Day et al., 1979). Part of this requirement appears to involve induction and/or maintenance of LH receptors (Grinwich et al., 1976; Day et al., 1979) and, possibly, maintenance of luteal cytosol estrogen receptors (Gibori et al., 1979; Keyes et al., 1979). PRL receptor may be induced by LH, PRL and/or estrogen (Richards, 1978; Waters et al., 1978; Ensor, 1978). This depends upon the hormonal environment the tissue is, and has been, exposed to, and the type of tissue.

Essentially, in the rat, LH, PRL and estrogen can be depicted as acting synergistically to preserve luteal structure and steroidogenic

competency. The mode of action of these hormones has not been delineated.

In the pig, the pituitary is necessary for prolonged CL maintenance (Denamur, 1968). LH is required during early pregnancy, at least (Spies et al., 1967). The increase in PRL binding sites with gestational age suggests a requisite for this hormone, as well. There are several hypothetical ways by which embryonic estrogens may effect these requirements: a) by direct induction of luteal tissue PRL receptors; b) by increasing pituitary release of PRL (Neill, 1974), which, in turn, induces its own receptor; c) by facilitating PRL-induced PRL receptor and/or LH receptor, since, chronic estrogen treatment was shown to increase the capacity of rat ovarian tissue to bind gonadotrophin (Lee and Ryan, 1975); d) by facilitating LH induction of PRL receptor, as it does in rat granulosa cells (Richards, 1978). PRL may also be important in maintenance of luteal cytosol estrogen receptor, as it is in the pregnant or pseudopregnant rat (Gibori et al., 1979).

Evidence strongly suggests that embryonic estrogens are associated with maternal recognition of pregnancy and extension of corpora lutea lifespan in swine. The mechanisms are unknown, but, may involve one or more of the theories presented. Undoubtedly, there is a delicate balance between luteolytic and luteotrophic mechanisms requiring the interaction of several endocrine factors acting in concert.

GENERAL MATERIALS AND METHODS

Hormone Radioimmunoassays

The radioimmunoassays (RIA) routinely employed in this laboratory have been previously described in detail (Connor, 1976). Following is a summary of these RIA including performance parameters pertinent to the determination of serum hormone concentrations in the present experiments. As well, a more detailed description of the prolactin (PRL) RIA is presented. Prior to these studies, porcine serum PRL had not been evaluated in this laboratory.

Estrogens. The RIA procedure used for serum estrogens (E) is basically that of Yu et al. (1974) without column chromatography. The specificity of the estradiol-17β (E_2-17β) antiserum (#029-14), obtained from B.V. Caldwell, Yale University, has been described by Wu and Lundy (1971). The E with the greatest cross-reactivity were estrone (63.7%), estriol (18.7%) and estradiol-17α (5.1%). The RIA employed had a sensitivity of 12.5 pg.

Assays were set-up to ensure that all serum samples taken from a single gilt during one cycle or treatment were assayed together. Determinations were done in duplicate. Pooled samples of gilt serum were included in each assay. For experiment I, the inter- and intra-assay coefficients of variation were 9.9% and < 10% respectively (n=12 assays). The four estrogen assays performed on samples from experiments II and III had an inter-assay coefficient of 12.9% and intra-assay coefficients of variation of < 8%. The mean percentage recovery of added ^{3}H-estradiol-17β (estradiol-17β-[6,7-^{3}H(N)]; New England Nuclear, Boston,

Mass.) was 68.8 ± 4.5% (n=12 assays) for experiment I and 70.3 ± 3.3% for the four assays of experiments II and III. Results were corrected for procedural losses.

Progesterone. Serum progesterone (P) was evaluated by the method of Abraham et al. (1971) as modified by Yuthasastrakosol (1975). The antiserum (GDN 337) was kindly supplied by G.D. Niswender, Colorado State University. It was raised in sheep in response to immunization with 11β - hydroxyprogesterone-BSA. The specificity of this antiserum was reported by Gibori et al. (1977). When reactivity of P was taken as 100%, the cross-reactivity was 139% with 11β-hydroxyprogesterone, 4.4% with 5α-pregnane-3, 20-dione and < 3% with other steroids. The sensitivity of this assay was 50 pg per assay tube.

Serum samples taken from an individual gilt during one cycle or treatment were assayed together. Samples were assayed in duplicate. Multiple estimations of P concentration in pooled samples of gilt serum were used to determine coefficients of variation. For experiment I, the inter- and intra-assay coefficients of variation, over 13 assays, were < 6% and < 4%, respectively. For experiments II and III the inter-assay coefficient of variation was 13.8% (n=5) and the intra-assay coefficients of variation were < 14%. Added ^{3}H-P (progesterone-[1,2-^{3}H (N)]; New England Nuclear, Boston, Mass.) was recovered by a mean percentage of 88.3 ± 5.8% (n=13 assays) for experiment I and 82.7 ± 3.8% over 5 assays of experiment II and III samples. Results were corrected for procedural losses.

The standards used in both E and P assays were from Mann Research Laboratories, Orangeburg, N.Y. and used without further chromatographic purification.

Luteinizing Hormone. The double-antibody RIA used for measuring serum luteinizing hormone (LH) was basically that of Niswender et al. (1969) as modified by Howland (1972). Determinations were performed in duplicate and expressed in terms of a porcine LH standard (LER 778-4) generously supplied by L.E. Reichert. Jr., Emory University Atlanta, Georgia. Purified oLH (LER-1056-CR for experiment I; NIAMDD-NIH-LH-21 for experiments II and III) was labeled with ^{125}I (as NaI from New England Nuclear, Boston, Mass.) in accordance with the procedures of Niswender et al. (1969). Anti-oLH serum (GDN #15) was supplied by G.D. Niswender, Colorado State University. The minimum detectable LH concentration, defined as 95% of initial binding, ranged from 0.05 to 0.06 ng /ml (n=4 assays). For statistical purposes, samples containing less than this concentration were assigned the minimum detectable level. By using samples of pooled gilt serum in each of the four assays the inter-assay and intra-assay coefficients of variation were determined to be 10.3% and $< 6\%$ respectively.

Prolactin. The double-antibody RIA for porcine prolactin (pPRL) followed the same basic procedure as that employed for serum LH. Both the purified pPRL (CH-I RR-2-75) and the anti-pPRL serum (RAS-pPRL B-4) were generously supplied by K.W. Cheng, University of Manitoba, Winnipeg Manitoba. The antiserum was used at a final dilution of 1:2000 and was able to bind 25 to 35% of the radio-labeled pPRL in the abscence of added hormone. Aliquots of 2.5 µg pPRL in 5 µl of distilled water were labeled with ^{125}I (as NaI, New England Nuclear, Boston, Mass.) by the

method of Thorell and Johansson (1971) employing lactoperoxidase and hydrogen peroxide.

The assays conditions were as follows. Each tube contained 100 ul ^{125}pPRL (8000 cpm) in 1% EW-PO_4-Azide buffer, 200 µl anti-pPRL in 0.5% RS-PO_4-EDTA buffer (pH 7.6), 400-450 µl of 1% EW-PO_4-Azide buffer (diluent) plus sample or pPRL standard to make a total primary reaction volume of 800 µl. Determinations were done in duplicate. After 5 days incubation at 4°C the primary reaction was terminated by adding 200 µl of goat antiserum to rabbit gamma globulin (Antibodies Incorp., Davis California) at a dilution of 3 parts antiserum to 7 parts PO_4Azide buffer. Tubes were incubated a further 24 h at 4°C during which time a white precipitate formed. Following this, tubes were vortexed, 1 ml of PO_4-Azide buffer was added and each tube was centrifuged (2000 RPM) for 25 minutes. The supernatant was decanted and both supernatant (free radioactivity) and precipitate (bound radioactivity) were counted. A standard curve was constructed from the results of assays tubes containing 0.2 to 16 ng of the purified pPRL (CH-I RR-2-75). The standard points were assayed in triplicate.

Prior to evaluation of pPRL in serum samples, inhibition curves were established. Figure 1 depicts the RIA inhibition curves plotted as % bound ($\%B_o$) on a logit scale and concentration on a log scale. The decrease in $\%B_o$ caused by increasing amounts of purified pPRL standard paralleled that of gilt serum. Similarly, increasing volumes of cycling gilt serum and lactating sow serum also showed parallellism. Addition of large amounts (20, 50 or 100 ng/tube) of FSH (NIH-FSH-P_2) or pLH (LER778-4) did not decrease the $\%B_o$.

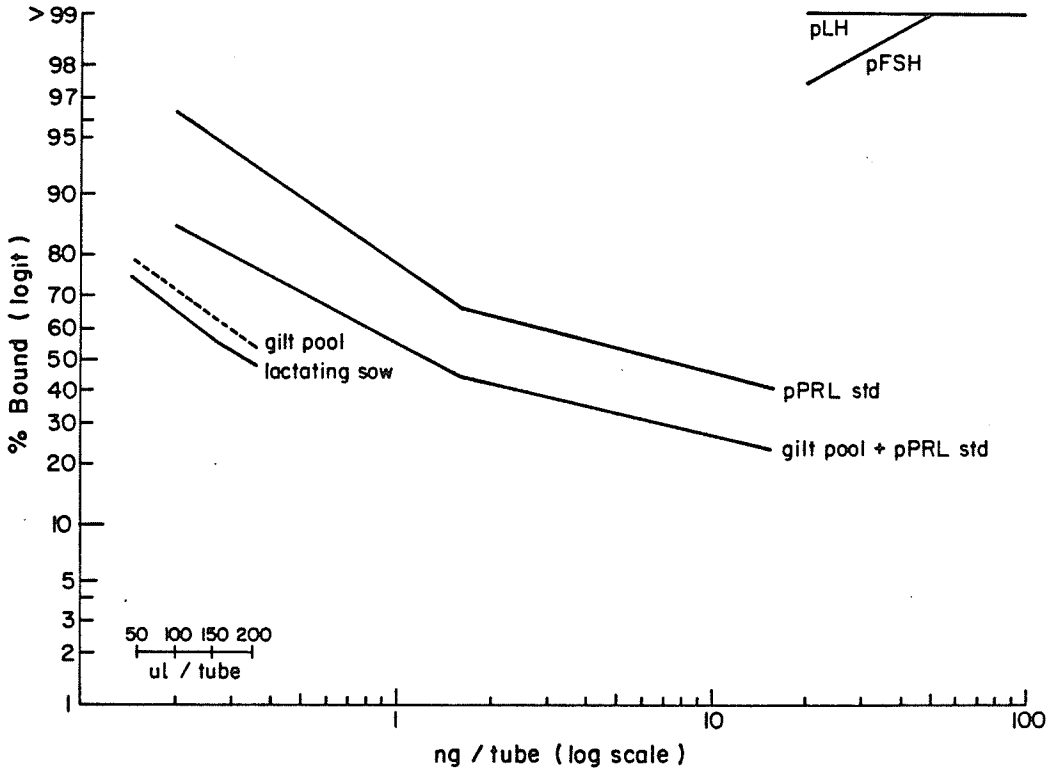

Figure 1. Inhibition curves obtained with serial dilutions of pPRL standard, gilt serum (pool), lactating sow serum, pLH and pFSH.

The sensitivity of the standard curve was such that the minimum detectable PRL concentration, defined as 95% of initial binding, ranged from 0.15 to 0.18 ng/tube. The inter- and intra-assay coefficients of variation were < 10% and < 8% (n=5 assays), respectively.

Statistical Procedures.

Hormone concentrations following treatments were analyzed by analysis of variance. Student-Newman-Keuls' test was used to detect differences between means. Comparisons of cycle lengths (experiment I), hormone concentrations at ovariectomy (experiments II and III), differences in ovarian characteristics (experiments II and III) and differences in specific binding of hormone (experiments II and III) were tested by Student's t-test. These were done in accordance with procedures prescribed by Steel and Torrie (1960).

EXPERIMENT I

Effect of Single or Multiple Injections of Estradiol-17β on the Interestrus Interval and Hormone Levels in the Cycling Gilt

The ability of exogenous estrogens to extend the interestrus interval in pigs has been known for over 20 years. Kidder et al., (1955) demonstrated that a single injection of DES (3 mg, im) on day 11 (day 1 = 1st day of estrus) significantly lengthened the estrous cycle of gilts by 6 days. Multiple injections started during the luteal phase, reportedly extended corpora lutea lifespan to between 53 and 111 days (Nishikawa & Waide, 1958, as reported by Denamur, 1968). Denamur (1968) summarized the requirements for estrogen-effected maintenance of pig corpora lutea as daily injection of a minimum effective dose (which varied from 1 mg for DES to 10 mg for estrone) for 5-7 days starting no later than day 11. Single injections of estrogens (Kidder et al., 1955) as well as daily injections started as late as day 14 of the cycle (Garbers and First, 1969) have been reported as being luteotrophic.

The sudden onset of estrogen synthesis by pig blastocysts between days 10 to 12 post mating has been suggested as an embryonic signal to prolong luteal function. Presumably, exogenous estrogens may act similarly. However, the mechanism by which estrogen exerts its luteotrophic effect is unknown. Stimulation of LH release has been suggested (Kidder et al., 1955), but the observed antagonism of prolonged elevated serum P on estrogen-induced LH release (Foote et al., 1958) challenges this idea. Other observations that multiple estradiol-17β (E_2-17β)

injections started during the luteal phase maintained CL P concentration, inhibited ovarian follicular growth and increased pituitary FSH and LH levels (Garbers and First 1969; Chakraborty et al., 1972) were interpreted by one group (Garbers and First, 1969) that estrogen blocked LH release, and by the other group (Chakraborty et al., 1972) to suggest estrogen stimulated LH release.

The pituitary is necessary for elaboration of the estrogen luteotrophic response (Anderson et al., 1967; Denamur et al., 1968). LH is required for maintenance of corpora lutea of early pregnancy (Spies et al., 1967). In vitro, LH can stimulate P synthesis by luteal tissue from gilts at mid-cycle (days 8-10) or early pregnancy (Cook et al., 1967) but requires simultaneous estrogen injections to prolong corpora lutea function in hypophysectomized sows (Denamur, 1968). PRL, the other pituitary hormone often associated with maintenance of luteal activity in sheep (Kann and Denamur, 1974) and rats (Nalbandov, 1973) did not affect porcine luteal P production in vitro (Cook et al., 1967). Rolland et al., (1976) demonstrated specific binding of oPRL by porcine luteal tissue which increased in early pregnancy, but any relevance to CL function is unknown. Estrogens have a well known stimulatory effect on PRL secretion in many species (Neill, 1974; Macleod, 1976), but, Garbers and First (1969) could detect no significant change in pituitary PRL content in gilts treated with E_2-17β . Material was limited, however, and results were variable. None of these experiments measured blood levels of hormones during estrogen treatment.

At the time this experiment was conceptualized, few reports were available concerning how long the interestrus interval could be extended

by exogenous estradiol treatment; nor, to our knowledge, had anyone examined the serum hormone profiles during or after estrogen administration. As well, no reports of daily serum PRL concentrations during the porcine estrous cycle were available.

Therefore, this experiment was designed to study the effects of mid-cycle single or multiple injections of E_2-17β on estrous cycle length and serum concentrations of estrogens (E), P, LH and PRL in the gilt. In addition, levels of serum PRL during the normal estrous cycle were of interest.

Observations, treatments and blood collections were conducted from May to December, 1977.

Materials and Methods

Experimental Animals

Crossbred gilts 6 to 8 months of age were housed in group pens and checked twice daily for estrus at 0830 h and 1700 h. The first day of estrus, determined by response to back pressure, was designated day 0 of the cycle. Only gilts which exhibited two consecutive estrous cycles of normal length were used for this study. During the course of the experiment gilts were penned individually in metal-framed crates (0.6m x 1.8 m), were exercised daily and observed for estrus. Each gilt was fed approximately 2 kg per day of a balanced ration (ca. 13% Crude Protein) and had access to water at all times.

Experimental Treatments

Treatment 1 (T 1). Each of the four gilts used served as their own con-

trol for one estrous cycle prior to the treatment cycle. At 0800 h on day 10 of the treatment cycle each gilt was injected intramuscularly (im), with 10 mg of E_2-17β (β-estradiol, Sigma Chemical Co., St. Louis, Mo.) in 2 ml peanut oil. A blood sample was taken immediately prior to the E_2-17β injection and subsequently every hour for 14 h. Otherwise, blood was collected daily at 0900 h. The same blood sampling regime was followed throughout the previous control cycle. Vehicle only (2 ml peanut oil) was injected im at 0800 h on day 10 of the control cycle. Collection of blood samples was discontinued at the first estrus following E_2 - 17β administration.

Treatment 2 (T2). The four gilts used for T2 also went through one control estrous cycle prior to the treatment cycle. Each animal was injected, im, with 10 mg E_2-17β in 2 ml peanut oil daily at 0800 h for five days starting on day 10 of the treatment cycle. Blood was collected just prior to each injection then hourly for 14 h. Daily samples were taken prior to day 10 and after day 14 until the next estrus. Collection of blood throughout the control cycle followed the same frequency. Vehicle only (2 ml peanut oil) was injected according to the same schedule as E_2 during the control cycle.

Blood Collection and Handling.

One to three days before the beginning of the control cycle an indwelling jugular cannula (silastic; 1.50 mm I.D., 4.50 mm O.D.) was surgically implanted into each animal. The only exception was gilt #978

(TI) which had a catheter implanted about 6 weeks prior to this experiment. In each case, the cannula was externalized on the center back at the shoulders and secured in place. Sterile saline containing 6 IU of sodium heparin per ml was placed in each cannula between sample collections.

Each sample (15 to 30 ml) was drawn into 15 ml vacutainers. Samples were stored at 4°C, then centrifuged within 24 h, at 3000 RPM, for 20 minutes. The serum was removed and aliquots were stored in screw cap vials at -20°C until assayed for P, E, LH and PRL.

Hematocrit readings were done regularly, particularly during the hourly blood collections on day 10 (T1) and days 10 through 14 (T2).

Results and Discussion

T1. The daily mean hormone concentrations throughout the T1 control cycle are presented in Fig. 2. The profiles of serum P, E and LH are similar to those described previously (Connor, 1976, et al., 1976; Henricks et al., 1972; Tillson et al., 1970).

P levels rose rapidly within 2 days of estrus (day 0) reaching 24.0 $\pm$ 5.5 ng/ml (mean $\pm$ S.E.) by day 6. Subsequent to a peak of 31.2 $\pm$ 6.8 ng/ml on day 12, P declined rapidly to basal levels by 3 to 4 days before the subsequent estrus, at which time P concentrations were generally $<$ 1 ng/ml.

After estrus, mean E levels remained between 18.5 and 35.0 pg/ml during the luteal phase. In general, serum E began to rise after peripheral P levels had begun to drop. Maximum mean E concentration of 80.4 $\pm$ 20.7 occurred on day -1.

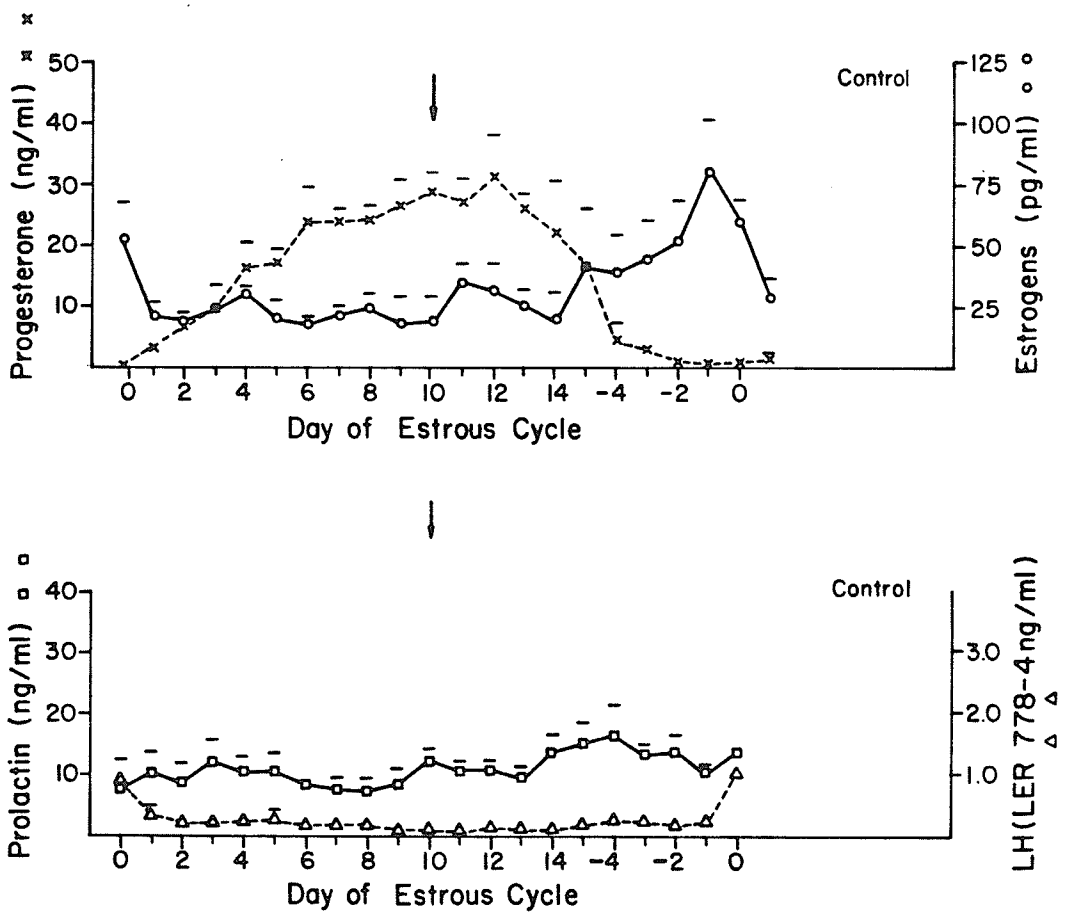

Figure 2. Mean (±SE) serum concentrations of progesterone, estrogens, PRL and LH in 4 gilts before and after peanut oil, im, (↓) on day 10 of the control estrous cycle. Day 0 = first day of estrus.

Basal concentrations of LH during the luteal phase were generally below 0.6 ng/ml in all gilts. The lowest levels observed were on days 9 to 14, followed by a sustained increase after P had begun to fall. As expected, the highest LH level in each animal was observed at estrus. Values in these animals did not exceed 1.6 ng/ml, which is within the reported ranges of 0.6-4.6 ng/ml (Henricks et al., 1972) and 0.4-6.3 ng/ml (Connor et al., 1976). The once daily blood collection schedule, in the present study, could account for failure to detect higher concentrations at estrus.

Serum levels of PRL fluctuated slightly. During most of the luteal phase of the cycle mean PRL concentrations were between 7.4 $\pm$ 2.5 ng/ml and 12.5 $\pm$ 1.8 ng/ml. Individual PRL values were generally between 4 and 16 ng/ml during this time. These are in agreement with the recent findings of Dusza and Krzymowska (1979) that basal PRL levels in 4 sows oscillated between 3 and 20 ng/ml. Earlier, Brinkley et al., (1972) sampling only every second day, reported that the average PRL concentration, excluding the day of estrus, was 14 ng/ml; at estrus, levels were highest at 36 ng/ml. A sharp elevation of PRL at estrus was also observed in several sows by Dusza and Krzymowska (1979). They collected blood four times between 0700 h and 1900 h each day. In the animals in this study, no estrus surge was noted. Either PRL did not increase in these gilts at estrus or the elevation was missed since only one daily blood sample was taken.

In the present study, the lowest levels of PRL were on days 6 through 9. An increasing trend appeared from day 13 reaching 16.6 $\pm$ 4.6 ng/ml on day -4. In 2 of the gilts, an increase in PRL to 20 and 30

ng/ml occurred 5 or 6 days before estrus. In the other two animals no elevation was obvious. The highest PRL levels observed by Dusza and Krzymowska (1979) were 4 days before onset of estrus and was attributed to plasma E presumably rising at that time. A similar PRL surge, attributed to E stimulation, occurs in sheep just before estrus (Kann and Denamur, 1974). From our data no obvious relationship with other hormone patterns is apparent. An increasing trend from day 14 on may be associated with the declining P levels, but the increase in PRL preceded the rise in blood levels of E.

The daily hormone levels throughout the treatment cycle of the same four gilts are depicted in Fig. 3. Treatment with 10 mg E_2-17β (im) on day 10 significantly extended ($P < .01$) the interestrus interval from 20.00 $\pm$ .41 days (control) to 26.25 $\pm$ 0.63 days (E_2 - treated). Prior to day 10, the pattern and concentrations of P, PRL and LH were similar to the corresponding control period, although in two animals, E tended to be higher from day 0 than in the control cycle. On day 8, serum from one animal had an unexpected E concentration of 151 pg/ml which accounts for the day 8 spike seen in Fig. 3. Pre-injection values on day 10 were similar to those on days 1 through 7.

After E_2-17β injections, the hormone profiles of P, LH and PRL resemble those of the control cycle; the main difference being the time period over which the relative changes occur.

Mean serum E was 192.8 $\pm$ 91.9 pg/ml by day 11, declined rapidly over the next 2 days but remained elevated during days 14 to 17 in three of four gilts. By day 18, levels (44.5 $\pm$ 10.9) had dropped and approached the preinjection mean value. This was followed immediately by a

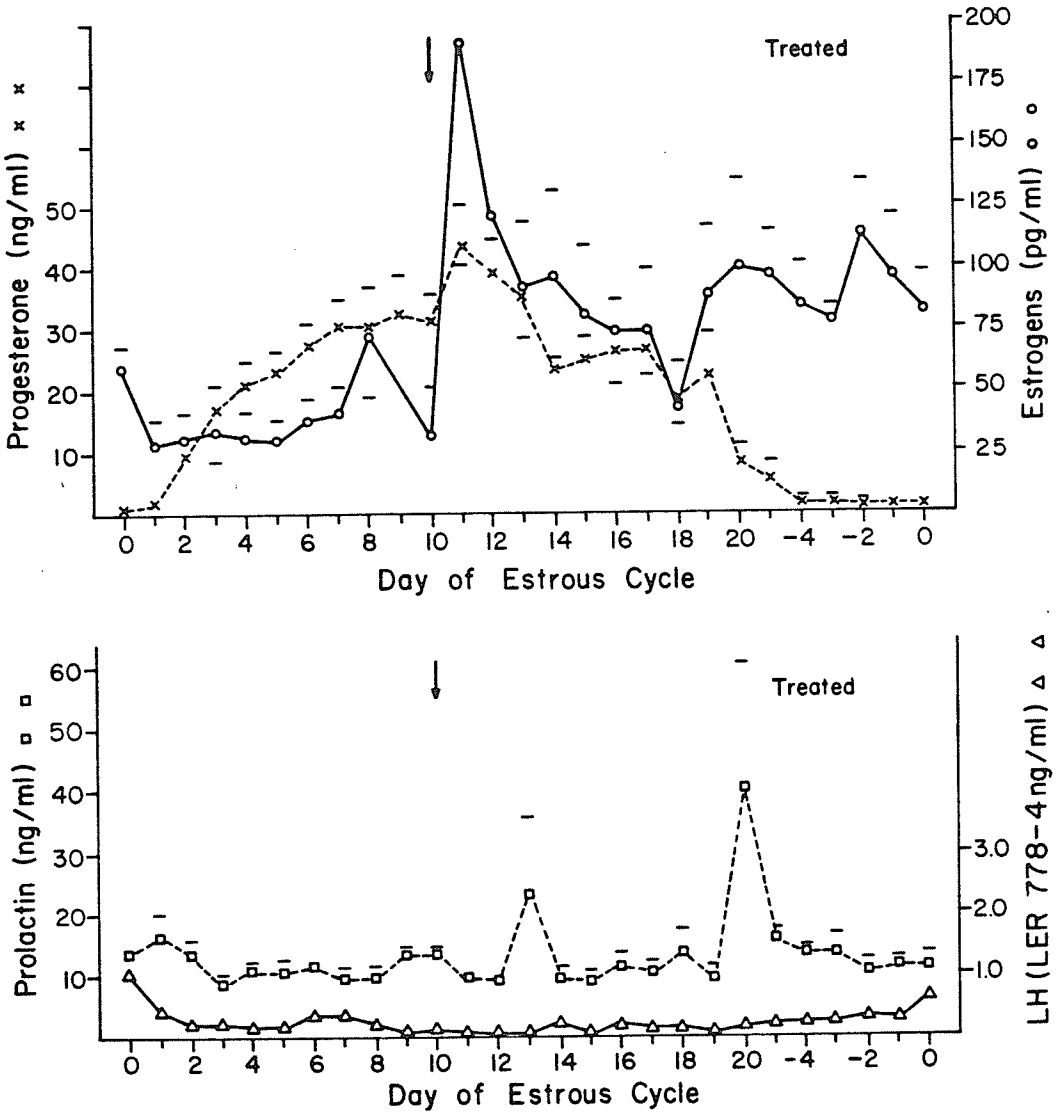

Figure 3. Mean (±SE) serum concentrations of progesterone, estrogens, PRL and LH in 4 gilts before and after 10 mg E_2-17β, im, (↓) on day 10 of the estrous cycle. Day 0 = first day of estrus.

sustained, though variable, increase of E in all animals, concurrent with rapidly declining P levels. Presumably, from about day 18 onward we were measuring predominantly endogenous estrogens secreted by maturing follicles.

Serum P reached a peak of 44.0 $\pm$ 6.7 ng/ml on day 11 afterwhich it dropped over the next two days. It then plateaued between 23.4 $\pm$ 2.4 and 26.6 $\pm$ 3.7 ng/ml during days 14 to 17, before the rapid descent observed during days 19 through -4 which is indicative of luteal regression. The more gradual initial decline of peripheral P, than seen in the control cycle, suggests sustained luteal activity. In pregnant gilts, P levels are not maintained at peak levels but decline from maximal levels until about day 28, then very gradually decline throughout gestation (Robertson & King, 1974).

On a daily basis, LH concentrations appear relatively unaffected by E_2 treatment. The levels were lowest (days 11-13) when P was maximal. After day 19, LH increased gradually to estrus.

Similarly, in three of the four gilts, PRL did not change following E_2 injection. For one gilt, a surge to 60 ng/ml occurred on day 13. E levels in this animal were low on both day 12 (42.0 pg) and day 13 (45.9 pg). The peak mean value seen on day 20 is, again, attributable to a surge in one animal, which was not associated with a corresponding change in the other hormones measured. The other three gilts had PRL concentrations between 10-18 ng. As in the control cycle, there was a trend towards increasing PRL 5-6 days before estrus.

The changes in individual hormone concentrations on day 10 in control and treatment cycles are presented in Figs. 4 and 5. In the control

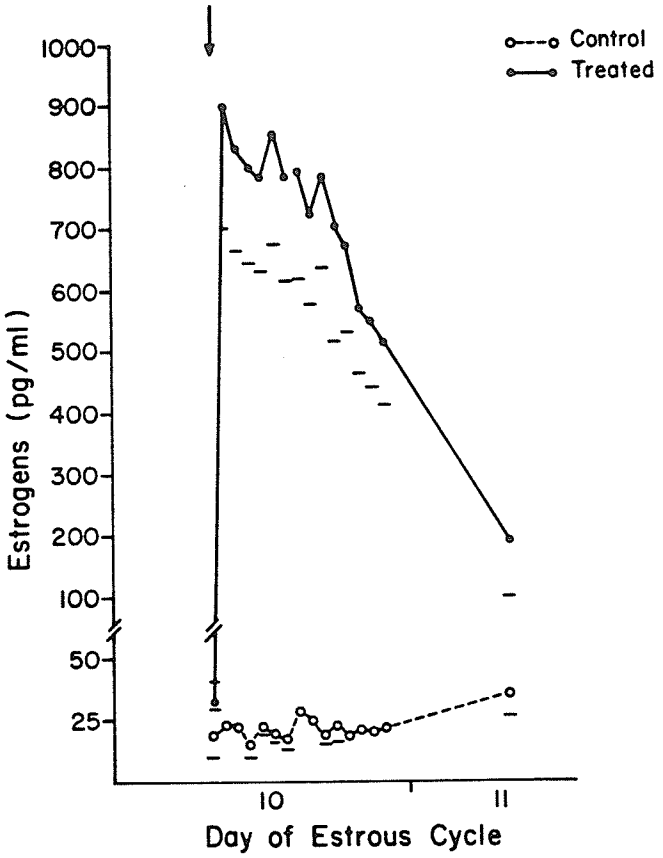

Figure 4. Mean (±SE) serum concentrations of estrogens in 4 gilts before and after peanut oil (Control) or E_2-17β (Treated) on day 10 10 of the estrous cycle. Blood samples were collected immediately before im injection (↓) at 0800 h, then hourly for 14 h.

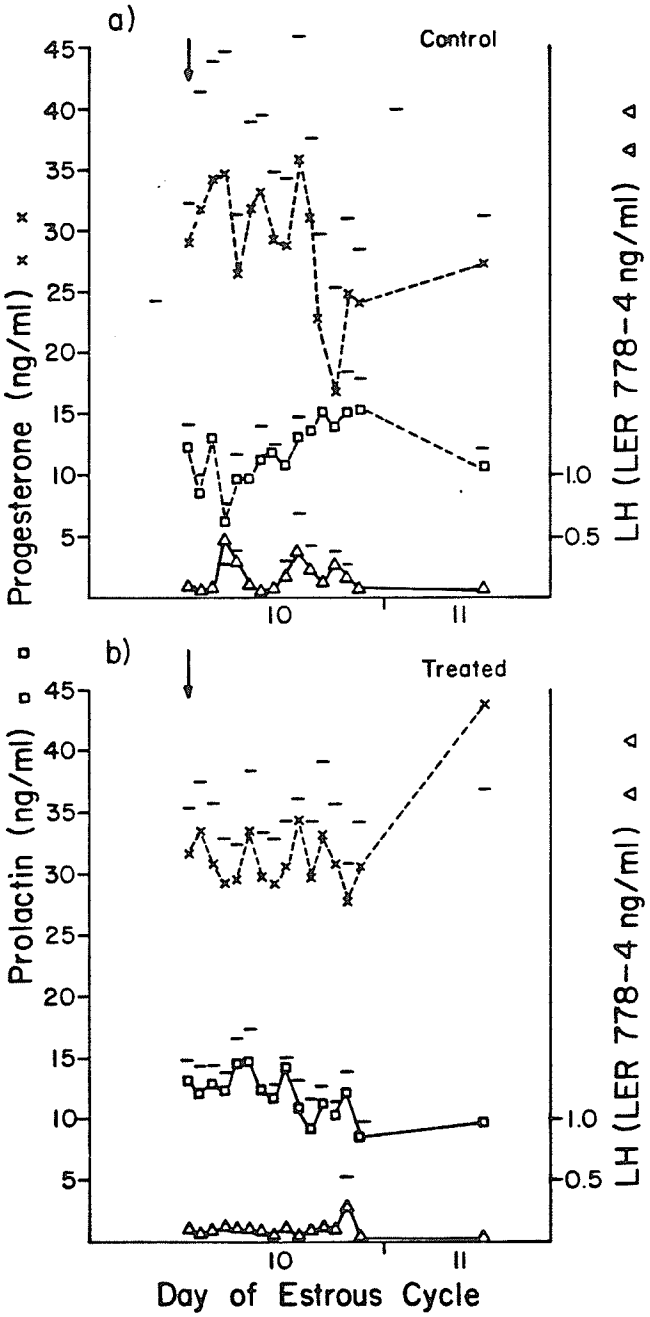

Figure 5. Mean (±SE) serum concentrations of progesterone, PRL and LH before and after peanut oil (Control) or E_2-17β (Treated) on day 10 of the estrous cycle. Blood samples were collected immediately before im injection (↓) at 0800 h, then hourly for 14 h.

period, serum E fluctuated little from the pre-injection mean of 19.4 ± 9.0 pg/ml (Fig. 4). Within an hour of E_2-17β injection, serum E had increased ($P < .01$) from 33.2 ± 8.0 pg/ml to 902 ± 204.8 pg/ml. A gradual decline occurred over the next 14 h. By 0800 h on day 11, E was still elevated at 192.8 ± 91.9 pg/ml. There were no significant changes in blood P, PRL or LH concentrations in the control or treatment cycle on day 10 (Fig. 5). Although LH concentrations did not differ significantly from pre-injection means, the random fluctuations seen in LH levels in the control cycle were not as obvious following E_2 treatment in the treatment cycle. This trend became more apparent in the T2 gilts given multiple E_2 injections (Fig. 10.)

T2. Gilts in the T2 group exhibited a control estrous cycle length of 21.04 ± .41 days. Hormone patterns throughout the control cycle (Fig. 6) were much like those in T1 controls (Fig. 2). The maximum P concentration of 35.5 ± 5.3 ng/ml was attained on day 13. Estrogens generally remained constant between 11 to 25 pg/ml from day 1 through 15, throughout the luteal phase. Peak estrogen values of 56.3 ± 10.6 and 94.6 ± 20.6 were reached at the first and second estrus, respectively. These coincided with LH concentrations of 1.28 ± 0.66 ng/ml and 0.53 ± 0.29 ng/ml. As in T1 controls, the lowest levels of LH were observed during mid-cycle (days 10-13).

All four gilts in the T2 control group exhibited an elevation in PRL levels 3 to 5 days before estrus, which lasted for 1 or 2 days. Until day 16 (day -5) PRL fluctuated between 8-17 ng/ml and was within this range again by day -2. However, no surge was noted at estrus.

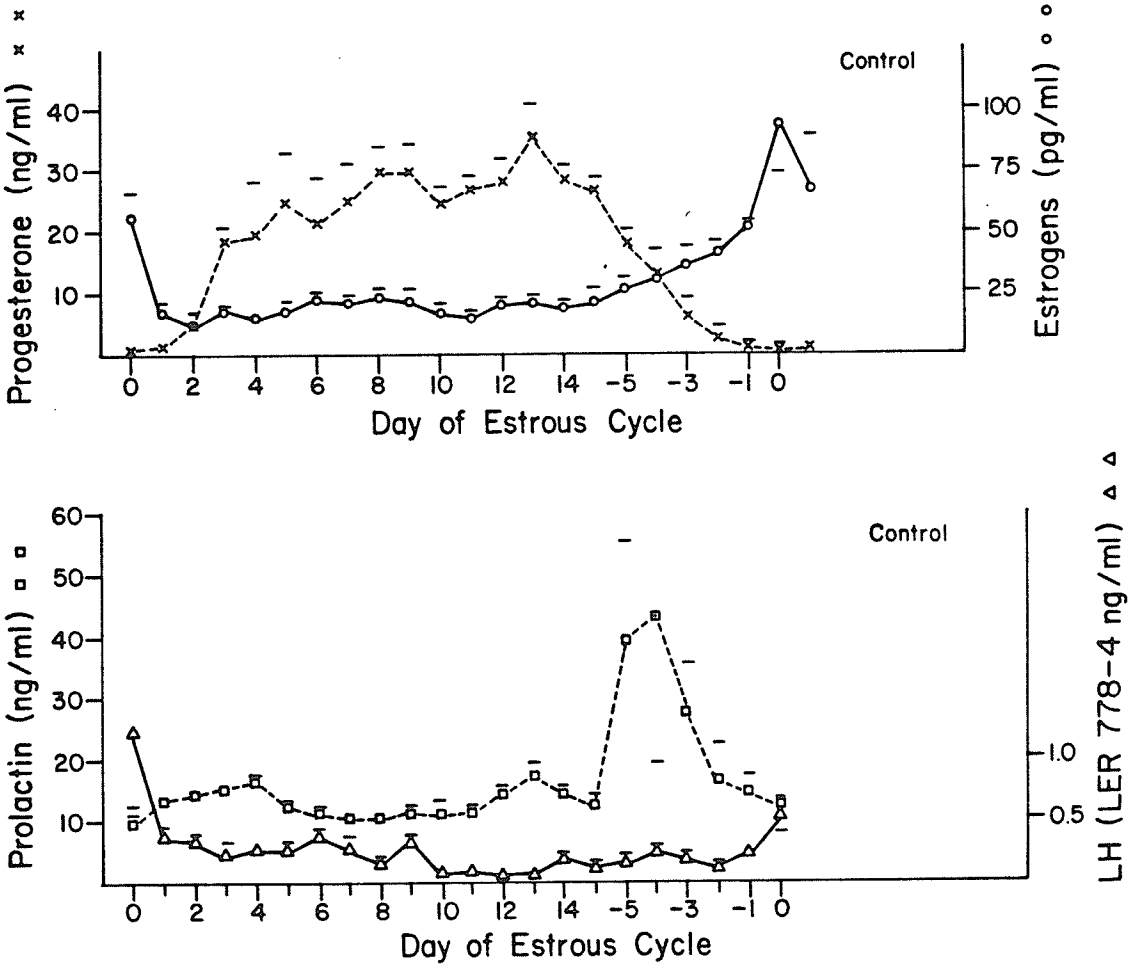

Figure 6. Mean (±SE) serum concentrations of progesterone, estrogens, PRL and LH in 4 gilts before and after peanut oil, im, on day 10 through 14 of the control estrous cycle. Day 0 = first day of estrus.

Treatment with 10 mg E_2-17β on days 10 through 14 extended the cycle length to 28.75 ± 1.11 days ($P < .01$) (Fig. 7). The four individual cycle lengths were 28, 26, 30, and 31 days. However, this extension was not significantly longer than that observed following a single E_2-17β injection on day 10. With the exception of PRL, the hormone profiles prior to day 10 in the treatment cycle (Fig. 7) were similar to the same period in the control cycle. High PRL levels of 60 ng and 75 ng in each of two gilts, one on day 2 and one on day 3, are responsible for the elevated mean PRL levels seen on these days. These were not associated with observable differences in P, LH, or E, and occurred 2 and 3 days after the estrus E peak in these animals. After day 3, the PRL concentrations were similar to the control cycle.

In three of four gilts, E levels before day 10 were higher than the control cycle, but the concentration was not unusually high.

The main effect of E_2-17β treatment appeared to be a prolongation of the mid- to late luteal-phase pattern of hormone secretion (Fig. 7). The daily serum P concentrations and profiles on days 10 through 14 are similar to the control cycle and were not altered by E_2-17β administration. Subsequent to a peak on day 13 (32.5 ± 2.8 ng/ml) the decline to basal levels was more gradual than in the control period, taking 6 days to fall below 5 ng/ml in the control cycle and 12 days in the E_2 - treatment cycle.

The daily PRL profile, days 10 through 14, is not unlike the control cycle during the same period. These levels were sustained until about day 21. In all gilts, an elevation in PRL occurred 4 to 7 days before the return to estrus and lasted 2 to 3 days in three gilts and 1 day in

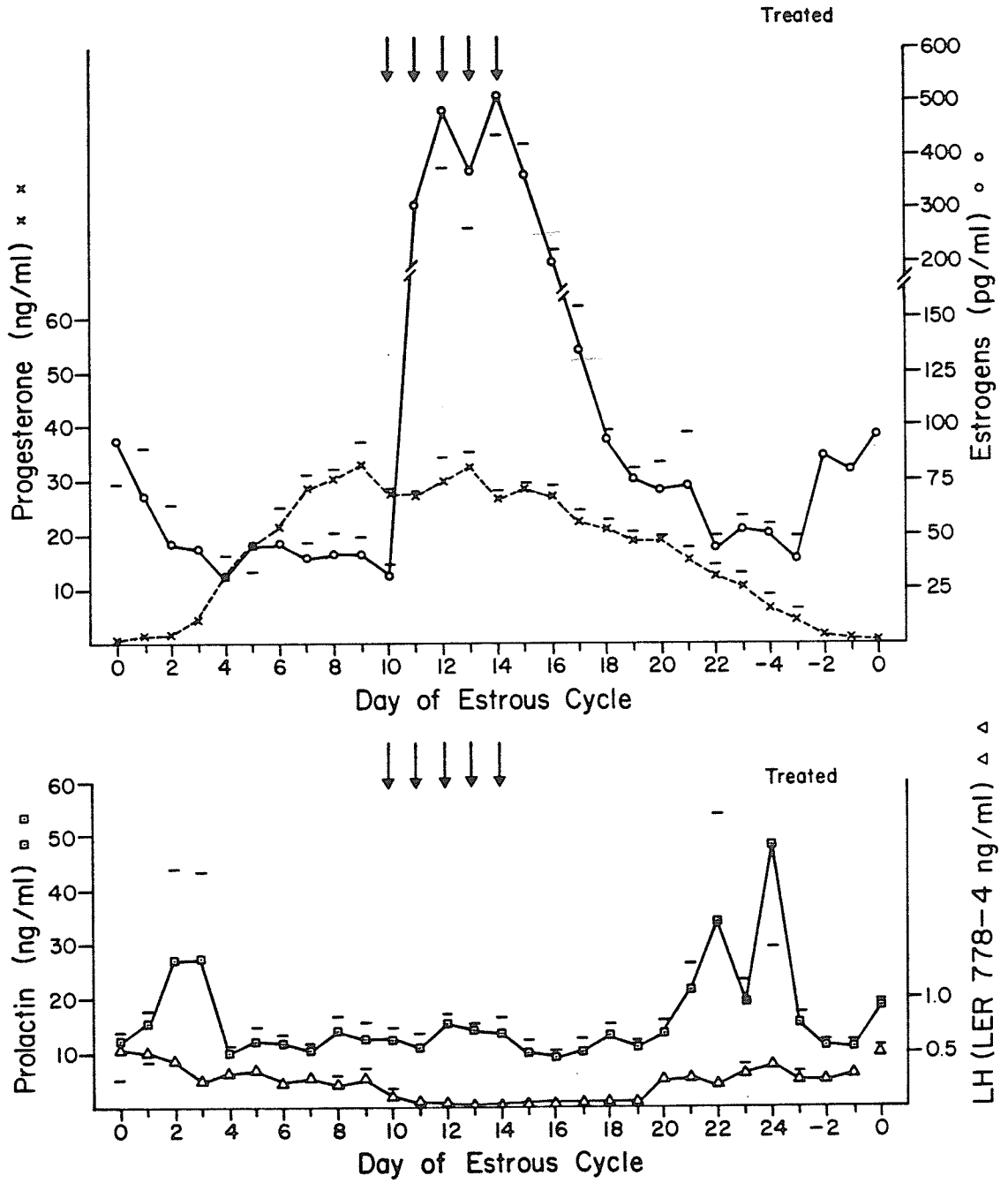

Figure 7. Mean (±SE) serum concentrations of progesterone, estrogens, PRL and LH in 4 gilts before and after 10 mg E_2-17β, im, (↓) on days 10 through 14 of the estrous cycle. Day 0 = first day of estrus.

one animal. Peak values, ranging from 18 to 92 ng/ml, were 2 to 5 fold greater than concentrations on the day preceding the observed increasing trends. This did not appear to be associated with changes in E at that time but since this is based on only daily sampling, it is not conclusive. PRL was again at luteal phase concentrations by day -3. In two gilts, PRL concentration approximately doubled between day -1 (13.0 ng/ml and 10.7 ng/ml) and day 0 (27.2 ng/ml and 19.6 ng/ml).

Mean LH concentrations during E_2 treatment remained below 0.07 ng/ml and stayed below 0.08 ng/ml until day 20. The sustained elevation during the 8 days before estrus is similar to that seen during the 7 days preceding estrus in the control cycle.

E levels declined rapidly over the 5 days following the last E_2-17β injection on day 14. Levels then remained somewhat elevated before approaching the pre-injection mean on day 22 (44.3 $\pm$ 5.4 pg/ml). By this time the P concentration was 12.1 $\pm$ 3.9 ng/ml and falling.

Frank et al., (1977) extended the interestrus interval of gilts from 19 $\pm$ .6 days (control; mean $\pm$ SE; n=4) to 73.7 $\pm$ 24.2 days (treated; n=3) with daily injections (sc.) of 5 mg EV on days 11 through 15. Maximum E_2 levels of 210 pg/ml, observed on day 17, were followed by a decline to 40 pg/ml on day 25. Levels appeared to be < 100 pg/ml by day 20. These concentrations, from days 20 to 25, are not unlike the ones observed in this study (by day 25 (day -3) E was 39.9 $\pm$ 9.3 pg/ml). As was noted in the present experiment, Frank and coworkers (1977) also observed a fall in peripheral P from maximum values until day 19. However, in their gilts, P was maintained at 10 to 15 ng/ml (similar to pregnant animals) between days 20 to 25 when blood collection stopped.

The reasons for the discrepancy in extended cycle lengths observed here and those of Frank et al., (1977) is unknown. They started treatment a day later (day 11) than we did, but maximum P concentrations were reached a day later and the concentration on day 19 (16 pg/ml) was similar to that reported here (19.2 $\pm$ 1.8 ng/ml). Although EV injected sc. would effect a slower release and maintain lower, yet sustained, elevated E_2 concentrations, the peripheral E concentrations on days 20 to 25 appear similar between the two studies.

The hourly changes in serum E over the 15 collection periods on each of days 10 through 14 of the control and treatment cycles are shown in Fig. 8. As expected, each E_2-17β injection resulted in a significant ($P < .01$) increase in levels of E. Maximum concentrations, detected 1 h (days 10, 13, 14) to 4 h (day 11) post-E_2-17β injection, were generally followed by a rapid fall during the rest of the day. The highest level observed was 1380.3 $\pm$ 164.7 pg/ml on day 13. Pre-injection means from days 11 through 14 did not fall below 235 pg/ml in three of the four animals. In one gilt, E was consistently lower and pre-injection concentrations on days 11-13, were < 150 pg/ml. By day 15, E concentrations were similar in all gilts (361.8 $\pm$ 56.4 pg/ml).

Serum concentrations of P, PRL and LH during the frequent collection period, days 10 through 14, for the control and treatment cycles are depicted in Fig. 9 and Fig. 10 respectively.

In the control cycle (Fig. 9), each hormone fluctuated considerably but there were no differences ($P > .05$) over the 15 h collection periods. With the exception of the day 13 peak, P generally fluctuated between 19-

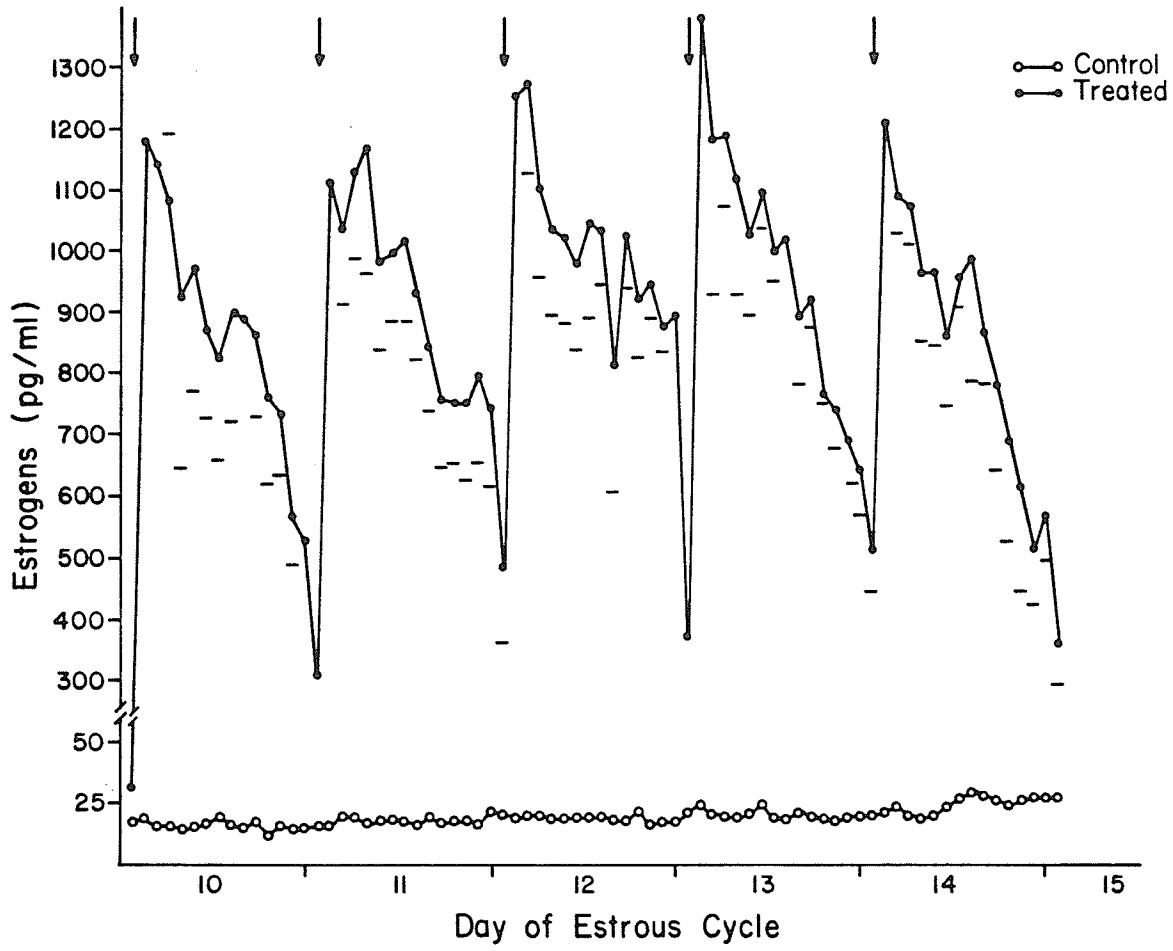

Figure 8. Mean (±SE) serum concentrations of estrogens in 4 gilts immediately before and for 14 h following peanut oil (Control) or E_2-17β (Treated), im, (↓) at 0800 h on days 10 through 14 of the estrous cycle.

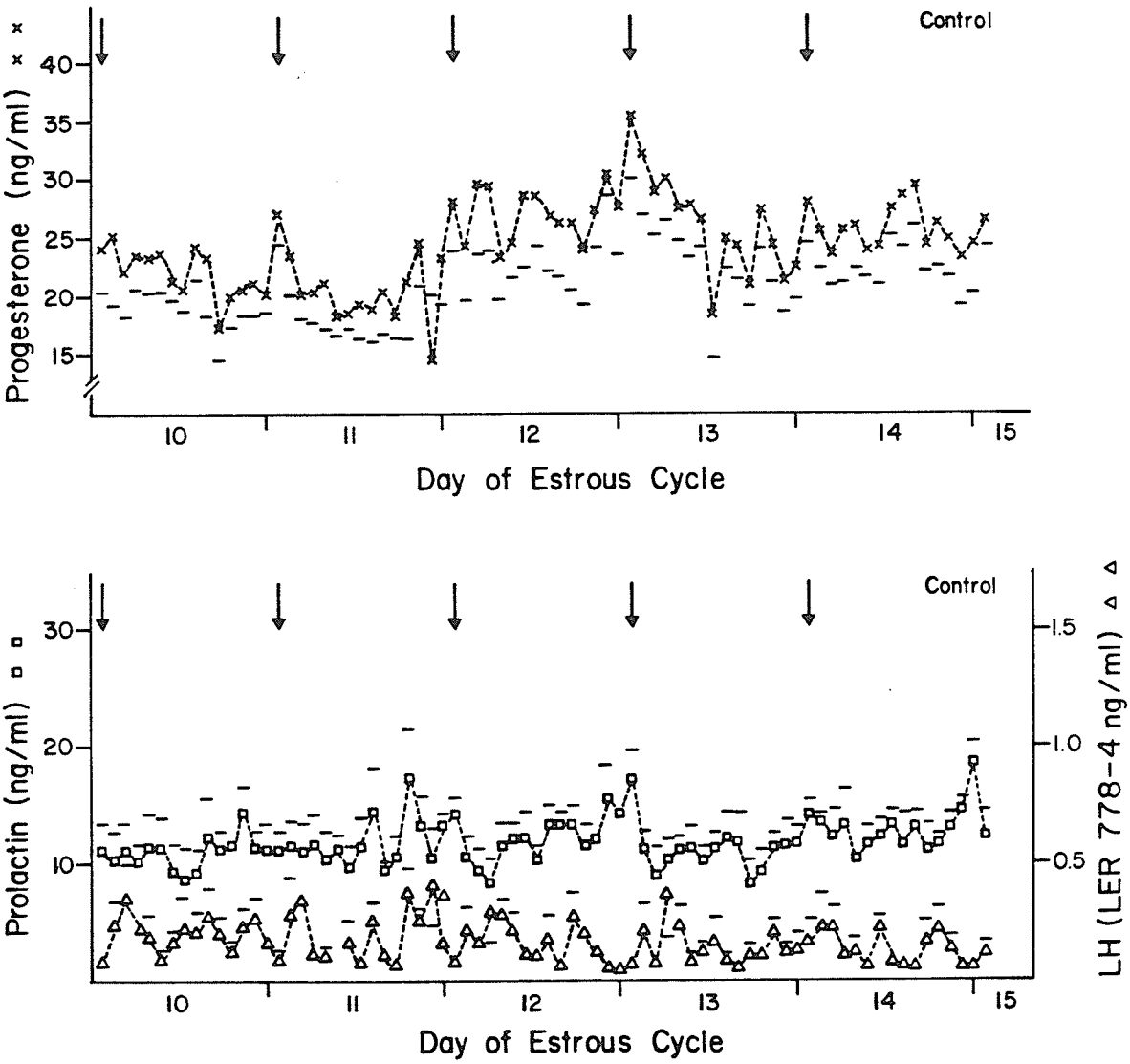

Figure 9. Mean (±SE) serum concentrations of progesterone, PRL and LH immediately before and for 14 h following peanut oil, im, (↓) at 0800 h on days 10 through 14 of the estrous cycle.

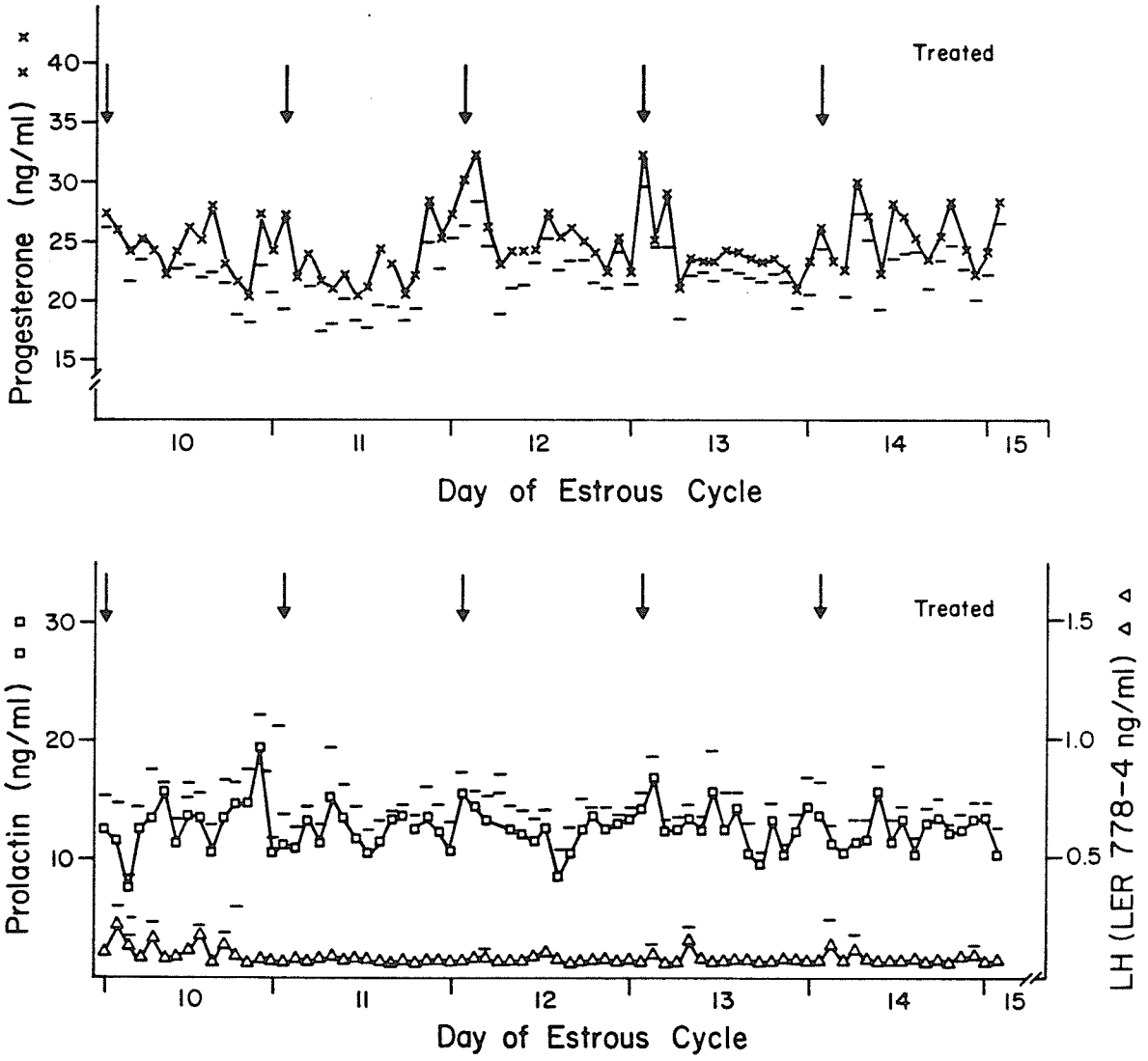

Figure 10. Mean (±SE) serum concentrations of progesterone, PRL and LH immediately before and for 14 h following E_2-17β, im, (↓) at 0800 h on days 10 through 14 of the estrous cycle.

29 ng/ml in the four gilts. Mean PRL levels remained between 8.4 $\pm$ 2.1 ng/ml and 18.8 $\pm$ 1.7 ng/ml. As well, LH oscillated considerably over the 5 day period between 0.06 ng/ml and 0.42 $\pm$ .18 ng/ml.

During the E_2-17β treatment period (Fig. 10) there were no signif-icant changes ($P > .05$) from pre-injection means in serum P or PRL con-centrations. However, there was a signicant difference ($P < .05$) in LH concentrations on day 10. With few exceptions, the mean LH levels were < 0.08 ng/ml from 1600 h on day 10 until the end of the frequent sampling period on day 14. As Fig. 7 reveals, LH remained at this basal level until day 20.

Therefore, the main effect of E_2-17β on the hormones measured, appeared to be a suppression of LH pulsatile secretion. These results coincide with the theory of Garbers and First (1969) that the increased pituitary LH content observed in E_2-17β treated gilts (days 14-24) was due to an estrogen block of LH release. As well, they confirm the earlier report of Foote et al. (1958) concerning the inability of exo-genous estrogen (20 mg E_2-17β) to stimulte LH release in the presence of sustained high blood P levels, as would be present during mid-cycle. Similar observations have been made in sheep (Bolt et al., 1971; Howland et al., 1971; Cummings, 1975) and cattle (Hobson and Hansel, 1972).

Circulating LH levels were at a nadir during mid-cycle when P levels were maximal. This is also noted in ewes (Hauger et al., 1975; Baird et al., 1975) and cows (Beck et al., 1976) in which the results of replace-ment therapy following ovariectomy suggest basal LH release is under joint repression by P and E (Hauger et al., 1975; Beck et al., 1976). Consequently, in the experiments reported here with gilts, exogenous E_2

may have further enhanced an existing endogenous E-P suppressive effect on tonic LH release.

The lack of an observed increase in serum PRL levels during E_2-17β treatment may also be related to the high luteal phase P levels. P has been suggested to inhibit the stimulatory action of E on PRL release in ovariectomized rats (Chen and Meites, 1970) and from rat pituitary cells in culture (Haug and Gautvik, 1976). As well, Garbers and First (1969) could detect no significant change in pituitary PRL content of gilts in which E_2-17β treatment was started during the late luteal phase (day 14).

Conclusions

As indicated by peripheral P concentrations, corpora lutea of gilts injected with E_2-17β were maintained beyond the normal lifespan. The interestrus interval was significantly lengthened ($P < .01$) by either a single injection of 10 mg E_2-17β, im, on day 10 (26.25 $\pm$ .63 vs. 20.00 $\pm$ .41 days) or 10 mg E_2-17β, im, on each of days 10 through 14 (28.75 $\pm$ 1.11 days vs. 21.04 $\pm$ .41 days). Five daily injections did not lengthen the estrous cycle significantly more than did a single injection. E_2-17β treatment caused prolonged luteal P production, initially depressed serum LH levels, but did not affect peripheral PRL concentrations.

Daily PRL levels in cyclic gilts remained fairly constant throughout the luteal phase. An increasing trend appeared 3 to 7 days before estrus. Levels were basal prior to the first day of estrus. A minor PRL surge at estrus was observed in only 2 of 4 gilts following E_2 treatment (T2).

EXPERIMENT II

Effect of Estradiol-17β on Hormone Levels, Ovarian Characteristics and Luteal Tissue Receptors for Prolactin and LH

Prolongation of the lifespan of porcine corpora lutea by exogenous estrogen administration requires an intact hypothalamo-pituitary system (Anderson et al., 1967; Denamur, 1968). Estrogen-stimulated hypophyseal luteotrophin release, as this might suggest, was not apparent following E_2-17β treatment in experiment I.

Part of the estrogen mechanism of action may involve a synergism with a pituitary luteotrophic substance(s). Hypophysectomized sows treated with a combination of LH and E_2 maintained fully active corpora lutea until at least day 20 (Denamur, 1968). Cytosol E receptors have been noted in porcine corpora lutea (Cook and Hunter, 1978). In vitro, E augmented HCG-stimulated P production (Goldenberg et al., 1972), and enhanced ^{125}I-LH binding (Nakano et al., 1977), to porcine granulosa cells. The observation of increasing luteal tissue receptor sites for PRL during early gestation in the pig (Rolland et al., 1976) suggests a dependency on this hormone as pregnancy advances. In the pregnant rat model, evidence strongly supports a luteotrophic synergism between LH, PRL and E (Gibori and Richards, 1978; Day et al., 1979; Keyes et al., 1979), part of which involves induction and maintenance of luteal receptors for these hormones. A co-operativity between these hormones in maintenance of porcine corpora lutea beyond the normal cyclic lifespan

may be possible. However, at present, the paucity of information concerning the factors regulating luteal function in swine prevents this idea from being little more than conjecture.

Experiment I demonstrated that E_2-17β was luteotrophic, as assessed by the interestrus interval and serum P concentrations. But, a possible mode of action remained elusive. The present experiment was designed to further investigate a possible mechanism of action of E at the ovarian level. Parameters of ovarian function evaluated included: follicular development; numbers and weights of corpora lutea; and luteal tissue receptor binding of LH and PRL. Serum hormone concentrations of E, P, LH and PRL, during and after treatment, were included to complement results of experiment I and to provide information on the hormonal environment at the time of tissue assessment.

This experiment was conducted from mid-April to mid-June, 1980.

MATERIALS AND METHODS

Experimental Animals

A group of 20 gilts consisting of 7 Managra, 7 York and 6 Managra x York, ranging in age from 186 to 199 days, were moved from the Glenlea Research Farm to the campus swine barn for this experiment. They were maintained in group pens and observed twice daily at 0830 h and 1700 h for signs of estrus. Only gilts exhibiting one estrous cycle of normal length were to be used. During the time of the experimental treatments, the animals were individually penned and fed similar to those in experi-

ment I.

Experimental Protocol

Treatments This experiment was designed to have four groups of animals, with four gilts per group. Groups I and II were controls, injected with 2 ml of peanut oil at 0830 h on days 10 through 14 of the cycle (first day of estrus = day 0). Groups III and IV were injected daily at 0830 h with 10 mg E_2-17β (Sigma β estradiol) im on days 10 through 14. Ovariectomies were performed on day 15 of the cycle in Groups I and III gilts and on day 20 in Groups II and IV gilts. Of the original 20 animals, only 14 exhibited one normal estrous cycle required before being assigned to a treatment group. One of these died under anesthetic while an ear vein catheter was being implanted. Consequently, Groups I, III and IV each consisted of only 3 animals while Group II had 4 animals.

Blood Collection and Handling An indwelling catheter (Argyle, Intramedicut gauge 16; length 70 cm, Sherwood Medical Industries, St Louis, Mo.) was implanted into the ear vein of each gilt on or before day 9 of the cycle. Anesthesia was maintained throughout the catheterizing procedure with a 2.5% solution of Surital (sodium thiamylal, Parke-Davis). Between blood collections, catheters were filled with sterile saline containing 6 IU of heparin per milliliter.

Starting on day 10, blood was drawn (30 ml) into 15-ml vacutainers immediately before the gilts were injected with oil or E_2-17β at 0830 h. Subsequently, blood was sampled (15 ml) at 6 and 12 h after injec-

tion. This frequency was maintained until day 15. From day 15 until ovariectomy on day 20, in Groups II and IV, blood samples were taken twice a day at 0830 h and 2030 h. Blood samples were taken at the time of ovariectomy. Samples were handled and stored in the same manner as experiment I.

Parameters Measured Periodic blood samples were used to establish serum hormone concentrations just before and during E treatment. Since experiment I had established the hourly hormone profile after E_2-17β treatment, the less frequent collection interval in this experiment was intended as a monitor to ensure that the E was having a similar effect in these animals. Serum collected at ovariectomy was intended to indicate the hormonal milieu which could be influencing the ovaries at that time. RIA were conducted as outlined under general materials and methods.

Of particular interest in this experiment was the effect that E_2-17β treatment would have on ovarian characteristics. The ovarian characteristics evaluated for each animal at ovariectomy included the weight of the ovaries, the number of medium (3-7 mm) and large (>7 mm) follicles, the number of corpora lutea, the weight of luteal tissue, and luteal tissue receptor binding of LH and PRL.

Collection and Handling of Ovarian Tissue

Ovariectomies were done with gilts under general anesthesia (Surital; sodium thiamylal, Parke-Davis). A blood sample was taken from the ear vein or ear vein catheter at the time of surgery. Upon removal, ovaries were immediately placed in iced saline (0.9% NaCL). Subsequent

evaluation was done in a cool room at 4°C. All buffers were at 4°C as well. Within 2 h of collection, ovaries were washed with 0.3 M sucrose-25 mM Tris-HCl buffer, pH 7.6, trimmed of connective tissue, patted dry and weighed. After weighing, ovaries were kept on cheesecloth soaked in the sucrose-Tris-HCl buffer while being examined for numbers of medium (3-7 mm) and large (> 7 mm) follicles and number of corpora lutea, or corpora albicantia, where no corpora lutea were obvious.

Tissue Receptor Preparation

As much of the preparation as possible was done at 4°C, otherwise, tissue was kept on ice at all times. Corpora lutea from the ovaries of each animal were decapsulated, dissected, weighed and placed in cold 0.3M sucrose-25mM Tris-HCl buffer, pH 7.6, at a ratio of 1:3 (wt:vol). Where corpora lutea were not visible (day 20, Group II gilts), corpora albicantia were treated in the same manner as corpora lutea. Homogenization was performed using a polytron PT 10 homogenizer (Brinkman Instruments, Inc, Westbury, N.Y.) at a maximum speed for 1 minute. The homogenate was centrifuged at 15,000 x g for 20 minutes at 4°C. The supernatant was filtered through one layer of cheesecloth then centrifuged again at 100,000 x g for 90 minutes at 4°C to obtain the total microsomal pellet which contains most of the broken cell membranes (Shiu et al. 1973). Following centrifugation, the supernatant was drained off and the pellets resuspended in ice-cold 25mM Tris-HCl buffer, pH 7.6, containing 10 mM $MgCl_2$, at a concentration of 1 ml buffer per gm of starting tissue. Each resuspended sample was divided into aliquots, in 10 x 75 mm glass

culture tubes, (Kimble Co Ltd.) for subsequent receptor and protein determinations. Tubes were covered with parafilm and stored at -20°C. Protein content, using the method of Lowry et al. (1951), was determined just before the receptor binding studies were conducted. This was within 6 months of collecting the tissue.

Hormone Preparations

Both the oLH (NIAMDD-NIH-LH-21) and oPRL (NIH-P-S13) used in the specific binding studies were supplied by the NIAMDD of the National Institutes of Health, Bethesda, Md.

Radioiodination The oLH (NIAMDD-NIH-LH-21) was labeled with ^{125}I (as NaI, New England Nuclear, Boston, Mass) using chloramine T, in accordance with the procedure of Niswender et al. (1969). The specific activity of the ^{125}I-oLH was 63 µCi/µg. The oPRL (NIH-P-S12) was radiodinated with ^{125}I by the lactoperoxidase method of Thorell and Johansson (1971). The resulting specific activity was 129 µCi/µg. The ^{125}I- oPRL was kindly supplied for this study, from the laboratory of H.G. Friesen, University of Manitoba, Winnipeg, Manitoba.

Assay Procedures

Specific binding studies were done with receptor preparations from each animal.

Specific Binding of ^{125}I-oLH. With the exception of the luteal receptor preparations from gilts in Group II, these studies were conducted with 0.51 $\pm$ 0.07 mg of protein per tube. The amount of protein used for

Group II was 0.12 ± .06 mg. The incubation mixture consisted of 200 µl 25mM Tris-HCl buffer, pH 7.6, containing 10 mM $MgCl_2$ and 0.1% BSA (incubation buffer), 100 µl receptor preparation (ca. 0.50 mg protein), 100 µl ^{125}I-oLH (50,000 cpm) in incubation buffer and 100 µl of incubation buffer containing 0 or 1 µg of unlabeled hormone, for a total volume of 500 µl. Samples were incubated in 10 x 75 mm glass culture tubes (Kimble Co. Ltd.) at room temperature for 16 h. The reaction was terminated with 3 ml of ice-cold incubation buffer. The bound (Bo) and free hormone were separated by centrifugation at 2000xg, for 30 min, at 4°C. The supernatant was aspirated with a pasteur pipette. The membrane-bound ^{125}I-oLH in the precipitate was determined by counting radioactivity in a Searle (model 1185) autogamma counter.

Binding of ^{125}I-oLH was determined in triplicate for each receptor preparation. Nonspecific binding (NSB), defined as the amount of radioactivity bound in the presence of a large excess (1 µg) of unlabeled hormone (oLH), was determined in duplicate for each sample. The percentage of specific binding %SB of ^{125}I-oLH to luteal tissue receptor preparations was calculated from the formula according to Cheng, (1975):

%SB = [cpm bound to the tissue in the absence of unlabeled hormone - cpm bound in the presence of a large excess (1 µg) unlabeled hormone] x 100 / total cpm put into the tube.

Specific Binding of ^{125}I-oPRL. Except for Group II samples, the ^{125}I-oPRL binding studies were conducted with 0.90 ± 0.24 mg of

protein per tube. The tubes for Group II animals contained 0.18 $\pm$ 0.12 mg protein. The assay procedure was the same as that for binding of ^{125}I-oLH except that the total volume of the incubation mixture was 400 µl. Thus, each tube in the oPRL binding assay contained 100 µl incubation buffer (25mM Tris-HCl, pH 7.6, containing 10 mM $MgCl_2$ and 0.1% BSA) 100 µl receptor preparation (1 mg protein), 100 µl ^{125}I-oPRL (50,000 cpm) in incubation buffer and 100 µl incubation buffer containing 0 or 1 µg oPRL. The incubation procedure, reaction termination, separation of Bo from free hormone, determination of Bo-radioactive hormone and the calculation of %SB were conducted according to the method outlined for oLH.

Results and Discussion

The blood collection schedule was not completed for all animals. Blood samples were taken from one gilt in Group I (G I), four gilts in Group II (G II) and three gilts in Group IV (G IV). Attempts to implant ear vein catheters in two gilts in G I were unsuccessful. Catheters in Group III (G III) gilts had either been removed by the animals or were non-functional within 2 days of the beginning of the treatment. Initial blood samples were collected from two G III gilts following E_2-17β injection and were analyzed to check the effectiveness of treatment on E levels. Response, in terms of elevated serum E, was similar in these gilts to those in G IV. Blood was collected from G II and G IV gilts at the scheduled time on most days; exceptions were one animal in G II and one in G IV in which catheters lost patency after day 16. Whether or not

catheters remained patent, gilts were treated according to their assigned group and ovariectomized on the designated day of the cycle.

Results accumulated from one of the four G II gilts was eliminated from averaging and statistical analysis. P levels were nondetectable throughout the experiment and E levels were erratic and at times unusually high (50 - 495 pg/ml).

The mean (±S.E.) pretreatment estrous cycle length of the thirteen gilts was 20.9 ± 0.25 days.

The hormone profiles from day 10 until ovariectomy on day 20 for control G II gilts are presented in Fig. 11. The pattern until day 15 may be considered representative of that expected in control G I gilts, since these two groups were treated similarly during this period. In general, the profiles in Fig. 11, appear typical for this stage of the cycle. The P concentrations in these gilts were lower than those in experiment I, but are comparable to values reported previously (Connor et al., 1976). A maximum of 15.9 ± 2.1 ng/ml on day 13 was followed by a precipitous drop to < 1 ng/ml by 2030 h on day 17. Concentrations of LH were generally < 0.5 ng/ml until day 19. As well, mean E levels were predominantly between 31.8 pg/ml and 64.9 pg/ml.

Except at 2030 h on day 14, PRL remained between 13.5 ± 3.6 ng/ml and 20.0 ± 4.3 ng/ml on days 10 through 19 of the cycle. The rise on day 14 is attributable to a surge to 72 ng/ml observed in one gilt. E levels in this gilt had risen from 29.2 pg/ml at 0830 h to 53.2 pg/ml by 2030 h. Concentrations of P were declining in this animal and were 2.8 ng/ml at the time of the PRL spike. In experiment I there was no obvious PRL

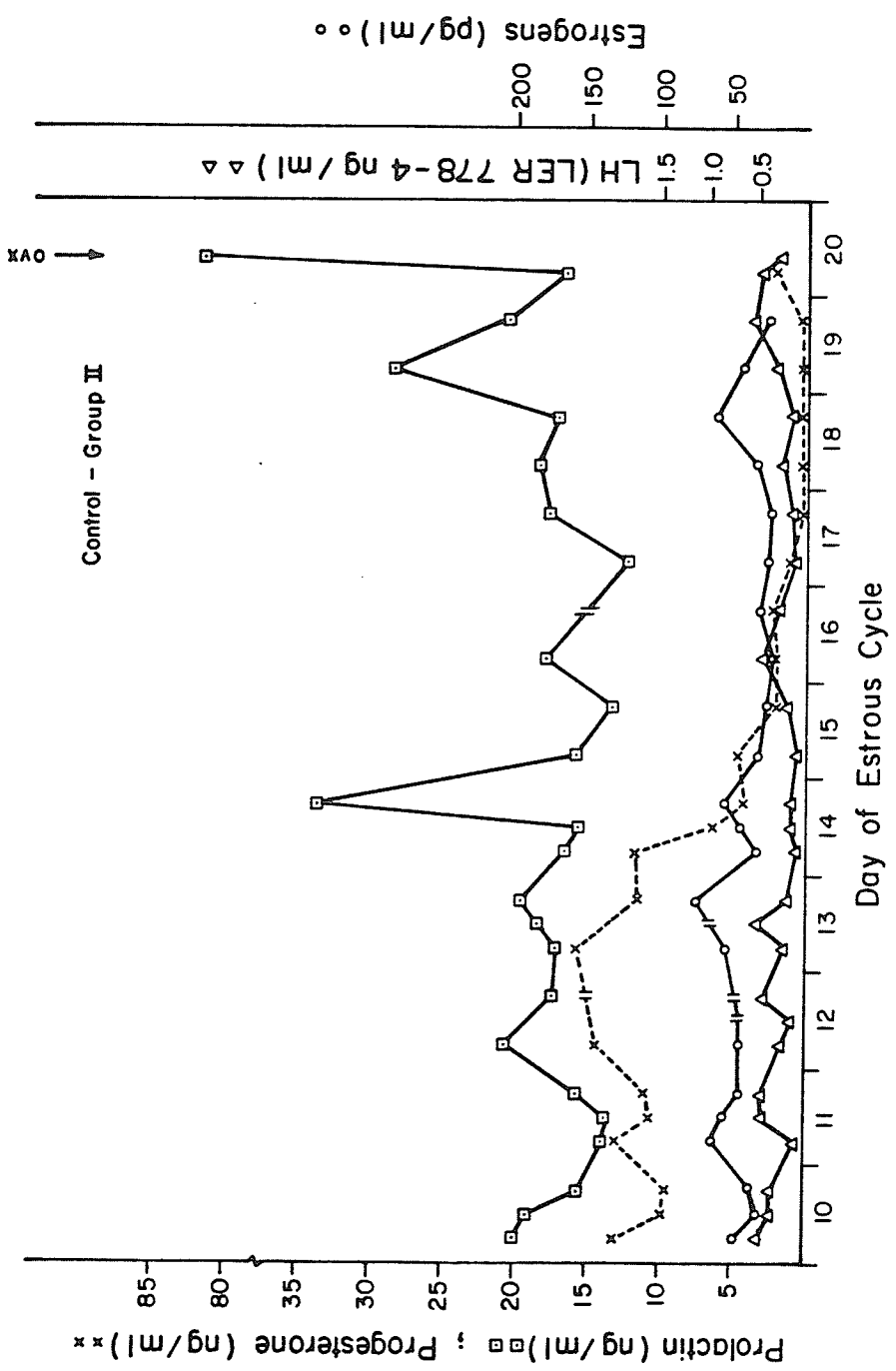

Figure 11. Mean serum concentrations of progesterone, PRL, estrogens and LH in gilts injected, im, with peanut oil on days 10 through 14 of the estrous cycle. Blood was collected immediately before injection at 0830 h then at 1430 h and 2030 h on days 10 through 14. From day 15 until ovariectomy (ovx) on day 20, samples were taken at 0830 h and 2030 h.

release in response to E_2 treatment during mid-cycle and less frequent sampling after day 14 precluded close appraisal of relative changes in PRL and E at proestrus. However, if high circulating P exerts an inhibitory effect on E-induced PRL release (Chen and Meites, 1970; Haug & Gautvik, 1976) then the declining P in this one G II animal may have allowed the rising E titer to stimulate PRL release.

Similarly, elevated PRL levels observed 1 to 3 days prior to ovariectomy would be 2 to 4 days before these gilts should have been in estrus. None of the G II gilts were in standing heat at 0800 h on day 20 but overt and behavioural signs indicated estrus could begin within the next 24 to 48 h. The surge to 81.5 $\pm$ 8.5 ng/ml at the time of ovariectomy is most likely a stress induced response (Ensor, 1978).

Mean hormone concentrations of E_2-17β-treated G IV gilts are depicted graphically in Fig. 12. From a pre-injection mean of 47.4 $\pm$ 15.5 pg/ml E remained above 224 $\pm$ 24.1 pg/ml until day 16. By the time of ovariectomy on day 20, the mean level was 64.1 $\pm$ 4.9 pg/ml.

There were no significant changes in serum P, LH or PRL concentrations following each E_2-17β injection on days 10 through 14. P changed little from the day 10 pre-treatment mean of 10.5 $\pm$ 3.6 ng/ml. Levels declined gradually from day 13 to 5.5 $\pm$ 2.6 ng/ml by day 17. At the time of ovariectomy on day 20, P was still at 6.2 $\pm$ 1.8 ng/ml. The observed concentrations are lower than in experiment I and those of Frank et al. (1977). However, the slower decline than controls and maintained elevation above basal values between days 17 and 20 are comparable, and suggest sustained luteal activity.

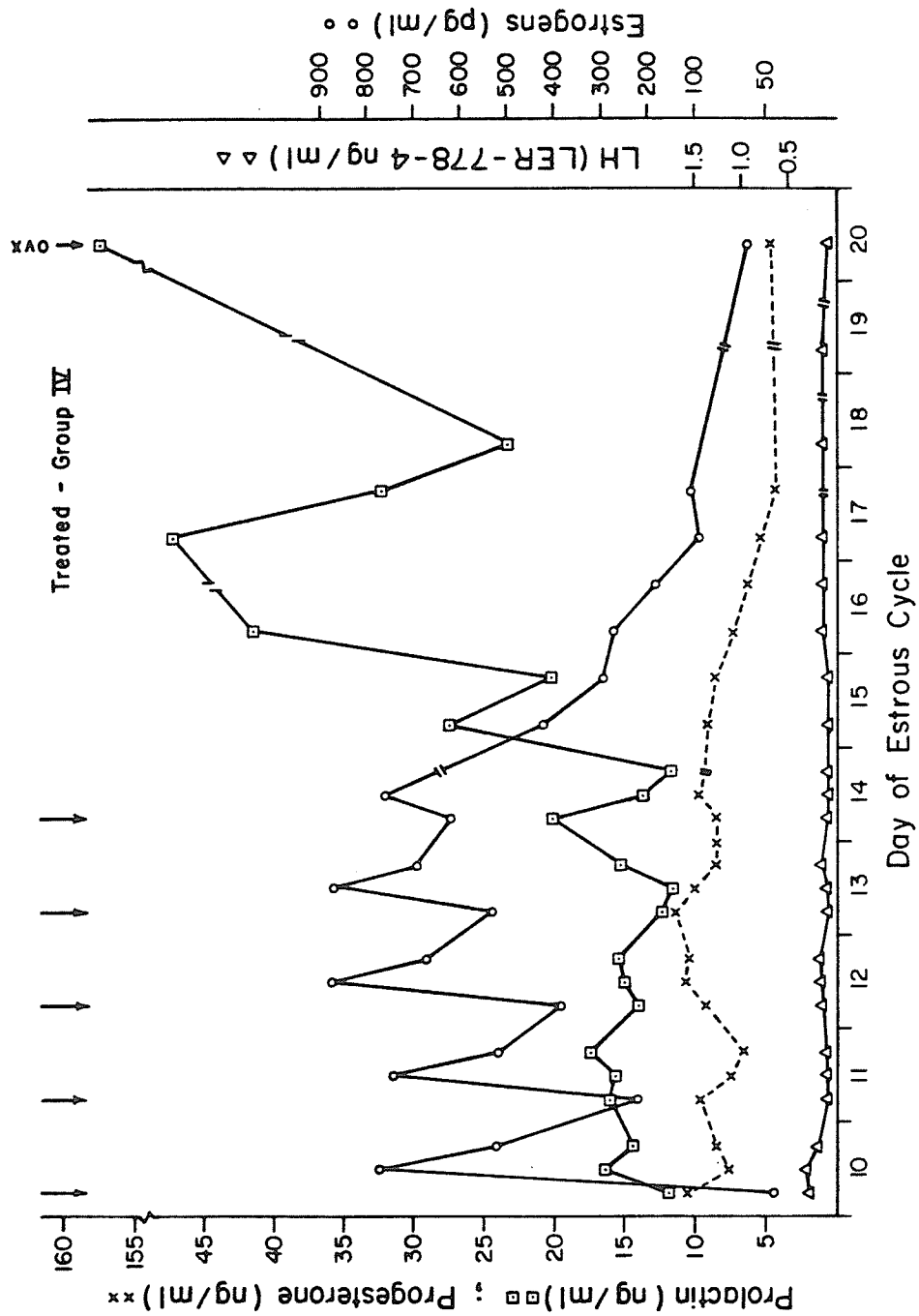

Figure 12. Mean serum concentrations of progesterone, PRL, estrogens and LH in gilts injected, im, with 10 mg E_2-17β (↓) on days 10 through 14 of the estrous cycle. Blood was collected immediately before injection at 0830 h, then at 1430 h and 2030 h on days 10 through 14. From day 15 until ovariectomy (ovx) on day 20, samples were taken at 0830 h and 2030 h.

As observed in experiment I, the LH concentrations following 10 mg E_2-17β appeared to oscillate less than in controls. Except for one gilt, at 2030 h on day 12, LH levels were < 0.2 ng/ml on days 11 to 20.

As in experiment I, concentrations of serum PRL on days 10 through 14 were unaffected by E_2 treatment. By the 0830 h collection on day 15, PRL levels were slightly elevated above the previous collection level in two animals and more than doubled in the third gilt. In this one gilt, PRL remained elevated on days 16 and 17 between 53 ng/ml and 80 ng/ml; while E was declining over this time period to 122.2 pg/ml on day 17 (2030 h). At ovariectomy a sharp rise in PRL to 157 $\pm$ 2.6 ng/ml was observed.

The situation with the serum PRL profile, at least, may be complicated by the severe heat conditions prevalent during part of this experiment. Ambient temperatures were 30°C to 37°C for several days. Signs of discomfort such as irritability and panting were frequently exhibited by several animals. During this period, the floors of the animal holding area were regularly sprayed with cold water and any animal showing signs of discomfort was treated similarly. As well, physiological compensation for the extreme heat may have been more difficult for the E_2-treated gilts since E can cause increased cardiac output and systemic hyperemia (Dickson, 1977). Consequently, in light of these factors, attempts to relate changes in PRL with other hormone changes must be done with reservation.

The hormone concentrations at ovariectomy in the four groups of animals are presented in Table 1. There were no significant differences in

Table 1. Serum Hormone Concentrations in Control and Estradiol-17β-treated Gilts at Ovariectomy on Day 15 (G I & III) or Day 20 (G II & IV) of the Estrous Cycle[1]

Hormone	Group I	Group II	Group III	Group IV
Progesterone (ng/ml)	4.80±0.48[a]	0.65±0.13[b]	6.68±1.14[a]	6.21±1.77[a]
Estrogens (pg/ml)	22.66±2.62[a]	42.81±10.27[ab]	183.32±96.59[ab]	64.08±4.94[b]
LH (ng/ml)	0.19±0.06	0.23±0.07	0.12±0.07	0.05±0
Prolactin (ng/ml)	46.47±10.34[a]	107.67±26.62[ab]	91.0±15.63[a]*	157.33±2.67[b]*

[1]Values expressed as mean ± S.E.

[a,b]Means with different superscripts across columns are significantly different (P<.01; *P<.025).

hormone concentrations between control and E_2-17β-treated animals ovariectomized on day 15 (G I and G III). In gilts ovariectomized on day 20 (G II and G IV), P was maintained at a significantly greater level ($P < .01$) in E_2-treated animals (G IV) than in controls (G II). Levels of P in E_2-treated groups were higher, but not significantly different from controls ovariectomized on day 15 of the estrous cycle.

Serum concentrations of E at ovariectomy were highest in G III gilts but were not statistically different from the other groups. In G IV, E levels were significantly greater than in G I ($P < .01$) but were not significantly elevated above their corresponding control G II. The concentrations observed in G IV on day 20 are similar to those seen on day 20 in E_2-treated gilts in experiment I, and those reported by Frank et al. (1977) for gilts in which the interestrus interval was extended by EV administration.

Although LH levels were consistently low at ovariectomy in E_2-treated animals, they were not different ($P > .05$) between groups.

PRL concentrations did not differ significantly between corresponding control and E_2-injected groups ovariectomized on the same day of the cycle. However, PRL levels were significantly higher in G IV gilts at ovariectomy on day 20 than in gilts ovariectomized on day 15 (G I, $P < .01$; G III, $P < .025$). In rats, the PRL release response to ether stress can be modified by sex steriods, being increased by E treatment and decreased by P (Reier et al., 1974). If this were applicable to pigs, then the lower PRL response to stress at ovariectomy in G I and G III gilts may have been due to a moderating influence of sustained elevated P

levels characteristic of the mid- to late luteal phase. Gilts in G IV did have higher E concentrations than G I animals ($P < .01$). Although P levels were not different between the groups, P levels in G IV had declined from the maximum mid-luteal phase values for several days. This may have allowed an E-enhancement rather than P-inhibition of PRL release. This idea is, of course, highly speculative. The high ambient temperatures, individual animal response to stress and E_2 treatment could all be involved. But, based on these limited observations, it appears that E_2 treatment did enhance the PRL release response at surgery in G IV gilts.

Results from the evaluation of ovaries collected from the four groups are presented in Table 2. Both control groups (G I and G II) had an average of 4 to 9 medium sized follicles (3-7 mm) and G II had 10.3 ± 1.4 large follicles (> 7 mm). None of the ovaries of E_2-treated gilts had follicles > 3 mm. These results agree with several earlier reports that E_2 administration suppressed development and maintenance of follicles beyond the 3 to 4 mm stage (Foote et al., 1958; Garbers and First, 1969; Chakraborty et al., 1972).

The number of corpora lutea was not affected by E_2-17β and no newly formed corpora lutea were noted. Gilts in G II did not have corpora lutea and only corpora albicantia (CA) were observed and collected. The number of CA and maintained corpora lutea in the other groups are similar.

Although corpora lutea from gilts treated with E_2 on days 10 through 14 and ovariectomized on day 15 (G III) were heavier, on a per CL

Table 2. Ovarian Characteristics of Gilts Treated with Vehicle or Estradiol-17β and Ovariectomized on Day 15 or Day 20 of the Estrous Cycle[1]

Group	Day of cycle	Number of Follicles		Corpora lutea	
		3-7 mm	>7 mm	Number	Individual weight (mg)
I	15	9.33±5.21	0	13.0±1.15	230.7±38.0
II	20	4.33±0.67	10.3±1.45	13.3±0.88*	67.7±16.8*
III	15	0	0	13.0±0	331.0±52.0
IV	20	0	0	11.7±2.18	283.7±3.5

[1]Values expressed as mean ± S.E.

*Corpora albicantia.

basis, than their controls (G I), this difference was not significant. Animals injected with E_2, and ovariectomized on day 20 (G IV) had average CL weights similar to both groups ovariectomized on day 15.

The results of binding experiments with subcellular particles of corpora lutea and albicantia are summarized in Table 3. Luteal tissue from E_2-treated gilts bound significantly greater ($P < .01$) amounts of oLH than did controls (G I) ovariectomized on day 15 of the cycle. Specific binding of oLH more than doubled after E_2-17β treatment. Recently, Ziecik et al., (1980) measured and characterized porcine luteal LH receptor during the estrous cycle and early pregnancy. Total LH receptor concentration in corpora lutea of pregnant pigs changed little between days 16 and 20, but, increased more than three fold compared to luteal tissue from day 16 non-pregnant pigs. Between day 20 and day 30 of gestation, a dramatic increase in concentration of LH receptor was noted; at a time when maximum quantities of estrone sulphate appear in the maternal circulation (Robertson and King, 1974). The present results demonstrate that administration of exogenous E can increase specific binding of LH, suggesting an increase in the number of available receptor sites.

Maintenance of the CL of early pregnancy in the pig depends, in part, on circulating LH. Administration of LH antisera on days 25 to 29 resulted in a prompt fall in P concentration (Spies et al., 1967). However, reports of serum LH levels during early pregnancy are conflicting (Tillson et al., 1970; Henricks et al., 1972; Ziecik et al., 1980). Whether LH levels are elevated, low, or similar to values in cycling

Table 3. Percent Specific Binding of oLH and oPRL to Porcine Luteal Tissue Receptor Following Vehicle or Estradiol-17β Administration[1]

Group	Day of cycle ovariectomized	% SB of oLH per mg protein	% SB of oPRL per mg protein
I	15	6.45 ± 1.0^{a}	2.51 ± 0.83^{a}
II	20	0^{b}	0^{b}
III	15	14.00 ± 3.90^{c}	2.44 ± 0.92^{a}
IV	20	16.70 ± 1.21^{c}	3.68 ± 1.65^{a}

[1]Values expressed as mean ± S.E.

[a,b,c]Means with different superscripts within a column are significantly different ($P<.01$).

animals is not certain.

During the estrous cycle in the pig (Ziecik et al., 1980) and ewe (Niswender and Diekman, 1979) the total number of luteal receptors for LH is greatest at the time of maximal P production, which coincides with basal serum LH concentrations. In the ewe, P production remains high during early pregnancy, serum LH is low and LH receptor concentration is not different from day 12 of the cycle to day 12, 16 and 20 of pregnancy (Niswender and Diekman, 1979). Therefore, while P production in the pig is maintained at less than maximum luteal phase levels during early pregnancy, a requirement for LH support of luteal function during this period need not be reflected by elevated serum LH. Thus, the low serum LH profile seen here following E administration may be comparable to the pattern in early gestation.

Consequently, part of the E_2 luteotrophic effect may be mediated by increasing available binding sites for LH. Chronic treatment with E was shown to increase the capacity of rat ovarian tissue to bind gonadotrophin (Lee and Ryan, 1975). This was, presumably, a result of elevated PRL. However, in the present study no elevation in PRL levels was observed following E_2 treatment. This does not preclude a possible facilitation of PRL induction of LH receptor, which would not require elevated serum PRL levels.

Administration of E_2-17β did not significantly alter corpora lutea binding capacity for oPRL (Table 3). The observed binding is similar to the specific binding of oPRL reported for luteal phase corpora lutea by Rolland et al., (1976), who also observed some specific binding by CA

(1.2%). In the present study CA did not show specific binding of PRL, but the quantity of tissue was limited and the amount per assay tube may have been insufficient.

Rolland and coworkers (1976) also noted that luteal tissue from early pregnancy (ca. days 32 to 46) bound approximately two to five times as much oPRL as corpora lutea of the estrous cycle. However, tissue from earlier in gestation was not examined, so, the time course of changes in luteal PRL receptor prior to day 30 are not known, at present.

Conclusions

The effects of five daily injections of 10 mg E_2-17β (days 10 through 14) on serum hormone profiles and porcine luteal function were consistent with the observations in experiment I. Luteal P production was maintained until ovariectomy on day 20, serum LH remained low and no immediate effect on PRL secretion was apparent. Hormone concentrations at ovariectomy on day 15, were not different ($P > .05$) between control and E_2-treated groups. In gilts ovariectomized on day 20, only P levels were significantly different ($P < .01$) between controls and E_2-treated animals; being maintained in the E_2 group at levels comparable to both control and E_2-treated gilts ovariectomized on day 15.

Administration of E_2-17β inhibited ovarian follicular growth beyond the 3 mm stage, maintained CL weight and more than doubled specific binding of oLH to luteal tissue receptor preparations. Specific binding of oPRL to corpora lutea receptor preparations was unaffected by E_2 treatment.

Therefore, at least part of the luteotrophic potential of E_2 in the pig appears to involve increasing available luteal binding sites for LH. The mechanism of this action is not clear, but a synergism between E, LH and PRL cannot be ruled out at this time.

EXPERIMENT III

Effect of Short-term Bromocriptine on Hormone Levels, Ovarian Characteristics and Luteal Receptors for Prolactin and LH.

The procine CL of the estrous cycle is commonly considered to function autonomously, without requiring hypophyseal support (Nalbandov, 1973, 1976). However, investigations utilizing hypophysectomized pigs indicated that, although CL development and P production following ovulation and luteinization may proceed normally until about the mid-luteal phase, pituitary luteotrophic support was necessary for attainment of maximum morphological and functional integrity beyond this point (Anderson et al., 1967; Denamur, 1968). The nature of this requirement has not yet been defined and the possibility that LH and/or PRL may be involved can not be excluded.

Porcine luteal tissue collected around mid-cycle (days 8-10, Cook et al., 1967; days 11-14, Watson and Leask, 1975, Watson and Wrigglesworth, 1975) responded to in vitro addition of LH by increased P production. More recently, Ziecik et al., (1980) demonstrated that porcine luteal concentration of LH receptor more than doubled between days 6 and 10 of the cycle and was maximal on day 12. These results support the concept of a physiological role of LH during normal porcine CL function.

There is little evidence implicating PRL as a luteotrophic hormone in the pig. However, the demonstration of increased specific binding sites for PRL in cyclic luteal tissue (Rolland et al., 1976) above that

in granulosa cells and corpus hemorrhagica (Rolland and Hammond, 1975) does suggest that PRL has a part in normal cyclic luteal activity.

To our knowledge, no one had attempted to inhibit PRL secretion in the cycling gilt and assess CL function. Therefore, this experiment was planned in an attempt to investigate the necessity of PRL secretion for maintenance of cyclic corpora lutea. Gilts were injected with bromocriptine, a specific inhibitor of PRL secretion, for 8 days starting on day 4 of the cycle. Presumably, by this stage of the cycle, corpora lutea would have reached approximately half maximal activity in terms of P production. On day 11, gilts were ovariectomized and the ovaries assessed for follicular development, numbers and weight of corpora lutea, and luteal tissue binding of oLH and oPRL. Blood samples collected throughout the trial were assayed for P, E, LH and PRL.

This experiment was conducted in June, 1980.

MATERIALS AND METHODS

Experimental Animals

Ten cycling gilts (6 Managra, 2 York, 2 Crossbred), 218 to 262 days of age, were brought into the campus swine barn from the Glenlea Research Station one to three days after estrus was observed. Five animals fitted with ear catheters were maintained in individual crates as in experiments I and II. The other five gilts were group-housed in an adjacent pen throughout the course of this experiment.

Experimental Protocol

Treatments. The gilts were divided into two treatment groups. Daily, at

0830 h, the five animals in the control group were injected, im, with 1 ml 60% ethanol-saline (vehicle) for 8 days starting on day 4 of the estrous cycle. Five gilts in the bromocriptine (Br) group were treated similarly with 10 mg Br (Bromocriptin-Mesilat; Sandoz Ltd., Basle, Switzerland) dissolved in 1 ml vehicle, on each of days 4 through 11 of the cycle. On day 11 of the cycle each animal was ovariectomized. Ovaries were evaluated and tissues prepared for receptor studies.

Blood Collection and Handling. Due to an unavailability of catheter material, only five gilts were successfully fitted with ear-vein catheters. Two control animals and three Br-treated gilts underwent ear-vein catheterization on day 3 of the cycle. The materials and procedures employed were the same as those for animals in experiment II. Blood (15-30 ml) was collected immediately before the morning injections at 0830 h and again at 2030 h on days 4 to 11. On day 11, the morning sample was taken as usual, before treatment. A sample was also taken from each of the 10 gilts at ovariectomy.

Samples were handled and stored according to the outline in experiment I.

Parameters Measured. These were basically the same as those in experiment II. Serum samples were assayed for P, E, LH and PRL by the RIA procedures reported in experiment I. As in experiment II, the ovarian characteristics examined included numbers of medium (3-7 mm) and large (> 7 mm) follicles, the appearance and number of corpora lutea, weight of luteal tissue and luteal tissue receptor binding of LH and PRL.

Evaluation of Ovarian Characteristics

Ovarian tissue was collected, handled, examined and prepared for luteal tissue receptor studies according to the procedures outlined in experiment II materials and methods.

Specific binding of ^{125}I-Labelled oLH and oPRL. The receptor preparations were assayed for specific binding in the same assays as tissue from experiment II. As with experiment II gilts, %SB was determined for individual receptor preparations. The amount of protein per tube was 0.48 $\pm$ 0.06 mg in the ^{125}I-oLH binding study and 0.93 $\pm$ 0.15 mg in the assay for SB of ^{125}I-oPRL.

Results and Discussion

The pattern of hormone secretion depicted for the two control animals in Fig. 13 (top) was consistent with the observations in experiment I and previous reports for this stage of the cycle (Tillson et al., 1970; Henricks et al., 1972; Connor et al., 1976). Mean concentrations of serum P rose from day 4 and stayed above 8 ng/ml until ovariectomy on day 11. E levels changed little during the collection period, usually remaining between 30 and 49 pg/ml. Oscillations in LH, resulting in a significant a.m.-p.m. interaction ($P < .05$) were most apparent from day 7 onward but concentrations were generally < 0.7 ng/ml. Levels of PRL fluctuated between 7.7 and 13.9 ng/ml from day 4 until day 11. At ovariectomy on day 11 a surge to 71 ng/ml occurred.

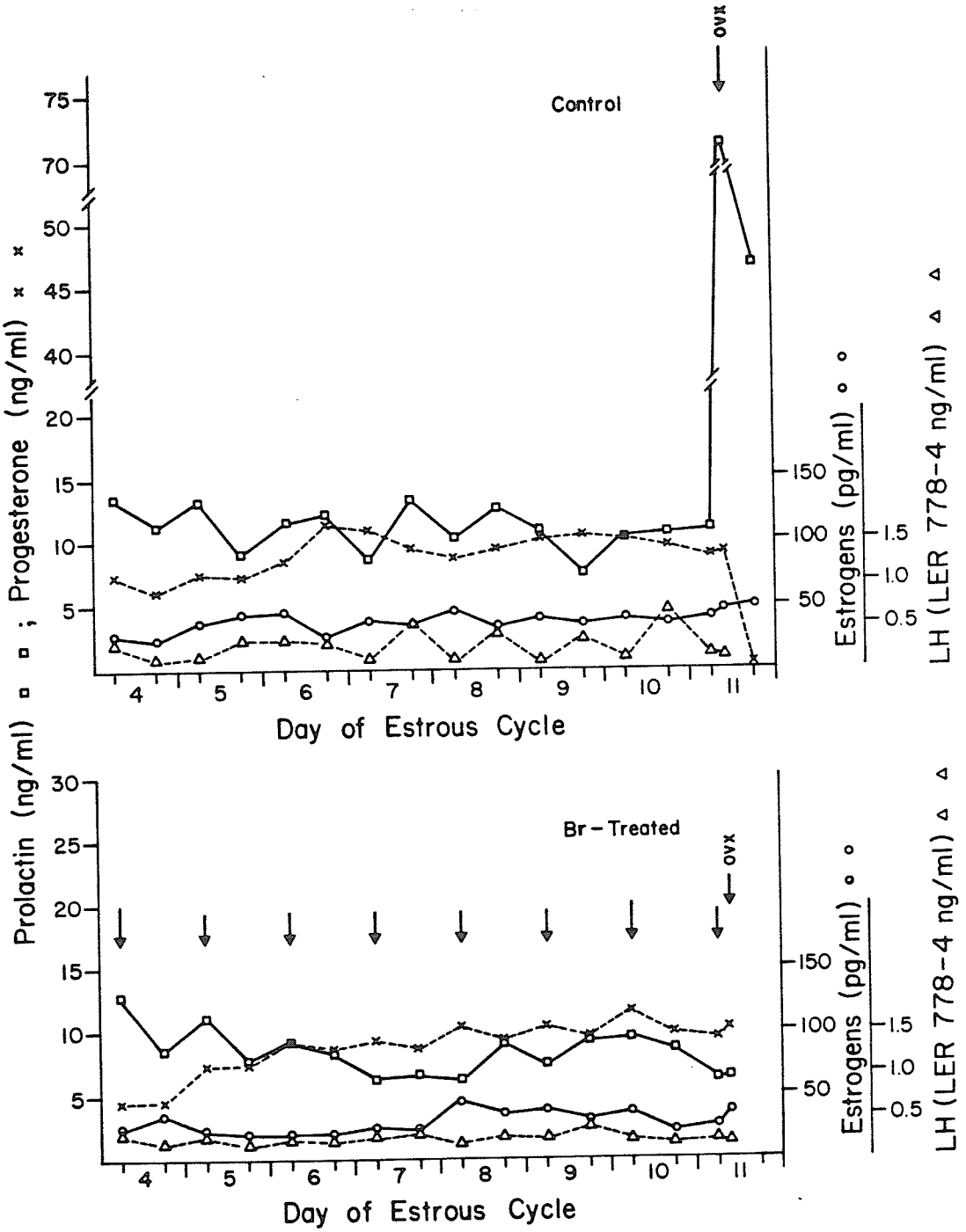

Figure 13. Mean serum concentrations of progesterone, PRL, estrogens and LH in gilts injected, im, with vehicle (Control; n = 2) or bromocriptine (Br-treated; n = 3) (↓) on days 4 through 11 of the estrous cycle. Blood was collected immediately before injection at 0830 h then at 2030 h. A sample was also taken at ovariectomy (ovx) on day 11.

Bromocriptine administered to three gilts on days 4 through 11 of the cycle did not significantly alter PRL secretion nor affect luteal function as assessed by peripheral P concentrations (Fig. 13, bottom). Serum P increased from day 4 in all animals, as it did in controls. Peripheral LH levels were not significantly affected by Br treatment but the a.m.-p.m. fluctuations observed in the control gilts were not obvious and mean values remained below 0.4 ng/ml.

Daily treatment with 10 mg Br did appear to effect some inhibition of PRL secretion, although it was not significant. Levels of PRL decreased from the day 4 pre-injection mean of 12.9$\pm$2.8 ng/ml and a declining trend was noted over the next two days. From day 6 until day 11, concentrations stayed below 9.3 $\pm$ 2.3 ng/ml. Levels below 4.2 ng/ml were not detected in any of the three gilts. The most obvious indication that Br was able to inhibit PRL release occurred at ovariectomy. None of the Brtreated gilts showed a surge of PRL at the time of ovariectomy (Table 4.), whereas, PRL levels in the control animals at this time were significantly elevated ($P < .01$) and were similar to the elevated PRL response observed at ovariectomy in experiment II. This observed suppression is consistent with the ability of Br to inhibit PRL secretion, in other species, under conditions when PRL output is normally stimulated by physiological, pharmacological or surgical means (Fluckiger, 1978).

Table 4 also shows that Br did not affect the concentrations of E, P, or LH as assessed in all animals at ovariectomy.

Therefore, it appears that bromocriptine can suppress PRL in gilts, but the dose employed was insufficient to decrease levels below 4 ng/ml.

Table 4. Serum Hormone Concentrations at Ovariectomy on Day 11 in Control and Bromocriptine Treated Gilts[1]

Group	Progesterone (ng/ml)	Estrogens (pg/ml)	LH (ng/ml)	Prolactin (ng/ml)
Control (n = 5)	11.1±0.93	45.7±6.6	0.13±0.03	96.2±16.1**
Bromocriptine (n = 5)	9.2±0.90	33.8±4.9	0.23±0.04	10.18±2.23**

[1]Values expressed as mean ± S.E.

**P<.01.

Lower serum PRL levels may have been achieved before the 12 h post-injection blood sample, but the presence of substantial amounts of circulating PRL at the times sampled indicates that any significant suppression was short-lived.

In ewes, daily doses of 0.5 to 2 mg of CB-154 (ergocriptine) effectively inhibited PRL secretion (Kann and Denamur, 1974; Land et al., 1980). Similarly, at this institution, 2 to 4 mg of Br per day was effective in rams (Sanford, unpublished observations). As well, Kiser et al., (1979) reported that 60 mg CB-154 depressed serum PRL in bulls from 64 ng/ml to 3.1 ng/ml within 6 h of injection. Consequently, at the outset of this experiment, it was felt that 10 mg Br would be adequate to suppress PRL in the gilts used. The only report available using the pig model dealt with lactating sows (Kraeling et al., 1979). The dose of 120 mg CB-154, seemed excessive for the present experiment, when results in other domestic species and the physiological status of the gilts in this study were considered. In retrospect, this may not be so, since even the dosage used for lactating sows did not suppress PRL levels to below 7 to 10 ng/ml from a pre-injection mean of 45 $\pm$ 13 ng/ml (Kraeling et al., 1979).

Evaluation of ovaries collected on day 11 did not reveal any consistent effect of Br on gross morphological characteristics (Table 5). Both corpora lutea numbers and weights were not different between control and treated groups. Similarly, ovarian follicular development up to the 7 mm stage was similar between groups. However, two of the five Br-treated gilts had follicles > 7 mm. The ovaries of one of these gilts contained

Table 5. Ovarian Characteristics of Control and Bromocriptine-Treated Gilts on Day 11 of the Estrous Cycle[1]

Group	Number of follicles		Corpora lutea	
	3-7 mm	>7 mm	Number	Individual weight (mg)
Control (n = 5)	4.4±1.9	0	12.1±1.1	363.2±69.6
Bromocriptine (n = 5)	4.6±1.4	4.2±2.6	13.4±1.2	419.6±50.3

[1]Values expressed as mean ± S.E.

one cystic follicle and 3 of 10 corpora lutea also appeared cystic. Prolonged administration of CB-154 to dogs has been reported to result in cystic follicles and cystic corpora lutea (Griffith and Richardson, 1975), but we are not aware of any such effects accompanying short-term Br treatment in large domestic animals.

Table 6 summarizes the results of the specific binding studies. Specific binding of oLH to luteal receptor preparations was similar in control and Br-treated groups. As well, oPRL binding to luteal tissue was not significantly altered by Br treatment, although, specific binding tended to be lower in the Br group.

Circulating PRL levels were still within the physiological range for the luteal phase (experiment I and II; Dusza and Krzymowska, 1979) during Br treatment and no effects on ovarian characteristics were noted. Therefore, any conclusions regarding a luteotrophic requirement for this hormone during cyclic porcine luteal development and function remain speculative. Presumably, if Br administration had been successful, a requirement for PRL may have been reflected by decreased P concentrations, a decline in luteal LH receptor (Grinwich et al., 1976, Waters et al., 1978) and/or a change in PRL receptor binding (Waters et al., 1978).

Conclusions

Daily injections of 10 mg Br to gilts for 8 days starting on day 4 of the estrous cycle had no significant effect on peripheral PRL concentrations, nor on luteal P production. Serum PRL levels did decline following Br administration but remained above 4.2 ng/ml, as assessed at

Table 6. Percent Specific Binding of oLH and oPRL to Porcine Luteal Tissue Following Bromocriptine Treatment[1]

Group	Number of animals	Day of cycle ovariectomized	% Specific binding per mg protein	
			oLH	oPRL
Control	5	11	10.70±2.84	2.32±0.59[2]
Bromocriptine	5	11	10.46±1.18	1.57±0.26

[1]Values expressed as means ± S.E.

[2]n = 4.

12 h intervals. Bromocriptine effectively blocked the PRL response to stress at ovariectomy on day 11. Corpora lutea numbers, weights and receptor binding of LH and PRL were not significantly affected by the 8-day Br-treatment.

GENERAL DISCUSSION

Results presented herein agree with the documented ability of exogenous estrogen administration, begun around mid-cycle, to prolong porcine cyclic corpora lutea lifespan (Kidder et al., 1955; Nishikawa and Waide, 1958; Gardner et al., 1963; Garbers and First, 1969; Chakraborty et al., 1972; Frank et al., 1977, Mahaboob Basha et al., 1980; Ford and Magness, 1980). In addition, these investigations characterized the serum hormone profiles of E, P, LH and PRL during and after treatment with E_2-17β and effects on luteal receptor binding of LH and PRL, areas which hitherto have not been studied in the pig. The maintained CL weight and serum P pattern following E_2-17β treatment are similar to that seen in the early stages of pregnancy in swine. The luteotrophic action of E is, in fact, considered by some to mimic the embryonic signal for maternal recognition of pregnancy which occurs around day 12 in the pregnant pig, coincident with the onset of blastocyst E synthesis (Perry et al., 1976; Cook and Hunter, 1978: Heap et al., 1979).

This concept of E-induced pseudopregnancy gains support from the findings that EV injections (sc) on days 11 through 15 results in a serum P profile, elevated estrone concentrations, utero-ovarian vein PGF concentrations (Frank et al., 1977) and qualitative make-up of uterine protein secretions (Mahaboob Basha et al., 1980) similar to early pregnancy. As well, Ford and Magness (1980) recently observed that intra-uterine infusion of E_2-17β (days 11-15) resulted in an increase in uterine blood flow similar to that seen in sows on days 12-13 of pregnancy (Ford

and Christenson, 1979). Although several theories have been proposed, the mechanism of the exogenous or embryonic E luteotrophic response remains elusive.

One of the proposed mechanisms involves E-elicitation of LH release (Kidder et al., 1955; Perry et al., 1976; Cook and Hunter, 1978). Although an absolute requirement for this hormone for maximum cyclic CL competency is not confirmed, its importance as a luteotrophin for sustained luteal activity beyond the normal lifespan, such as during pregnancy, is accepted. Corpora lutea P production promptly declines and pregnancy is terminated in gilts injected with LH antiserum on days 24 to 29 of pregnancy (Spies et al., 1967). Luteal tissue from early (Lemon and Loir, 1977; Watson and Maule Walker, 1978) through late (Lemon and Loir, 1977) pregnancy responds to LH by increased P production. Assuming that E_2-17β treatment can elicit physiological events that are generally associated with early pregnancy, the idea that the requisite for LH may be reflected by elevated serum LH levels is not supported by the present experiments. As suggested by increased pituitary LH content, concomittant with E-induced luteal maintenance in gilts (Garbers and First, 1969; Chakraborty et al., 1972), E_2 administration in the present experiments appeared to suppress serum LH levels, although not significantly. At present, it is uncertain whether this mimics the serum LH profile in early gestation. Reports of both elevated (Henricks et al., 1972; Ziecik et al. 1980) and depressed (Tillson et al., 1970) peripheral LH concentrations have appeared in the literature. However, an early investigation by Melampy and coworkers (1966) noted that

pituitary LH concentration remained high during days 18 to 25 of pregnancy, which supports the current findings reported here.

Low circulating LH may not be contrary to a requirement for this hormone during early pregnancy since elevated levels are not observed in sheep (Niswender et al., 1968) or cattle (Lukaszewska and Hansel, 1980). Elevated LH might, actually, be detrimental to maintenance of CL function by desensitization or down-regulation of luteal LH receptors or adenyl cyclase, thereby rendering corpora lutea unresponsive to LH (Hunzicker-Dunn et al., 1978; Catt et al., 1979).

A possible way in which E could facilitate the physiological role of LH was demonstrated by the significant increase in available LH binding sites in luteal tissue subsequent to E_2-treatment (experiment II). That this is not unlike the situation in early pregnancy is suggested by the recent observations of Ziecik et al. (1980). Total concentration of luteal receptor for LH in the pregnant pig on days 16 and 20 was 2 to 3 fold greater than on days 14 and 16 in non-pregnant animals. Receptor concentration then increased dramatically between days 20 and 30 coinciding with the time of peak estrone sulphate levels in the maternal circulation (Robertson and King, 1974; Robertson et al., 1978).

Since control gilts were ovariectomized on day 15 (experiment II), when luteal concentration of LH receptors reportedly is declining (Ziecik et al., 1980), it is not certain whether E_2 treatment resulted in an increase in LH receptor above mid-cycle levels, reflecting an increased requirement for LH, or if mid-cycle levels were maintained. In experiment III, corpora lutea from gilts ovariectomized on day 11 did

demonstrate greater specific binding of LH than did those in experiment II ovariectomized on day 15. In addition, E_2-treated animals had higher luteal binding of LH on days 15 and 20 than did either of these groups.

The increase in specific binding of LH may have been mediated by E-facilitation of PRL-induction and/or maintenance of LH receptor. Part of the PRL requirement during pregnancy in the rat is met by this means (Grinwich et al., 1976; Day et al., 1979). Although, in the present experiments E_2 did not provoke immediate PRL release as was expected (Neill, 1974), peripheral PRL concentrations and luteal tissue receptor binding of PRL were maintained in E_2-treated animals. The preservation of specific binding sites for PRL similar to luteal phase levels here, and as reported previously (Rolland et al., 1976) is provocative evidence that PRL serves a physiological function in maintaining porcine corpora lutea competency following E_2 treatment or early pregnancy, as well as during the estrous cycle. The role of PRL in CL function in the pig has not been defined and attempts to suppress PRL secretion (experiment III) failed to prove any more illuminating.

The additional possibility that LH and PRL have the potential to be anti-luteolytic is suggested by observations of antagonism to the lytic influence of $PGF_{2\alpha}$ when porcine luteal tissue from early pregnancy were superfused <u>in vitro</u> with LH (Maule Walker and Watson, 1977; Watson and Maule Walker, 1978) or PRL (Maule Walker and Watson, 1977). The concentration of utero-ovarian vein PGF obvserved in pregnant (Moeljono et al., 1977) or EV treated (Frank et al., 1977) gilts, on days 13 to 17,

were significantly lower than in non-pregnant animals, but, it is not known whether PG levels are too low to be effective or are, in some manner, neutralized. Consequently, during the critical period of maternal recognition of pregnancy, LH and/or PRL may provide a safeguard against the potential retrogressive influence of uterine or luteal (Guthrie et al., 1979) PGF. This protective function could be mediated by E-effected enhancement or maintenance of luteal receptor sites for LH and PRL such as was observed following E_2 treatment in experiment II.

While not investigated in the present studies, there is evidence that estrogens, either of exogenous or embryonic origin, may exert a direct anti-luteolytic effect by inhibition or redirection of uterine PGF production. Bazer and Thatcher (1977; Frank et al., 1978) have argued that in the pregnant uterus, as in the uterus exposed to EV treatment (Frank et al., 1978), E cause a redirection of PG flow away from the utero-ovarian vein and into the uterine lumen. As well, Perry et al. (1976) suggested that inhibition of the uterine luteolytic mechanism may be effected by maintenance of low uterine tissue concentrations of E_2-17β. Endometrial conversion of E_2 to estrone sulphate does not decline during early pregnancy (Perry et al., 1976) as it does during the late luteal phase in the cyclic pig (Pack and Brooks, 1974). The present experiments did not specifically measure peripheral estrone concentrations. However, Frank et al. (1977) noted that gilts treated with EV (days 11-15) had significantly elevated plasma estrone levels which indicated that E administration results in sustained uterine enzyme activity similar to early pregnancy.

Therefore, there is considerable support for the concept that

exogenous E administration, begun at mid-cycle, evokes physiological responses similar to those occurring during early pregnancy in the pig. However, the exact nature of E-induced luteal persistance remains an enigma. Evidence presented here indicates that part of this luteotrophic mechanism includes an increase in the number of corpora lutea binding sites for LH and maintenance of luteal receptor sites for PRL, yet the respective roles of these hormones in regulation of porcine CL function have not been defined. Although not investigated in the present studies, it is probable that E exert an anti-luteolytic effect on the uterus. As well, a direct luteotrophic action of E on the CL cannot be excluded at this time.

SUMMARY

Administration of 10 mg E_2-17β to gilts on day 10, or on days 10 through 14, of the estrous cycle significantly extended the interestrus interval ($P < .01$), prolonged luteal P production, initially depressed the pulsatile serum LH profile, but did not affect peripheral PRL concentrations. Five daily injections of E_2-17β, started on day 10, did not extend the estrous cycle length significantly more than did a single injection on day 10.

Evaluation of ovaries and luteal tissue from gilts treated with peanut oil (control) or 10 mg E_2-17β on days 10 through 14, then ovariectomized on day 15 or day 20, revealed that E_2 treatment inhibited ovarian follicular growth beyond the 3 mm stage, maintained corpora lutea weight, and increased ($P < .01$) luteal receptor specific binding of oLH by more than 2 fold above controls ovariectomized on day 15. There were no significant differences in ovarian characteristics between E_2-treated gilts ovariectomized on day 15 and those ovariectomized on day 20. The percent specific binding of oPRL to luteal receptor preparations was similar between control gilts ovariectomized on day 15 and E_2-17β treated gilts ovariectomized on day 15 or 20.

Results suggest that part of the E_2 luteotrophic action involves an increase in the available corpora lutea binding sites for LH and possibly maintenance of luteal receptor sites for PRL. The mechanisms have yet to be delineated, but facilitation, augmentation or synergism between E, LH and PRL cannot be excluded.

Daily concentrations of PRL in cycling gilts remained fairly constant throughout the luteal phase and were not apparently affected by E_2 treatment. An increasing trend, which did not appear to be associated with serum E changes, occurred in all animals 3 to 7 days before estrus. Levels returned to basal values prior to the first day of estrus.

Bromocriptine injections (10 mg/day, im) from day 4 through 11 of the estrous cycle did not significantly lower serum PRL concentrations in gilts, but did prevent the stress-induced surge of PRL at ovariectomy on day 11. Administration of Br did not affect peripheral P concentrations, numbers or weights of corpora lutea nor luteal receptor binding of oLH and oPRL. These results do not allow any conclusions concerning a role for PRL in cyclic luteal function. However, the observations of specific binding sites for PRL in corpora lutea suggest a physiological requisite, the significance of which remains to be elucidated.

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APPENDIX TABLES

Table 1A. Experiment I: Mean (±SE) Serum Hormone Concentrations in Gilts (n = 4) during the Control Cycle, T_1.

Day of cycle[1]	Progesterone (ng/ml)	Estrogens (pg/ml)	LH (ng/ml)	Prolactin (ng/ml)
-2	0.48±0.33	47.24±13.48	0.12±.02	7.10±1.10
-1	0.27±0.11	47.92±17.34	0.16±.06	5.75±1.35
0	0.49±0.21	54.38±15.48	0.87±.38	7.83±1.24
1	3.42±3.84	22.35±3.32	0.36±.10	10.11±3.88
2	6.99±4.86	18.56±5.26	0.23±.12	8.73±3.08
3	9.76±3.56	24.65±0.45	0.22±.10	12.06±3.62
4	16.58±3.79	30.53±1.65	0.26±.13	10.30±2.82
5	17.43±2.30	20.44±7.05	0.29±.12	10.30±3.10
6	24.00±5.53	18.89±2.48	0.19±.03	8.23±0.39
7	24.14±2.09	21.42±3.73	0.18±.09	7.83±2.11
8	24.25±2.58	24.95±5.76	0.16±.03	7.45±2.51
9	26.62±4.35	18.51±7.70	0.10±.04	8.33±2.95
#1 10	29.08±3.27	19.36±9.01	0.10±.03	12.47±1.85
2	31.69±9.80	23.47±2.53	0.07±.01	8.55±1.65
3	34.40±9.65	22.28±3.82	0.08±.01	13.10±0.10
4	34.73±10.06	15.98±4.10	0.48±.21	6.30±2.30
5	26.58±4.76	22.39±3.48	0.31±.09	9.85±2.35
6	31.98±7.14	20.86±3.92	0.10±.01	9.90±0.50
7	33.47±6.04	18.08±4.43	0.07±.01	11.35±2.75
8	29.35±5.59	28.66±1.04	0.08±.01	11.90±0.70
9	28.97±5.41	25.59±1.66	0.19±.13	10.90±0.30
10	35.87±10.08	19.52±4.50	0.39±.28	13.15±1.55
11	31.04±6.63	23.29±7.23	0.24±.18	13.60±0.80
12	22.97±6.85	19.33±2.31	0.11±.05	15.20±.40
13	16.94±8.44	23.30±3.22	0.28±.18	14.00±0.20
14	24.92±6.18	21.53±0.18	0.16±.10	15.10±3.30
15	24.38±4.41	22.38±2.83	0.08±.02	15.40±2.20
11	27.42±3.89	35.05±8.45	0.07±.02	10.72±1.56
12	31.32±6.77	33.89±10.76	0.17±.10	10.85±1.44
13	26.18±2.57	25.59±8.04	0.12±.03	9.70±1.64
14	22.12±8.63	20.52±9.61	0.11±.05	13.75±2.49
15	17.37±8.70	41.05±15.98	0.18±.05	15.07±3.56
-4	4.59±3.06	39.52±14.35	0.27±.04	16.60±4.64
-3	3.21±1.20	44.97±15.12	0.22±.04	13.10±1.81
-2	1.36±0.44	52.87±14.46	0.17±.03	13.90±2.61
-1	0.99±0.52	80.43±20.74	0.22±.06	10.25±1.64
0	1.06±0.36	60.10±8.10	1.02±.21	13.92±0.80

[1]On day 10 samples were collected hourly from 0800h (1) until 2200h (15).

Table 2A. Experiment I: Mean (±SE) Serum Hormone Concentrations in Gilts (n = 4) during the E_2-17β Treatment Cycle, T_1.

Day of cycle[1]	Progesterone (ng/ml)	Estrogens (pg/ml)	LH (ng/ml)	Prolactin (ng/ml)
1	1.83±0.45	29.69±7.36	0.44±.06	16.20±4.00
2	9.03±2.17	31.41±11.27	0.22±.03	13.57±2.47
3	17.13±4.02	34.62±11.81	0.22±.08	8.65±1.48
4	21.28±4.02	32.12±9.76	0.19±.05	10.90±1.06
5	23.51±2.87	30.84±8.36	0.18±.03	10.72±1.97
6	27.51±3.40	37.04±9.80	0.33±.07	11.25±0.90
7	30.71±5.46	42.31±10.84	0.32±.08	9.90±1.30
8	30.41±6.52	74.88±26.22	0.20±.07	9.72±1.40
9	32.97±6.77	61.32±19.27	0.09±.02	13.22±1.63
#1 10	31.84±3.67	33.24±8.00	0.10±.02	13.25±1.51
2	33.50±4.20	902.13±204.80	0.07±.02	12.30±2.22
3	30.96±4.05	830.50±165.15	0.09±.03	12.80±1.41
4	29.40±3.51	803.07±152.78	0.11±.05	12.47±1.33
5	29.65±2.98	786.40±144.94	0.10±.02	14.70±2.07
6	33.66±4.82	858.64±172.04	0.10±.03	14.95±2.40
7	29.97±3.53	787.42±166.57	0.09±.02	12.40±0.42
8	29.38±3.94	792.70±168.15	0.07±.01	11.72±1.56
9	30.52±3.72	728.95±146.43	0.10±.03	14.25±0.71
10	34.31±1.65	785.01±149.09	0.05±.00	11.00±2.07
11	29.87±4.31	707.90±191.15	0.09±.01	9.25±2.18
12	33.14±5.92	675.14±142.17	0.13±.04	11.37±1.26
13	30.93±5.73	571.78±107.35	0.10±.02	10.27±1.32
14	27.81±3.13	554.87±110.10	0.31±.24	12.42±1.80
15	30.55±3.64	518.26±101.10	0.06±.00	8.65±1.10
11	43.98±6.70	192.82±91.93	0.06±.00	9.73±0.81
12	39.23±6.06	122.34±50.29	0.05±.00	9.10±0.91
13	35.51±6.77	93.65±32.17	0.06±.01	23.35±12.22
14	23.40±2.44	96.67±36.20	0.21±.06	9.82±1.86
15	25.16±3.71	81.31±28.82	0.08±.01	9.12±1.79
16	26.15±4.66	74.98±13.37	0.20±.10	11.45±2.03
17	26.63±3.67	74.60±26.43	0.13±.08	10.52±1.75
18	18.93±5.80	44.50±10.91	0.13±.04	13.92±3.73
19	22.43±7.30	89.28±27.22	0.08±.02	9.77±2.18
20	8.06±3.44	100.56±36.19	0.15±.04	40.65±26.49
21	5.71±3.42	97.66±18.94	0.20±.04	15.82±1.28
-4	1.73±0.61	85.07±17.38	0.23±.04	13.65±1.15
-3	1.81±0.41	79.58±5.02	0.21±.03	13.80±2.76
-2	1.42±0.34	114.83±21.94	0.33±.10	10.67±1.65
-1	1.42±0.49	95.59±23.45	0.29±.05	11.60±1.12
0	1.38±0.48	83.30±13.83	0.60±.18	11.47±2.64

[1]Same as Table 1A.

Table 3A. Experiment I: Mean (±SE) Serum Hormone Concentrations in Gilts (n = 4) during the Control Cycle, T_2.

Day of cycle[1]	Progesterone (ng/ml)	Estrogens (pg/ml)	LH (ng/ml)	Prolactin (ng/ml)
-2	0.57±0.27	56.22±2.07	-	8.77±1.16
-1	0.65±0.13	50.15±2.78	.140±.005	12.23±3.21
0	0.95±0.44	56.27±10.58	1.28±.657	9.85±2.15
1	1.34±0.50	17.60±3.47	.367±.063	13.55±0.71
2	5.21±2.56	13.68±2.20	.328±.056	14.37±1.29
3	18.69±3.01	18.43±1.25	.247±.147	15.36±0.84
4	19.53±9.64	15.05±0.20	.270±.005	16.66±4.37
5	24.76±9.79	18.45±4.17	.253±.029	12.16±1.80
6	21.60±7.57	23.71±1.74	.359±.045	11.43±1.90
7	24.71±7.48	21.73±3.77	.282±.102	10.70±1.19
8	29.52±5.72	23.14±3.99	.160±.048	10.57±1.07
9	29.70±5.15	22.04±4.80	.317±.074	11.37±1.20
#1 10	24.19±3.76	17.46±3.31	.079±.014	11.12±2.11
2	25.09±5.79	18.78±2.97	.242±.108	10.60±2.29
3	22.18±3.91	15.96±2.51	.350±.151	11.02±2.17
4	23.54±2.73	16.15±3.96	.216±.077	10.20±1.15
5	23.44±2.74	14.26±3.39	.197±.089	11.67±2.57
6	23.99±4.43	16.15±3.78	.096±.030	11.62±2.81
7	21.49±1.66	16.95±3.80	.170±.057	9.67±2.18
8	20.82±1.98	19.13±3.68	.225±.140	8.92±2.33
9	24.33±4.04	16.55±2.51	.211±.092	9.27±2.04
10	23.22±4.67	15.21±1.20	.276±.136	12.30±3.78
11	17.36±2.86	17.69±2.90	.211±.070	11.10±1.86
12	20.25±2.69	12.89±2.51	.127±.034	11.55±0.42
13	20.95±2.70	15.79±1.56	.243±.069	14.60±2.04
14	21.15±2.72	14.59±1.60	.272±.067	11.55±1.27
15	20.30±1.48	15.96±3.06	.171±.081	11.32±2.24
#1 11	27.09±2.31	15.30±1.42	.094±.037	11.25±1.40
2	23.72±3.69	16.59±1.30	.298±.145	11.82±1.75
3	20.01±1.84	19.77±2.82	.350±.023	11.23±2.10
4	20.57±2.54	19.47±4.40	.120±.026	11.85±2.45
5	21.26±3.95	16.72±1.74	.101±.041	10.46±2.31
6	18.51±1.60	17.25±2.86	-	11.13±1.09
7	18.83±1.72	18.06±3.52	.174±.092	9.92±1.56
8	19.40±2.81	17.15±1.56	.080±.027	11.56±2.46
9	19.07±2.87	15.51±3.83	.250±.098	14.60±3.56
10	20.49±3.69	19.66±1.51	.104±.031	9.40±0.80
11	18.64±1.93	16.88±2.49	.080±.023	10.82±1.82
12	21.26±4.74	18.16±2.04	.390±.103	17.66±3.88
13	24.75±3.95	17.22±1.81	.251±.052	13.10±2.47
14	14.65±5.58	16.26±2.82	.417±.182	10.35±2.85
15	23.52±4.07	22.78±2.56	.356±.196	13.40±0.87
#1 12	28.03±4.06	20.56±1.24	.089±.018	14.35±1.43
2	24.60±4.92	19.54±3.92	.212±.091	10.90±1.23
3	29.87±6.08	20.28±3.10	.168±.113	9.83±1.58

Continued .....

Table 3A (Continued)

Day of cycle	Progesterone (ng/ml)	Estrogens (pg/ml)	LH (ng/ml)	Prolactin (ng/ml)
4	29.70±5.97	19.87±1.49	.309±.136	8.73±1.89
5	23.47±3.58	18.65±2.13	.290±.068	11.97±1.58
6	24.81±3.19	18.87±3.23	.219±.079	12.02±1.43
7	28.80±6.17	18.32±1.74	.109±.020	12.02±2.20
8	28.70±4.36	19.16±1.72	.110±.030	10.42±1.25
9	26.93±4.79	19.75±1.84	.180±.099	13.12±1.81
10	26.41±4.45	18.31±2.49	.056±.002	13.20±1.06
11	26.35±5.65	18.37±1.71	.287±.108	13.40±1.67
12	24.24±5.13	22.45±3.73	.200±.052	11.72±1.71
13	27.59±3.43	16.36±4.47	.125±.031	12.10±0.66
14	30.57±1.74	17.04±1.76	.060±.000	15.86±2.65
15	27.85±4.03	17.64±2.38	.056±.003	14.33±0.88
#1 13	35.49±5.33	21.62±3.80	.066±.005	17.05±2.59
2	32.18±5.17	24.48±5.15	.227±.102	11.12±1.71
3	29.00±3.89	20.87±4.90	.084±.027	9.00±2.36
4	30.02±3.25	19.38±3.57	.375±.189	10.27±1.73
5	27.61±2.73	19.42±3.83	.246±.081	11.05±1.02
6	27.97±4.88	20.96±4.25	.089±.032	11.30±1.89
7	26.81±2.66	24.78±5.96	.127±.041	10.37±1.10
8	18.63±3.87	18.93±2.09	.169±.115	11.22±1.57
9	25.19±2.70	18.30±0.41	.090±.037	12.20±2.09
10	24.70±3.15	21.30±4.13	.056±.002	12.00±2.25
11	21.12±1.81	19.23±2.19	.116±.063	8.37±2.13
12	27.48±3.12	18.81±1.42	.102±.034	9.62±1.89
13	24.40±2.86	17.56±1.83	.201±.061	11.60±1.27
14	21.51±2.97	19.90±4.00	.121±.030	11.70±2.12
15	22.66±2.74	19.73±4.30	.132±.071	11.97±1.35
#1 14	28.01±3.29	19.29±2.79	.171±.086	14.17±1.04
2	25.67±3.12	21.71±2.69	.229±.157	13.90±0.47
3	23.65±2.75	23.39±2.93	.219±.096	12.47±2.47
4	25.97±4.67	20.13±3.02	.101±.031	13.42±3.00
5	26.12±3.88	19.70±1.80	.132±.064	10.57±1.10
6	24.00±2.14	20.44±1.59	.062±.005	11.87±1.41
7	24.27±3.02	23.13±3.33	.239±.069	12.27±1.99
8	27.79±2.75	21.69±1.49	.085±.011	13.35±1.02
9	28.96±4.30	24.19±3.82	.060±.004	11.90±2.77
10	29.62±3.34	22.46±2.44	.057±.002	13.20±1.05
11	24.51±2.21	20.86±2.77	.186±.078	11.02±2.54
12	26.17±3.69	19.36±1.78	.236±.079	11.98±0.82
13	25.03±3.12	21.08±2.06	.140±.050	13.05±1.39
14	23.66±4.18	22.36±2.63	.068±.011	14.93±0.48
15	24.86±4.49	22.30±5.12	.070±.011	18.80±1.74
15	26.73±2.25	22.37±5.41	.125±.044	12.57±2.27
-5	18.07±2.02	27.31±5.49	.169±.075	39.45±17.86
-4	13.27±4.61	30.11±2.79	.224±.067	43.45±24.86
-3	6.60±3.24	37.26±7.94	.192±.011	27.70±7.68
-2	2.92±1.12	41.63±4.07	.134±.017	16.60±6.85
-1	1.21±0.74	51.73±2.85	.207±.008	14.95±2.72
0	0.63±0.27	94.63±20.65	.526±.295	12.05±1.77

[1]On days 10 through 14, samples were collected hourly from 0800h (1) to 2200h (15).

Table 4A. Experiment I: Mean (±SE) Serum Hormone Concentration in Gilts (n = 4) during the E_2-17β Treatment Cycle, T_2.

Day of cycle[1]	Progesterone (ng/ml)	Estrogens (pg/ml)	LH (ng/ml)	Prolactin (ng/ml)
-1	2.98±1.59	-	-	-
0	0.75±0.26	84.12±16.88	.526±.294	12.05±1.77
1	1.81±1.02	68.43±18.89	.515±.176	15.67±2.27
2	1.89±0.66	46.40±18.54	.442±.013	28.20±15.91
3	4.57±1.45	44.78±21.39	.260±.053	28.85±15.38
4	12.96±4.23	31.70±12.57	.316±.091	10.10±1.12
5	18.72±3.79	45.58±12.94	.330±.063	12.20±2.77
6	21.74±4.52	46.97±7.66	.225±.050	12.00±1.50
7	28.78±3.04	39.37±6.54	.278±.062	10.70±0.38
8	30.22±2.80	42.27±9.20	.206±.099	14.05±2.81
9	33.54±4.12	42.31±8.31	.261±.139	12.80±2.72
#1 10	27.46±1.39	31.43±6.99	.108±.038	12.80±2.21
2	26.07±1.86	1179.75±161.43	.224±.070	11.82±2.97
3	24.42±2.49	1143.92±155.97	.136±.034	7.73±0.98
4	26.19±1.40	1082.55±113.85	.092±.012	12.72±1.58
5	24.67±2.64	922.45±275.79	.165±.074	13.30±4.03
6	22.74±0.69	975.70±205.03	.084±.013	15.85±0.78
7	24.53±1.88	872.54±145.88	.096±.019	11.27±2.42
8	26.39±3.39	826.07±158.23	.107±.019	13.87±1.29
9	25.65±3.64	901.32±179.04	.179±.045	13.55±1.77
10	28.15±5.36	890.81±116.49	.061±.006	10.85±2.04
11	23.46±1.53	863.65±136.14	.146±.049	13.62±3.25
12	21.82±2.86	780.72±140.33	.095±.019	14.77±1.80
13	20.58±2.36	734.65±97.75	.056±.003	14.80±2.77
14	27.62±4.62	569.95±81.99	.070±.009	19.20±2.95
15	24.45±3.51	525.14±89.00	.062±.006	10.40±1.53
#1 11	27.43±8.06	311.45±83.63	.066±.009	11.10±2.78
2	22.14±0.51	1111.09±168.06	.079±.012	11.00±1.77
3	24.03±2.74	1035.53±127.19	.062±.009	13.35±1.31
4	21.93±4.38	1132.54±141.46	.085±.027	11.25±1.92
5	21.14±3.15	1168.14±203.83	.090±.021	15.07±4.19
6	22.43±2.25	981.44±142.69	.079±.025	13.20±2.91
7	20.67±2.00	994.14±112.78	.089±.012	11.92±2.48
8	21.07±3.32	1017.07±136.50	.079±.014	10.65±2.12
9	24.56±4.93	932.64±112.21	.055±.003	11.45±1.84
10	23.17±3.64	841.83±104.72	.055±.003	13.45±0.98
11	20.75±2.32	759.12±114.01	.061±.008	13.85±0.92
12	22.39±3.01	756.34±101.25	.056±.002	12.45±1.69
13	28.80±3.80	756.80±122.56	.064±.011	13.65±2.93
14	25.34±2.49	797.13±144.35	.067±.011	12.20±2.40
15	27.67±2.17	744.74±125.70	.061±.005	10.80±2.69
#1 12	30.24±4.05	481.65±117.77	.061±.006	15.55±1.63
2	32.66±4.06	1257.06±170.32	.082±.009	14.00±1.43

Continued .....

Table 4A (Continued)

Day of cycle	Progesterone (ng/ml)	Estrogens (pg/ml)	LH (ng/ml)	Prolactin (ng/ml)
3	26.19±1.45	1270.79±145.67	.090±.024	13.05±2.04
4	23.07±4.15	1101.58±145.93	.064±.007	15.55±1.57
5	24.45±3.22	1037.80±143.29	.057±.002	12.67±1.90
6	24.42±3.01	1023.52±142.60	.065±.009	12.27±1.83
7	24.49±1.39	978.99±140.34	.087±.024	11.62±1.87
8	27.60±2.27	1045.45±133.49	.100±.029	12.75±1.08
9	25.62±2.75	1034.78±88.39	.072±.016	8.65±2.25
10	26.44±3.09	814.54±209.27	.056±.002	10.45±2.20
11	25.17±1.70	1025.14±82.17	.062±.007	12.56±2.79
12	24.12±2.55	921.85±97.48	.068±.014	13.80±0.30
13	22.60±1.59	944.92±52.21	.065±.010	12.73±1.62
14	25.74±1.68	875.31±40.36	.057±.003	13.00±0.98
15	22.64±1.07	897.97±76.46	.068±.014	13.53±0.67
#1 13	32.54±2.77	372.00±116.84	.056±.002	14.07±1.27
2	25.24±0.66	1380.26±164.69	.095±.045	16.93±1.79
3	29.09±4.84	1181.39±252.57	.053±.003	12.33±0.81
4	21.02±2.51	1189.81±121.13	.057±.003	12.60±1.03
5	23.75±1.53	1119.38±191.66	.152±.062	13.66±1.35
6	23.69±1.07	1023.78±125.56	.072±.012	12.60±1.11
7	23.67±1.86	1094.61±62.30	.057±.003	15.86±3.15
8	24.57±1.64	1000.77±49.15	.053±.003	12.16±3.12
9	24.02±1.74	1020.81±14.76	.053±.003	14.26±1.18
10	23.81±1.87	894.59±113.90	.065±.010	10.33±2.68
11	23.54±1.76	921.07±44.98	.053±.003	9.93±1.27
12	23.83±1.36	767.09±18.48	.055±.005	13.20±1.40
13	22.99±1.16	740.42±81.43	.068±.013	10.16±0.86
14	21.05±1.44	691.21±72.78	.067±.017	12.46±1.96
15	23.65±2.86	642.39±67.55	.055±.005	14.43±2.72
#1 14	26.30±2.10	516.16±77.07	.057±.003	13.73±2.96
2	23.38±0.18	1210.18±62.34	.142±.092	11.40±1.40
3	22.79±2.64	1088.80±66.57	.058±.008	10.66±0.35
4	30.23±5.64	1071.41±71.09	.118±.063	11.43±1.96
5	27.11±2.04	964.02±107.80	.077±.022	11.73±1.36
6	22.17±2.98	963.77±116.47	.053±.003	15.93±1.92
7	28.43±4.98	859.56±117.21	.053±.003	11.46±1.73
8	27.40±3.43	957.28±48.88	.053±.003	13.26±0.89
9	25.53±1.39	989.74±200.50	.065±.015	10.30±1.50
10	23.74±2.75	865.99±83.28	.053±.003	13.00±1.03
11	25.50±2.10	781.60±139.96	.053±.003	13.56±1.08
12	28.85±3.95	691.91±166.74	.053±.003	12.06±0.78
13	24.31±1.65	618.25±174.43	.088±.036	12.46±1.44
14	22.32±2.19	518.35±94.61	.095±.040	13.20±1.74
15	24.34±2.21	571.29±77.80	.053±.003	13.26±1.42
15	28.51±1.80	361.80±66.37	.056±.004	10.17±2.37

Continued .....

Table 4A (Continued)

Day of cycle	Progesterone (ng/ml)	Estrogens (pg/ml)	LH (ng/ml)	Prolactin (ng/ml)
16	27.36±2.77	200.76±20.26	.057±.002	9.00±0.86
17	22.74±2.60	135.58±19.38	.056±.004	10.12±2.55
18	21.13±2.03	94.82±7.80	.057±.005	13.02±2.16
19	19.17±1.83	76.21±7.59	.079±.011	11.46±0.93
20	19.39±0.96	72.31±14.49	.259±.068	13.70±2.30
21	15.86±3.65	74.96±24.45	.270±.080	21.80±4.51
22	12.10±3.92	44.30±5.42	.210±.074	34.20±19.46
-5	10.41±2.56	53.00±5.78	.312±.112	19.40±3.78
-4	6.54±3.73	51.17±4.32	.399±.010	48.45±19.33
-3	4.38±2.67	39.86±9.27	.260±.076	15.55±2.23
-2	1.82±0.57	87.42±26.63	.254±.055	11.23±0.63
-1	1.23±0.51	80.88±17.58	.282±.039	11.07±1.20
0	1.39±0.77	97.18±15.00	.495±.135	18.73±5.15

[1]On days 10 through 14, samples were collected at 0800h (1) then hourly until 2200h (15).

Table 5A. Experiment II: Mean (±S.E.) Serum Hormone Concentrations in Control (Group II) and E_2-17β-Treated (Group IV) Gilts

Day of cycle[1]	Group II				Group IV			
	Progesterone (ng/ml)	Estrogens (pg/ml)	LH ng/ml	Prolactin (ng/ml)	Progesterone (ng/ml)	Estrogens (pg/ml)	LH ng/ml	Prolactin (ng/ml)
10 #1	13.27±.63	49.40±36.67	.47±.21	20.00±4.27	10.53±3.57	47.40±15.53	.20±.11	11.80±2.27
2	9.75±1.71	31.85±7.91	.35±.09	19.20±4.40	7.81±2.34	778.97±210.57	.21±.10	16.60±5.33
3	9.45±.95	37.76±19.09	.32±.10	15.66±2.59	8.59±2.28	528.75±120.99	.14±.05	14.40±2.41
11 #1	13.18±1.12	64.91±50.15	.09±.01	14.00±1.96	9.84±1.56	224.00±24.15	.07±.01	16.20±1.67
2	10.77±.17	57.07±37.10	.46±.25	13.80±.61	7.68±1.83	749.00±131.13	.05±.00	15.80±5.17
3	11.09±.15	48.45±30.37	.48±.22	15.86±1.21	6.92±2.44	521.18±59.16	.06±.01	17.73±2.77
12 #1	14.64±1.36	47.67±28.06	.26±.10	20.66±2.49	9.42±2.94	383.05±50.70	.12±.03	14.00±1.70
2	- -	- -	.15±.10	- -	11.03±.65	879.77±74.36	.12±.03	15.00±1.80
3	- -	- -	.45±.21	17.50±4.50	10.54±.26	672.62±7.49	.16±.09	15.40±5.43
13 #1	15.92±2.15	58.83±24.21	.22±.11	17.06±4.03	11.92±1.57	534.96±11.06	.07±.02	12.40±1.80
2	- -	- -	.50±.44	18.40±10.60	10.00±.02	870.60±117.08	.05±.00	11.73±2.09
3	11.97±1.08	81.38±42.08	.18±.04	19.66±3.39	8.65±1.04	696.95±12.96	.12±.04	15.26±3.40
14 #1	11.94±4.68	30.70±5.14	.10±.01	16.53±2.52	8.69±1.96	621.48±59.24	.06±.01	20.13±1.87
2	6.55±2.11	45.52±10.32	.14±.00	15.66±5.76	9.71±.92	762.62±144.86	.05±.00	13.80±1.55
3	4.45±1.63	57.29±3.30	.15±.05	33.46±19.26	- -	- -	.08±.03	11.80±2.80
15 am	4.76±2.91	31.30±6.46	.10±.01	15.93±3.41	9.06±1.57	425.30±93.36	.05±.00	27.46±9.46
pm	2.34±1.84	22.63±6.44	.19±.08	13.46±3.64	8.09±.93	296.87±7.91	.08±.03	20.80±1.20
16 am	2.47±1.97	20.98±7.88	.45±.22	18.00±5.80	- -	275.63±29.47	.10±.15	41.73±19.38
pm	2.32±1.82	31.46±0.79	- -	- -	6.41±.60	188.70±4.68	.08±.18	- -
17 am	1.07±.57	28.05±3.13	.14±.05	12.40±2.20	5.46±2.64	99.80±26.82	.09±.04	47.10±26.30
pm	.50±.00	26.97±1.92	.12±.01	17.90±3.10	4.83±2.17	101.24±21.00	- -	32.60±20.40
18 am	.50±.00	34.90±18.04	.26±.11	18.60±1.80	- -	- -	.10±.05	23.30±8.70
pm	.50±.00	63.45±1.41	.15±.03	17.20±0.40	- -	- -	- -	- -
19 am	.50±.00	43.52±9.09	.30±.06	28.50±19.50	- -	- -	.18±.13	- -
pm	.50±.00	28.09±4.17	.56±.29	20.50±9.90	- -	- -	- -	- -
20 am	2.24±1.74	- -	.44±.20	16.70±4.90	- -	- -	- -	- -
ovx			.27±.10	81.50±8.50	6.21±1.77	64.08±4.94	.05±.00	157.33±2.66

[1]Blood samples collected at 0830h (1), 1430h (2) and 2030h (3) or 0830h (am) and 2030h (pm).

Table 6A. Experiment III: Mean (±S.E.) Serum Hormone Concentrations in Control (n = 2) and Bromocriptine (Br)-treated (n = 3) gilts

Day of cycle[1]	Progesterone (ng/ml)		Estrogens (pg/ml)		LH (ng/ml)		Prolactin (ng/ml)	
	Control	Br-treated	Control	Br-treated	Control	Br-treated	Control	Br-treated
4 am	7.34±0.29	4.77±2.58	29.37±2.85	25.07±.83	.29±.01	.30±.03	13.65±2.05	12.96±2.76
pm	6.34±0.20	4.87±2.02	24.08±9.88	35.40±10.13	.12±.04	.21±.04	11.45±3.75	8.60±1.34
5 am	7.81±1.42	7.58±2.26	35.29±5.95	23.58±5.82	.16±.05	.28±.01	13.35±.25	11.16±3.00
pm	7.75±0.35	7.65±2.19	41.62±3.55	21.39±6.34	.37±.10	.19±.08	9.05±3.85	7.86±2.48
6 am	8.11±0.40	9.31±1.70	46.98±1.39	22.26±5.00	.38±.02	.25±.10	11.50±.70	9.26±2.31
pm	11.54±1.09	8.86±2.35	27.97±5.53	21.17±6.29	.32±.12	.23±.07	12.40±3.60	8.46±1.32
7 am	11.29±1.45	9.28±2.25	37.04±6.35	25.60±4.88	.15±.03	.25±.11	8.60±1.00	6.63±.91
pm	9.48±1.33	8.73±1.92	31.51±5.46	23.91±5.83	.55±.25	.32±.10	13.30±2.70	6.83±.74
8 am	8.83±0.29	10.51±1.67	47.58±11.53	46.66±14.19	.13±.08	.20±.03	10.15±1.75	6.46±1.52
pm	9.25±0.35	9.62±1.40	33.46±.02	35.38±11.87	.43±.18	.26±.05	12.75±.15	9.20±2.43
9 am	10.30±1.04	10.61±1.36	38.19±10.25	38.80±5.71	.10±.05	.25±.10	11.00±1.40	7.66±1.81
pm	10.51±0.19	9.85±1.91	36.25±3.31	34.72±5.81	.37±.00	.38±.21	7.75±.65	9.13±.56
10 am	10.09±0.38	11.77±2.05	39.32±8.98	39.37±8.73	.15±.05	.24±.03	10.40±3.00	9.20±.80
pm	9.85±1.77	10.01±2.49	35.25±11.66	22.97±4.37	.70±.58	.20±.05	10.60±3.40	8.43±.42
11 am	8.49±2.12	9.79±1.11	40.57±4.73	29.59±5.36	.18±.01	.23±.06	11.65±2.25	6.83±1.41
ovx	9.07±.03	10.27±.22	48.58±7.90	39.66±5.27	.13±.06	.20±.06	71.00±39.00	6.90±.25
pm	.44±.13	- -	51.19±.03	- -	.81±.58	- -	46.50±33.50	- -
12 am	N.D. -	N.D. -	35.98±3.41	23.32±6.25	.57±.12	.31±.08	15.10±4.90	9.30±2.60

[1]Blood samples collected at 0830h (am) and 2030h (pm).

N.D. = non detectable.