INFLUENCE OF ESTRADIOL ON SERUM LUTEINIZING HORMONE CONCENTRATIONS IN THE PREPUBERTAL GILT

By

THOMAS ANDREW HOAGLAND

Bachelor of Science
University of Connecticut
Storrs, Connecticut
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Master of Science
University of Connecticut
Storrs, Connecticut
1976

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Thesis Approved:

Robert P. Wettemann
Thesis Adviser

Richard Hintz

Milton Wells

E. J. Turman

Duane T. Gardner

Norman N. Durham
Dean of Graduate College

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CHAPTER I

INTRODUCTION

Age at puberty in farm animals is an economically important factor. Each day that an animal is unproductive because it has not attained puberty increases the maintenance charges and fixed costs that must be divided among all the animals in the herd. Intensively raised livestock, such as swine, are affected more dramatically than other species because of greater fixed costs. The total efficiency of the operation is decreased each day that puberty attainment is delayed. Confinement of swine has become an integral component of an efficient operation as production has intensified. Some gilts raised in confinement experience altered reproductive efficiency, namely delayed puberty (Jensen et al., 1970; Christenson and Ford, 1979). Therefore, information explaining the sequence of events which culminate in puberty is necessary for the development of treatment regimens to induce puberty in anestrous gilts.

Gilts usually ovulate more ova during their third estrus, compared to their pubertal estrus. A gilt that reaches puberty by 200 days of age will experience at least three estrous periods by 265 days of age and could be bred to farrow by 400 days of age. Gilts that farrow their first litter between 300 and 400 days of age compared to gilts that farrow their first litter after 400 days of age will have a longer

productive life and be able to raise more pigs per year (Chapman et al., 1978). If puberty could be induced by 5 to 6 months of age in gilts, their lifetime production would be increased. Information concerning the prepubertal period may permit breeding of gilts at younger ages and increase lifetime production.

An important physiological mechanism present in adult cycling female mammals is the biphasic luteinizing hormone response to estradiol (Vilchez-Martinez et al., 1974). It is this mechanism that causes the synchronization of estrus and ovulation. This biphasic luteinizing hormone response to estradiol becomes operable prior to puberty attainment in female rats (Vilchez-Martinez et al., 1974). The age when estradiol can elicit a biphasic LH response in prepubertal gilts is not known. Therefore, these experiments were designed to determine what influence estradiol has on the release of luteinizing hormone from the anterior pituitary of gilts at approximately 10 and 20 weeks of age. A second objective was to determine the influences of estradiol on the gonadotropin releasing hormone induced release of luteinizing hormone from the anterior pituitary in prepubertal gilts.

CHAPTER II

LITERATURE REVIEW

Puberty in General

Puberty has been defined by Marshall (1922) as the period at which the organism becomes sexually mature. Crew (1931) and Asdell (1946) more clearly defined puberty as the time at which reproduction first becomes possible. Puberty in all further discussions in this dissertation will be defined in the female as the time of the first ovulation that is associated with estrus. When considering the male it is very difficult to determine when puberty has occurred. Therefore the various criterion used in the different experiments will be indicated when the male is discussed.

Age and weight at puberty of various mammals have been extensively studied. The age at first estrus in the bovine female is markedly influenced by breed and level of nutrition (Hansel, 1959). Similarly in bulls a low plane of nutrition delays the age to the first ejaculation (MacDonald, 1971). The attainment of puberty in boars and gilts can be delayed by restriction of protein and energy (Robertson et al., 1951; Haines et al., 1959; Goode et al., 1960; Zimmerman, et al., 1960). Season can also influence pubertal attainment in swine. Either the season of birth (Wiggins, et al., 1950; Robertson et al., 1951; Warnick et al., 1951; Haines et al., 1958), the season in which puberty is

attained (Mavrogenis and Robison, 1976) or changes in the length of photoperiod (Mahone et al., 1979) can retard or accelerate the attainment of puberty. Seasonal influences on puberty attainment are accentuated in seasonal breeding sheep. If lambs are born late during the lambing season, they will not reach puberty during their first year of life. Whereas, lambs born early during the lambing season will be able to grow sufficiently to reach puberty in their first year of life (Joubert, 1963). In addition to the environmental influences, age at puberty is controlled by genetic factors (Zimmerman et al., 1960). Growth, especially body weight, is related to puberty. In general, animals must reach a certain percentage of their mature weight before puberty occurs (Joubert, 1963). The physiological mechanisms involved in reproductive maturation during the prepubertal period are varied, complex and associated with general growth of the organism.

The Influence of the Central Nervous System on Puberty Attainment in the Rat

Harris and Jacobsohn (1952), in their classical experiment with hypophysectomized rats, observed that if pituitary tissue from immature rats was grafted beneath the median eminence of hypophysectomized adults, reproductive function was sustained. Therefore, the mechanisms initiating and maintaining reproductive activity reside primarily in the brain. Precocious puberty occurs in mammals as a result of lesions in various parts of the brain. Lesions in the anterior hypothalamus of rats advanced the age of vaginal opening (Donovan and van der Werff ten Bosch, 1959). They concluded that the lesions destroyed a hypothalamic center that tonically inhibited follicle stimulating hormone (FSH)

secretion. Numerous studies concur that lesions in the anterior hypothalamus cause precocious puberty (Donovan and van der Werff ten Bosch, 1959; Meijs-Roelofs and Moll, 1972; Sherwood and Timiaras, 1974). Perhaps these lesions destroyed specific estradiol receptors in the hypothalamus (Eisenfeld and Axelrod, 1967; Kato and Ville, 1967; Ginsburg et al., 1972; Plapinger and McEwen, 1973). Serum luteinizing hormone (LH) and FSH were not altered in immature female rats after lesions in the anterior hypothalamus (Advis and Ramirez, 1977). Yet, they observed an increase in uterine weight and histological evidence of follicular development, which indicate an increase in gonadotropin secretion. They offered as an alternate hypothesis, that the lesions increased the release of other pituitary hormones such as growth hormone or prolactin which could alter the uterine and ovarian responsiveness to unaltered concentration of gonadotropins. Flerko (1975) indicated that isolation of the hypophysiotropic area (HPA, part of the hypothalamus located above the median eminence) from higher brain areas induced slight changes in the function of the testis, thyroid and adrenal cortex of adult rats. Spermiogenesis was maintained, LH content of the anterior lobe of the female increased and castration cells were formed in the pituitary after gonadectomy. He concluded the HPA is able to maintain and regulate, to some extent, the basal (tonic) secretion of the tropic hormones. When the preoptic anterior hypothalamic area (PAHA) was separated from the HPA there were no ovulations or corpora lutea formation. Thus the ovulatory surge of LH was abolished. Control of the endocrine system by the central nervous system is an integral part of adult reproductive activity. Factors that alter the nervous system during the transient changes occurring before puberty

are not known. In some manner, the higher brain centers are changed at puberty.

Pituitary Involvement in the Attainment of Puberty

The pituitary gland is composed of three gross anatomical lobes; the anterior, intermediate, and posterior lobes. The anterior and intermediate lobes originate from the ectoderm of the roof of the mouth, whereas the posterior lobe arises from primitive nervous tissue. The anterior and intermediate lobes migrate toward the posterior lobe and eventually culminate as two separate functioning endocrine glands. The posterior pituitary is composed mostly of nervous tissue and is under direct nervous control. In contrast the anterior and intermediate lobes are very vascular and are controlled via the actions of releasing and inhibiting peptides produced by the hypothalamus. Hypothalamic portal blood vessels in the rat are present at 21 days of gestation and the median eminence capillary network is nearly complete by the 5th day after birth (Glydon, 1957). Luteinizing hormone releasing hormone concentration in hypothalamic tissue of 25 day old rats is similar to the concentration in adults (Ramirez and Sawyer, 1966) and follicle stimulating hormone releasing hormone activity was present by 10 days of age (Kragt and Dahlgren, 1972). Peripheral concentrations of gonadotropin releasing hormone (GnRH) were similar in heifers two months prior to the first ovulation and at the first ovulation (Gonzalez-Padilla *et al.*, 1975a).

Peripheral concentrations of LH and FSH in rats (Dohler and Wuttke, 1975) and sheep (Foster *et al.*, 1975; Lee *et al.*, 1976a) are

greater before puberty than after puberty. Serum LH, FSH and prolactin concentrations were greater before puberty than after puberty in heifers. Average serum LH concentrations in bulls increased steadily until puberty (Lunstra et al., 1978). Serum LH concentrations were greater and more erratic in prepubertal than postpubertal gilts (Pomerantz et al., 1975; Foxcroft et al., 1975). Therefore, gonadotropic hormones are synthesized and released in a more variable manner in the prepubertal animal when compared to the adult.

The prepubertal animal has an operable portal system and concentrations of releasing hormones similar to the adult animal. They also can synthesize and release pituitary hormones which can sustain reproductive function in the hypophysectomized adult. Therefore, I conclude that the immature pituitary is capable of synthesizing and releasing the hormones necessary for normal reproductive function.

Gonadotropin Concentrations During Development in the Rat

Serum prolactin concentrations in male rats increase gradually from about 25 days of age to adult concentrations between 55 and 70 days of age (Dohler and Wuttke, 1975). In female rats, prolactin concentrations increase as in the male until a few days prior to vaginal opening when prolactin concentrations become quite variable (Dohler and Wuttke, 1974; Wuttke, et al., 1976; Dohler and Wuttke, 1975; Parker and Mahesh, 1976). The increasing serum prolactin concentrations may inhibit phasic LH release which may prevent early puberty (Wuttke et al., 1976).

Follicle stimulating hormone concentrations in neonatal male rats are generally less than in adult males and less than in neonatal female

rats (Dohler and Wuttke, 1975; MacKinnon et al., 1978). Serum FSH concentrations in male rats increase between 25 and 45 days of age after which serum FSH decreases to adult concentrations (Parker and Mahesh, 1976; Ojeda et al., 1976). Follicle stimulating hormone concentrations are greater in the neonatal female as compared to adults. Follicle stimulating hormone concentrations increase between 7 and 20 days of age and decrease to adult diestrus values before vaginal opening (Dohler and Wuttke, 1975; MacKinnon et al., 1978). The increase in FSH and LH during days 7 to 20 after birth in the female do not occur in the male and may be the first indication of sex differences. Serum LH concentrations were low in the male rat with 4% of the animals possessing elevated concentrations (MacKinnon and Mattock, 1976). These elevations lasted about one hour and occurred at any time of the day. Whereas the prepubertal female rat possessed greater average LH concentration (Dohler and Wuttke, 1975) with 7% of the animals having elevated concentrations (Mackinnon and Mattock, 1976). An estrogen induced LH surge occurs in the female rat during the first proestrus and serum concentrations of LH are similar to those in adults (Meijs-Roelofs et al., 1975; Parker and Mahesh, 1976; Ojeda et al., 1976).

Gonadotropin Concentrations During Development in the Ovine

Plasma FSH concentrations in rams are low at birth and increase to a maximum that is greater than in adults, by 5 weeks of age (Lee et al., 1976a). Peripheral FSH concentrations in ewe lambs are also low at birth and gradually increase to a maximum at 20 weeks of age (Foster

et al., 1975). After reaching maximal values, FSH concentrations decrease in both sexes to adult concentrations by the initial estrus or 45 weeks of age. Serum LH concentrations follow the same trends as FSH concentrations; they increase to a maximum by five weeks of age in rams and 20 weeks of age in ewes. The maximum LH values obtained prepubertally were similar to concentrations in adult rams and less than the concentrations at the initial ovulatory surge in ewes. Lee et al. (1976b) observed increased release of FSH and LH after GnRH in five week old rams compared to younger and older prepubertal rams. This augmented gonadotropin release was coincidental with the increase in LH and FSH concentrations during development.

Gonadotropin Concentrations During Development in the Bovine

Plasma LH concentrations in bulls increase linearly until puberty, then average plasma LH remains constant (MacMillan and Hafs, 1968; Mongkonpunya et al., 1973; Lunstra et al., 1978). Luteinizing hormone concentrations in prepubertal heifers (Gonzalez-Padilla et al., 1975a) fluctuate similar to the fluctuations discussed in prepubertal female rats (MacKinnon et al., 1978). The variation in LH concentrations in prepubertal heifers was composed of increases lasting approximately one hour that reached adult concentrations (Gonzalez-Padilla et al., 1975a). One week prior to the first spontaneous estrus, LH concentrations declined and remained low (Gonzalez-Padilla et al., 1975a). Follicle stimulating hormone concentrations in these heifers were variable and prolactin increased gradually to a maximum eight days before estrus.

Gonadotropin Concentrations During Development in the Porcine

Average serum LH concentrations in prepubertal miniature boars remained constant for the first 12 weeks of life (Elsaesser et al., 1976). Serum LH concentrations were greater during the night with a tendency for more increases greater than 1 ng/ml at night than in the daytime in 60 and 160 day old boars (Elsaesser and Parvizi, 1979). Serum LH concentrations declined from birth to 10 weeks of age in prepubertal miniature gilts (Elsaesser et al., 1976). Spontaneous LH release was observed in two month old prepubertal gilts (Foxcroft et al., 1975). The release consisted of 1.3 LH peaks greater than 1 ng/ml per hour.

Testicular Development in General

Differential development of the gonads occurs during the first trimester of embryonic development. If the gonad is destined to be a testis, the cells of the germinal epithelium become organized within the medulla of the gonad. Ovarian development does not occur as soon as testicular development. Ovarian development begins when the cortex of the gonad accumulates nests of cells which are differentiated into ovarian follicles and each follicle contains an ovum. Removal of the gonad from the early embryo of either sex prior to the development of the genital ducts results in the development of female internal and external genitalia, regardless of the genetic sex of the embryo. Thus it appears that the testis secretes a substance which causes the development of the male tract.

The testis is composed of seminiferous tubules which are separated

by interstitial tissue and Leydig cells. At birth, the small seminiferous tubules have not formed a lumina. They contain only spermatogonia. Leydig cells are influenced by the dams endocrine system and are identifiable at birth but regress during the early postnatal period and do not become functional again until prior to the first ejaculation. If the gonads of immature animals are transplanted into mature animals they function as mature gonads. However, if the gonads from mature animals are transplanted into immature animals they become quiescent and atrophy. Therefore lack of responsiveness of the gonads to tropic hormones cannot account for the lack of sexual maturation.

Testicular Development in the Rat

Testicular weight in the male rat relative to body weight increases from about 20 days of age to a plateau between 40 and 80 days of age (Ewing et al., 1966; Desjardin et al., 1968). This weight increase is associated with increases in both width and length of the seminiferous tubules (Krueger et al., 1974; de Jong and Sharpe, 1977). The numbers of Sertoli cells and spermatogonia increase rapidly between 20 and 40 days of age. The number of spermatids also increase from 25 to 75 days of age and mature spermatogonia are present in the reproductive tract at 50 days of age (Clermont and Perey, 1957; Clegg, 1960; Swerdloff et al., 1971; de Jong and Sharpe, 1977).

Androstenedione is secreted by immature rat testes in greater quantities than testosterone (Nayfeh and Baggett, 1966; Moger, 1975). Testicular steroid metabolism changes at about 40 days of age and testosterone output increases relative to androstenedione.

Testicular responsiveness to gonadotropic hormones in rats varies with age (Odell and Swerdloff, 1976). Rats that were hypophysectomized and did not experience elevated serum FSH concentrations did not respond to LH administration with increased release of testosterone as they matured. Therefore, these workers concluded that the increased concentrations of FSH during 20 to 40 days of age stimulates testicular LH receptors (Greenstein, 1978).

Testicular Development in the Ram

Sertoli cells in the testes of developing rams mature during 17 to 21 weeks after birth in nonseasonal breeding sheep (Lee et al., 1976a) and the activation of spermatogenesis follows with the presence of spermatozoa by 39 to 42 weeks of age (Lee et al., 1975; Lee et al., 1976a). Plasma testosterone concentrations are low at birth and increase gradually from the fifth week to the twenty fifth week of age (Lee et al., 1975), which follow the maturational changes in the Sertoli cells and the increase concentrations of serum FSH and LH. Plasma testosterone then increases rapidly to 2.7-5.2 ng/ml by the forty first week of life (Lee et al., 1975; Lee et al., 1976a), at which time LH concentrations attain their maximum and spermatozoa were present in the seminiferous epithelium (Skinner et al., 1968; Lee et al., 1976a).

Testicular Development in the Bull

Average concentrations of serum testosterone increased linearly between 7 and 13 months of age and were related to an increase in average serum LH concentrations (Rawlings et al., 1972; Secchiari et al., 1976; Lunstra et al., 1978). Mongkonpunya and co-workers (1975)

indicated that the increases in serum LH concentrations prior to six months of age in Holstein bulls did not stimulate increased testosterone secretion. Leydig cells become more responsive to LH stimulation as puberty approaches (Mongkonpunya et al., 1975; Lunstra et al., 1978). Androgens are secreted by prepubertal bulls (Linder and Mann, 1960). Prior to 4 months of age androstenedione was the predominant steroid secreted by the testes (Lindner, 1959; Lindner and Mann, 1960). Androstenedione concentrations were 32, 17 and 7% of the testosterone concentrations in plasma of 2, 4 and 6 month-old bulls (Mongkonpunya et al., 1975). The Leydig cells differentiate after four months of age in bulls (Hooker, 1974). Spermatogonia and a small number of primary spermatocytes are present in the seminiferous tubules at two months of age, whereas, spermatozoa first appeared at 7 months of age (Schanbacher, 1979).

Testicular Development in the Boar

The seminiferous tubules in boar testes develop a lumen and the testes enlarge in volume, increase in weight and decrease in firmness with age until puberty (Esbenshade et al., 1979; Mahone et al., 1979). Primary spermatocytes first appeared at 3 months of age, secondary spermatocytes at 4 to 5 months of age and mature spermatozoa at 5 to 6 months of age (Hauser et al., 1952). Average plasma testosterone concentrations increased from birth until the second and third weeks after birth and subsequently declined until the fifth week of age (Colenbrander et al., 1978). Then average plasma testosterone increased gradually until the 18th week of age, after which it increased rapidly to adult concentrations. Changes in prepubertal testosterone concentrations are compatible with changes in testicular testosterone

concentrations (Booth, 1975; Segal and Raeside, 1975), steroid-histochemical activity (Worbel et al., 1973; van Straaten and Wensing, 1977) and morphological differentiation (van Straaten and Wensing, 1977) of the testis.

Ovarian Development in the Rat

Rat ovarian weight, relative to body weight, increases slowly from birth to about 20 days of age (MacKinnon et al., 1978). This increase in ovarian weight is due to an increase in interstitial tissue and not to follicular growth (Peluso et al., 1976). After day 20, ovarian weight increases more rapidly and attains a plateau near puberty, at about 40 days of age (MacKinnon et al., 1978). In the rat ovary at birth, all oocytes are in the resting prophase and are enclosed in small follicles. Soon after birth, some follicles begin to grow; this follicular growth can occur at any age (Peters, 1976).

Response of the immature rat ovary to gonadotropins varies with age (Rennels, 1951). Ovaries of infantile rats (0 to 6 days of age) fail to respond to pregnant mare serum gonadotropin (PMSG) while ovaries of young rats (7 to 15 days of age) become heavier and synthesize steroids when exposed to PMSG. Before puberty (16 days of age to the first ovulation) PMSG injections cause an increase in follicular growth, estrogen synthesis and ovulation (Rennels, 1951; Hillensjo et al., 1974). The binding of LH to immature rat ovarian granulosa cells increases with advancing age, but FSH binding remained relatively constant between day 5 and 33 with a significant increase between days 33 and 38 (Peluso et al., 1976). The responsiveness of ovarian adenyl cyclase to LH, FSH and prostaglandin E, was acquired by day 10 which

coincides with the onset of the ability to produce steroids in response to gonadotropins (Hunzicker-Dunn and Bernbaumer, 1976). Total plasma estradiol is lower during 20 to 40 days of age than in adult rats (Dohler and Wuttke, 1975). Less plasma estradiol is bound to alfa-fetoprotein as the animal matures thus increasing the concentration of unbound active hormone while total plasma estradiol concentration decreases (Raynaud et al., 1971). The increasing free estradiol concentrations may cause an increase in the number of granulosa cells and thus an overall increase in FSH receptors per ovary (Louvet and Vaitukaites,1976; Richards et al., 1976).

The administration of prolactin to female rats causes the advancement of the onset of puberty (Voogt et al., 1969; Wuttke and Gelato, 1975; Wuttke et al., 1976). When prolactin was implanted in the medial basal hypothalamus it inhibited endogenous prolactin secretion and increased LH and FSH release (Clemens et al., 1969; Voogt and Meites, 1971; Voogt et al., 1969). Thus prolactin may have a direct stimulatory effect on release of gonadotropin releasing hormone and reduce the age to puberty. More recently, it was indicated that prolactin may sensitize the ovary to endogenous gonadotropins (Advis and Ojeda, 1978). Hyperprolactinemia in prepubertal rats may also stimulate adrenal progesterone production (Advis and Ojeda, 1978). Increasing estradiol and prolactin concentrations in the immature rat may increase the sensitivity of the ovary to gonadotropins which stimulates the ovary to ovulate and luteinize.

Ovarian Development in the Ewe

Ovaries in prepubertal ewes synthesize estradiol and very little progesterone (Foster et al., 1972 and 1975). Superovulation can be

induced in ewe lambs by treatment with gonadotropins (Mansour, 1959). Although there was no response when gonadotropins were given at one week, by four weeks of age follicular stimulation occurred. Ovulation was induced at 8 weeks of age when PMSG and human chorionic gonadotropin (HCG) were both administered and at 16 weeks of age by PMSG and/or HCG administration. A sustained gonadotropin (LH and/or FSH) release was induced by infusion of GnRH for 72 hours in 5 month old ewe lambs but no significant changes in ovarian morphology occurred (Mayar et al., 1978). Yet, treatment of ewe lambs with pituitary extracts for 72 hours resulted in increased ovarian weight, increased numbers of corpora lutea and increased numbers of follicles greater than 8 mm. Ova aspirated from 4 to 7 month old ewe lambs following PMSG administration did not develop past the early blastocyst stage. However, ova from adult ewes treated similarly developed into blastocysts and had a significantly increased cleavage index (Wright et al., 1976). The reasons for the differences in the ova and the ovarian response to endogenous gonadotropins in prepubertal ewes as compared to the adult ewes is not known.

Ovarian Development in the Cow

Plasma estradiol concentrations in heifers average about 30 pg/ml about 50 days prior to the initial estrus and gradually decreased to 20 pg/ml by the first estrus (Gonzalez-Padilla et al., 1975a). Progesterone concentrations are low and increases less than 1 ng/ml occur about 15 days prior to estrus (Gonzalez-Padilla et al., 1975a). Puberty can be induced in some heifers with either gonadotropins (Foote, 1972) or steroids (Gonalez-Padilla et al., 1975c). The

response to treatment to initiate puberty is usually better as the heifers are nearer the age of spontaneous puberty. These data are consistent with the view that a certain steroidal environment is required to alter the pituitary release of gonadotropins (Greenstein, 1978) and increase the sensitivity of ovarian tissue to gonadotropins (Goldenberg et al., 1972).

Ovarian Development in the Gilt

The ovary of the prepubertal gilt is similar to other species in that it can be stimulated to develop mature follicles and ovulate (Casida, 1935; Bhalla et al., 1969). Circulating gonadotropins are at concentrations similar to those in adults, however, they are not released in the typical adult cyclic pattern (Foxcroft et al., 1975; Pomerantz et al., 1975; Elsaesser et al., 1976). Circulating estradiol values between 5 and 37 pg/ml were observed one week prior to spontaneous puberty (Shearer et al., 1972). Therefore the ovaries of these gilts responded to endogenous gonadotropins and were synthesizing estradiol. Ovulation can be induced in 155 day old gilts with PMSG followed by either HCG or a GnRH analog (Rampacek et al., 1976). When gilts were mated, the number of gilts pregnant at 25 days increased as the average age at treatment increased to 175 days. Progesterone and estradiol administered after induced ovulations in prepubertal gilts increases the number of gilts pregnant at 25 days of gestation (Shaw et al., 1971; Ellicott et al., 1973; Segal and Baker, 1973; Rampacek et al., 1976). This indicates that the ovaries of immature gilts are capable of responding to gonadotropins yet the necessary steroid hormones are not produced to maintain pregnancy. Removal of the

uterus allowed the induced corpora lutea to function normally (Rampacek et al., 1976) and significantly less uterine tissue was required to cause luteolysis in prepubertal gilts than adult cycling gilts (Puglisi et al., 1978). The data indicate that the prepubertal gilt can respond to exogenous gonadotropins with ovulation and estrus but, certain unknown maturational changes must occur in the ovary before spontaneous puberty is attained.

Adrenal Influences on Puberty Attainment in the Rat

Endocrine glands other than those normally considered as part of the reproductive system may be involved in sexual maturation. The adrenal glands can synthesize androgens, progesterone and estrogens. Since if the adrenals are removed before 26 days of age puberty is delayed (Ramaley, 1973), the adrenals may have a role in pubertal development.

Estrogens from the prepubertal adrenal gland behave similar to standard preparations of estradiol and other estrogens during column chromatography and in some radioimmunoassays, but lacked biological activity when assayed in a uterine cytosol estrogen receptor system (Weisz and Gunsalus, 1973). The role of adrenal progesterone in puberty attainment is not clear (Ramaley and Bartosik, 1974; Meijs-Roelofs et al., 1975a). Meijs-Roelofs and Moll (1978) in their review of sexual maturation and the adrenal gland summarized the adrenal contribution as not being an essential element in the maturation of the reproductive system. However, they indicated that since the adrenal

gland contributes to the general metabolic process, it may be important in reproductive maturation. The rat has a biological clock, under the direction of circadian rhythms in the pituitary-adrenal axis, which becomes functional at 26 days of age (Ramaley, 1973; 1975). The timing of waves of follicular growth, estradiol secretion, LH release and ovulation become more exact during the prepubertal period and can be linked to a biological clock (Ramaley, 1979). Whether or not the development of adult biorhythmicity plays a role in puberty onset requires more research.

Relationship of Steroid Receptors to Puberty

Determination of tissue hormone receptors in addition to plasma hormone concentrations may improve the understanding of physiological processes (Thomas, 1973; Gorski and Gannon, 1976). Studies to determine the relationship of hormone receptors to the attainment of puberty have been initiated in some species.

Most steroidal hormones penetrate the cell membrane and are bound tightly but reversibly to a cytoplasmic receptor protein. This activated complex then translocates to the nucleus and gene expression is modified (Greenstein, 1978). Estrogen receptors in the hypothalami of rats are first measurable between days 12 and 20 of postnatal life (Eisenfeld, 1972). The number of hypothalamic receptors are similar to that in adults by day 22 of age, at which time the positive estrogen feedback system becomes operable (Ginsburg et al., 1972). Concentrations of tritiated estradiol bound to the hypothalami, preoptic areas and amygdala of 24 day old rats are similar to those in adult animals. This

increase in estrogen binding correlates with the disappearance of α-fetoprotein from developing female rats and the increase in free estradiol concentrations (Greenstein et al., 1977).

Androgen binding proteins have been found in the cytosol of the prostates and seminal vesicles from 20 day old rats and uptake of tritiated testosterone in brain tissues of 11 day old males was similar to that in adults (Eisenfeld, 1972). Dihydrotestosterone binding proteins were found in the cytosol from hypothalami of 7 day old male rats (Kato, 1975). Receptors for sex steroids seem to be present well before the onset of puberty. Thus it seems unlikely that any sudden change in rat steroid receptor activity precipitates the onset of puberty.

Relationship of Gonadotropin Receptors to Puberty

Gonadotropin receptors are located on the cell membrane (Greenstein, 1978). When the receptor is occupied by a specific gonadotropic hormone the adenyl cyclase system is stimulated. The subsequent alteration in the production of cyclic adenosine monophosphate causes changes in protein kinase activity (Catt and Dufau, 1976; Ryan and Lee, 1976). When ^{125}I-HCG was injected into female rats of 5 to 30 days of age, it was bound mainly to thecal and interstitial cells of the ovary and was present in very low concentrations in granulosa cells (Presl et al., 1972). Estrogen stimulation increases the number of cells that respond to FSH (Goldenburg, et al., 1972) but not the number of receptors per cell (Reiter et al., 1972). If the number of cells with FSH receptors increase after estrogen exposure then the responsiveness of the ovary to FSH will increase.

The number of LH receptors in isolated porcine granulosa cells increases 35-fold per cell as the follicle enlarges from 1-2 mm to 6-12 mm (Kammerman and Ross, 1975; Lee et al., 1976a). Estrogen stimulated its own follicular receptors and decreased LH receptors (Richards et al., 1976). Estrogen and FSH treatment results in an increase in both estrogen and FSH receptors. There seems to be an interaction between granulosa and thecal cells under the influence of estradiol and FSH (Greenstein, 1978). The net result may be the stimulation of sufficient LH receptors on the follicles to cause luteinization of those follicles which are at a critical stage of development. Atresia results if the follicle loses receptors for estradiol, FSH and LH (Greenstein, 1978).

The testes of the developing rat contains receptors for both LH and FSH. The binding of ^{125}I-LH was localized on interstitial cells of the testes of immature rats (deKretser et al., 1969). Whereas, Sharpe et al. (1973) were not able to detect specific binding of ^{125}I-LH in testicular homogenates from 22 day old animals. This may be related to the limited number of Leydig cells in 20 day old rats (Knorr et al., 1970).

Binding of FSH to testicular tissue increases from 5 to 15 days of age in rats (Desjardins et al., 1974), then no further increases occur until puberty. Follicle stimulating hormone receptors appear to be confined to the Sertoli cells (Desjardins, et al., 1974). Testicular membrane bound receptors are differentiated early in the rat testes. Greenstein (1978) indicated that the prepubertal changes in the testes may be an increase in intracellular enzymes necessary for spermatogenesis.

Pituitary Sensitivity to GnRH in the Male

The magnitude and pattern of the LH response to GnRH in immature male rats at 10 or 20 days of age are similar to those in adult rats (Odell and Swerdloff, 1976). Follicle stimulating hormone was released in greater amounts in response to GnRH in immature male rats as compared to adult rats.

Peripheral concentrations of LH and FSH in the developing ram increase during the fifth week of age (Lee et al., 1976a). There is also an increase in pituitary gonadotropin content in rams after the sixth week of life (Skinner et al., 1968). The postcastration increase in LH in rams is greater at 60 days compared to adult rams (Crim and Geschwind, 1972). Therefore, pituitary sensitivity to steroid hormones or GnRH in developing rams seems to change with age. Two to three month old rams responded to LH-RH with greater LH and FSH release as compared to younger and adult rams (Lee et al., 1976b). They suggested that this time period may represent the time of initiation of the pubertal process in the ram.

Bulls will respond to GnRH and release LH during the early prepubertal period (Mongkonpunya et al., 1973). Gonadotropin releasing hormone administered to 100 day old bull calves (Mosley et al., 1979) elicited a release of LH similar in maximum concentration and duration to the release in mature bulls (Minton et al., 1979).

In the prepubertal male pig, LH-RH administration resulted in a similar maximum release of LH as in the adult boar or castrated adult boar (Pomerantz et al., 1974). The duration of LH release was longer

for the adult boar than the prepubertal boar or adult castrated boar. This suggests that testicular hormones may influence pituitary responsiveness to GnRH in boars.

Pituitary Sensitivity to GnRH in the Female

The response to LH-RH in the prepubertal female rat is greatest at 15 through 25 days of age and decreases until 35 days of age (Debeljuk et al., 1972). Ovariectomy abolished the enhanced response at 15 days, indicating the enhancement might be steroid mediated (MacKinnon et al., 1978).

Release of LH in prepubertal ewe lambs was observed after the administration of GnRH or an LH-RH analog (Rippel et al., 1974; Mayar et al., 1978). However, ovarian alterations due to this hormonal release were not observed.

Gonadotropin releasing hormone will elicit a release of LH from heifers that are 60, 120 or 270 days of age (Moseley et al., 1979). The maximum LH concentrations obtained and the duration of the release were similar for the different ages. However, the time required to reach the maximum concentration tended to be less in the younger heifers when compared to the two older groups of heifers.

Gonadotropin releasing hormone induces an LH increase in the prepubertal gilt which is comparable to the LH increase in adults prior to ovulation (Chakraborty et al., 1973). However the induced prepubertal increase in LH had no significant effect on the ovary.

These data are conclusive in that the prepubertal animal, male and female, can release LH, and in the rat FSH, after the administration of GnRH.

Testicular Responsiveness to Gonadotropins

Gonadal responsiveness to endogenous and exogenous gonadotropins usually increases with advancing age. When the gonads of immature animals are transplanted into mature animals they function in a mature manner. Yet, when mature gonads are transplanted into immature animals the gonads become quiescent and atrophy (Gier and Marion, 1969).

Follicle stimulating hormone administered for 10, 20 or 25 days to immature male rats, that were hypophysectomized 5 days earlier, stimulated a progressively larger response of the prostate gland to a constant dose of LH (Odell and Swerdloff, 1976). Also the release of testosterone after LH injection in these rats was increased 10-fold by FSH pretreatment. Follicle stimulating hormone or FSH and estradiol stimulates the formation of LH receptors and increases the number of Leydig cells (Greenstein, 1978). As the male rat matures the testosterone response to LH administration increases (Odell et al., 1974). Therefore, circulating FSH titers may stimulate Leydig cell growth and maturation of LH receptors in the developing male rat.

Plasma testosterone concentrations increase gradually between the fifth and twenty-fifth weeks of age in rams and then testosterone increases rapidly to adult concentrations by the forty-first week after birth. These increases in testosterone coincide with increases in serum LH concentrations (Lee et al., 1976a).

The testes of the prepubertal bull (0-4 months of age) secrete greater quantities of androstenedione than testosterone (Lindner, 1959; Lindner and Mann, 1960). This difference in testicular secretion is reflected in plasma concentrations of androgens (Rawlings et al., 1972).

Bulls less than 6 months of age do not secrete testosterone in response to naturally occurring episodic LH release (Lacroix et al., 1977) or GnRH induced LH release (Mongkonpunya et al., 1973). However, bulls 2, 4 or 6 months of age did respond to GnRH induced release of LH with increased plasma androstenedione concentrations (Mongkonpunya et al., 1973). Binding of gonadotropins at the testes probably is not the rate limiting step for the initiation of testicular function in young bulls and changes that are necessary for further testicular development probably are related to changes in the intracellular testicular enzyme concentrations.

Plasma concentrations of androgens, testosterone and dihydrotestosterone, were unaltered in boars from birth to 5 weeks of age (Elsaesser et al., 1976). However, Colenbrander et al. (1978) indicated a slight increase in plasma androgens by the third week of life. Thereafter, plasma androgens remained constant until 10 weeks of age (Elsaesser et al., 1976; Colenbrander et al., 1978) and increased steadily to adult values from 10 to 24 weeks of age.

Ovarian Responsiveness to Gonadotropins

Ovarian responsiveness to LH administration, as measured by uterine growth, increases as the female rat matures (Odell and Swerdloff, 1976). As in the male, FSH stimulated similar increases in gonadal weight in 21 and 75 day old hypophysectomized rats (Odell and Swerdloff, 1976). Various hormonal treatments of FSH, LH, growth hormone and prolactin have not been able to explain the insensitivity of the immature female rat to the effects of LH administration.

Follicular development and ovulation in ewe lambs at one week of age cannot be induced by treatment with PMSG, progesterone and HCG (Mansour, 1959). However, at 4 weeks of age, follicular stimulation occurred with subsequent ovulation and fertilization when ewes were given the same treatments (Mansour, 1959). The success rate for induction of puberty with exogenous hormone therapy is related to the age of the lamb at treatment (Foote, 1972). An analog to LH-RH induces LH release in ewes but ovarian morphological changes do not occur (Mayar et al., 1978). This suggests that insufficient gonadotropins are released since a crude ovine pituitary extract increased the number of large follicles in ewe lambs of similar age and condition.

Prepubertal heifers will respond to exogenous gonadotropins. If calves are given PMSG and HCG at 1 to 5 months of age, ovulation will occur, however, treatment of calves less than a month of age does not alter ovarian weight or cause ovulation (Larsen et al., 1971). In the heifers that ovulated, the older heifers ovulated more ova than did the younger animals. Plasma estradiol concentrations increase in heifers between 5 and 9 weeks before their first estrus (Gonzalez-Padilla et al., 1975a). Therefore, the ovaries possessed the ability to respond to endogenous gonadotropins and follicular maturation occurs with estradiol synthesis and release.

Prepubertal gilts will respond to exogenous gonadotropins (Casida, 1935; Rampacek et al., 1976). Only primary follicles were found in gilts until the seventh day of life and secondary follicles were first evident at 42 days of age (Bhalla et al., 1969). Vesicular follicles were first encountered at 11 weeks of age and at that time exogenous gonadotropin treatment could elicit ovulation (Bhalla et al., 1969).

When puberty is induced in gilts with exogenous gonadotropins before 200 days of age, corpora lutea formed do not maintain pregnancy as in the adult pig (Rampacek et al., 1976). Maturational changes of the immature swine ovary must occur before the induced corpora lutea function as in the adult.

Exogenous gonadotropins will stimulate ovarian follicular growth and ovulation before puberty in all species discussed. The success of gonadotropin treatment depends on the age of the animal. If gilts are nearer in age and size to that normally observed at spontaneous puberty, there is a greater probability that ovulation and pregnancy will occur.

Influence of Steroid Hormones on Gonadotropin Secretion

Steroid hormones exert inhibitory and/or stimulatory feedback effects on gonadotropin synthesis and/or secretion from the pituitary. Plasma estradiol concentrations increase at proestrus in all species. Initially, estradiol has an inhibitory effect on LH secretion which is followed after a specific time period by a stimulatory effect on gonadotropin release. Estradiol is a regulator of the ovulatory surge of gonadotropins in cycling females. This biphasic effect (negative and positive feedback) of estradiol on gonadotropin secretion can be elicited in females and castrated males; whereas, intact males only have negative feedback. Maturation of the estradiol feedback mechanism is an integral part of puberty attainment.

Influence of Steroid Hormones on Gonadotropin Secretion in Males

Plasma LH and FSH concentrations increase within two hours after castration of male rats when they are at least 10 days of age (Swerdloff et al., 1971). The same amounts of testesterone are required to reduce the post-castration increase in LH concentrations in 10 and 21 day old rats as in 73 day old rats (Odell and Swerdloff, 1976). The effects of testosterone treatment on serum FSH concentrations in 21 and 73 day old castrated male rats is similar to that on LH.

Peripheral serum concentrations of LH increase from birth to 14 days of age in ram lambs (Foster et al., 1975). Castration results in increased serum LH and similar amounts of steroid hormone are required to decrease the LH concentration to precastration values in prepubertal and adult rams.

Serum LH increases gradually in bulls between birth and 13 months of age (Gombe et al., 1973; Mori et al., 1974; Schanbacher, 1979). A temporary increase in serum LH in bulls at 5 months was indicated by Lacroix et al., (1977). Whereas, average LH concentrations were similar from birth to adulthood when blood samples were taken more frequently (Karg et al., 1976). Serum FSH concentrations in bulls were not influenced by age and were similar between 3 and 13 months of age.

Luteinizing hormone concentrations were not altered after castration in neonatal male miniature pigs (Elsaesser et al., 1978) or in new born domestic male pigs (Ford and Schanbacher, 1977). When testosterone propionate was administered to castrated male pigs at one or 12 weeks of age, serum LH was significantly depressed in the younger pigs

and not influenced in the older boars (Elsaesser et al., 1977). However, administration of estradiol to these castrated boars resulted in no change in LH concentrations. This indicates that the effects of testosterone are probably not due to aromatization to estradiol. Since administration of testosterone at a constant amount per kg body weight to young and old boars gave different responses, this indicates that the pituitary becomes less sensitive to the negative effect of testosterone as the animals mature.

Influence of Steroid Hormones on Gonadotropin Secretion in Females

Steroid hormones inhibit gonadotropin secretion in female rats at very young ages (Goldman et al., 1971; Ojeda and Ramirez, 1972; Meijs-Roelofs et al., 1975a). During the first six hours after estrogen treatment, the inhibitory effect on LH release occurs in part at the pituitary; whereas, during 6 to 12 hours after estrogen treatment the inhibitory effect on LH secretion occurs at the hypothalamus because GnRH administration results in LH release. Castration of female rats after 10 days of age elicits an increase in serum LH and FSH concentrations (Caligaris et al., 1972). The post-castration increase is related to the pre-castration concentration of free estrogens (MacKinnon et al., 1978).

Progesterone inhibits serum LH and FSH concentrations in pre-pubertal female rats before and after castration (Caligaris et al., 1972; Odell and Swerdloff, 1976; MacKinnon et al., 1978). Progesterone administration to 20 day old rats after pretreatment with estrogen resulted in a significant increase in plasma LH and FSH within 3 days (Caligaris et al., 1972).

A single injection or implant of estradiol stimulates LH release in female rats that are greater than 22 days of age (Caligaris et al., 1972; Ojeda et al., 1976; MacKinnon et al., 1978). Release of LH and FSH after GnRH is enhanced by estrogen pretreatment (MacKinnon et al., 1978) if it is given at least 16 hours prior to GnRH administration. The first ovulatory estrus (puberty) and subsequent estruses in rats are characterized by a proestrous increase in plasma estrogen. Gonadotropin release after GnRH is greater at estrus as compared to the rest of the cycle (Castro-Vazquez and Ojeda, 1975). The effects of steroid hormones on gonadotropin release are probably mediated by the hormone receptor populations.

Estrogen treatment of prepubertal (5-6 weeks of age) and ovariectomized ewes will induce an LH release, which is similar to the preovulatory LH surge (Land, 1978). Castration of ewes greater than 2 months of age results in increased serum gonadotropins. The post-castration increase in gonadotropins can be inhibited by estrogen treatment (Foster et al., 1975). Estrogen administered to intact ewe lambs and ovariectomized ewes inhibits LH release for approximately 12 hours (Scaramuzzi et al., 1971; Coppings and Malven, 1976). Similar to studies in rats, Coppings and Malven (1976) indicated that the first six hours of inhibition of LH release occurred at the pituitary and perhaps also at the hypothalamus. During the second six hours of the inhibition of LH release, estrogens exerted their inhibitory effect at the hypothalamic GnRH releasing neurons. However, at ten to twenty two hours after treatment estrogen stimulated an LH release similar to a normal ovulatory surge of LH (Scaramuzzi et al., 1971). During the period when estrogen has a stimulatory effect on LH release, GnRH

induced LH release is also enhanced (Coppings and Malven, 1976).

Estrogen treatment also has an initial inhibitory, then a stimulatory effect on LH secretion in prepubertal heifers (Gonzalez-Padilla et al., 1975b; Swanson and McCarthy, 1978; Forrest et al., 1978; Griffin and Randel, 1978; Kiser et al., 1978; Rhodes III and Randel, 1978). The inhibition of LH and FSH occurs for approximately 10 hours after estrogen administration (Gonzalez-Padilla et al., 1975b; Kiser et al., 1978). The stimulatory effect on LH release begins at 12 hours after estrogen treatment and continues for six to ten hours (Gonzalez-Padilla et al., 1975b; Swanson and McCarthy, 1978; Forrest et al., 1978; Griffin and Randel, 1978; Rhodes and Randel, 1978); whereas, serum FSH concentrations were not significantly altered (Gonzalez-Padilla et al., 1975b). The biphasic LH response to estradiol in immature heifers is similar to the response in ovariectomized heifers (Beck and Convey, 1977) and in intact mature heifers approaching ovulation. The cause of the enhanced LH release could be increased GnRH release and/or increased sensitivity of the gonadotrophs to GnRH.

Prepubertal gilts will respond to chronic or acute estradiol treatment with reduced basal and episodic releases of LH for approximately 20 hours (Pomerantz et al., 1975; Foxcroft et al., 1975; Elsaesser and Parvizi, 1979). Luteinizing hormone release after GnRH in 2 month old gilts was also inhibited as compared to controls for approximately 4 hours after estrogen treatment (Pomerantz, et al., 1975). Augmented release of LH similar to the preovulatory surge in adults became evident 60 to 80 hours after estradiol administration (Elsaesser and Foxcroft, 1978; Elsaesser and Parvizi, 1979). This stimulation was not

noted in 6 day old gilts but was evident in 60 and 161 day old gilts given 60 μg of estradiol benzoate per kg body weight. The increase in LH concentrations in the 60 day old gilts was more variable with a slightly lower maximum concentration than in the 161 day old gilts. An explanation for the differences in positive estrogen effects in the early and late prepubertal gilts is not obvious. The concentrations of nuclear and cytoplasmic estrogen and progesterone receptors in hypothalmi and pituitaries were similar in 1, 2.5, 4, 5.5 month old gilts and for gilts just prior to their first ovulation (Diekman and Anderson, 1979). They further determined that numbers of pituitary receptors for estradiol and progesterone in mature cycling gilts were 15-fold greater than in any of the prepubertal gilts.

Based on the above data, animals require a certain maturation of the hypothalamus and pituitary to be responsive to steroid hormones. The inhibitory effects of estradiol and progesterone on gonadotropin secretion become apparent at an earlier age than the stimulatory effects. Yet, the positive effects of estradiol on LH secretion occur before puberty in rats, sheep, heifers and gilts.

CHAPTER III

MATERIALS AND METHODS

General

Yorkshire gilts, born during November and December or February and March, were used in the following experiments. Gilts were fed a 16 percent protein diet composed of corn and soybean meal ad libitum for the duration of the experiment.

Procedure

Experiment I: Preliminary Experiment to Determine the Effect of Dosage of Estradiol on Serum Luteinizing Hormone Concentrations in Prepubertal Gilts

A preliminary 2 x 2 factorially designed experiment (Table I) was performed using four gilts at 9 weeks of age and 4 gilts at 19 weeks of age to determine the amount of estradiol and the bleeding schedule necessary to evaluate the inhibitory and stimulating effects of estradiol on serum luteinizing hormone (LH) concentrations. Also, the use of the cannulation technique described by Kreider (1975) for post-pubertal gilts was evaluated for smaller gilts. Canulae were placed in gilts one day prior to treatment and gilts were placed in individual

TABLE I

EXPERIMENTAL DESIGNS AND THE NUMBER OF ANIMALS IN EXPERIMENTS I, II AND III

Experiment I Treatments

Estradiol (50 µg)		Estradiol (200 µg)	
9 Week	19 Week	9 Week	19 Week
2	2	2	2

Experiment II Treatments

Control (oil)		Estradiol (50 µg)	
10 Week	20 Week	10 Week	20 Week
4	5	5	5

Experiment III Treatments

Oil Saline		Oil GnRH (25 µg)		Estradiol (50 µg) GnRH (25 µg)	
9 Week	19 Week	9 Week	19 Week	9 Week	19 Week
5	5	5	5	5	5

crates (60 x 150 cm) for the duration of the intensive bleeding period. Estradiol (50 or 200 μg in one ml corn oil; 1, 3, 5 (10)-estratrien-3, 17βdiol) was injected intramuscularly at 0830 hr.

Blood serum samples (6 ml) were obtained from the older gilts every 0.5 hour for 2 hours prior to estradiol treatment and for 36 hours after treatment. However, serum samples were only taken at hourly intervals from the younger gilts. Serum was obtained after the samples were allowed to clot for 24 hours at 5 C and centrifugated at 1200 x g for 20 minutes. Blood plasma samples (10 ml) were obtained, immediately prior to estradiol treatment and at 1, 2, 4, 6, 8, 12, 24 and 36 hours after treatment. The blood plasma samples were mixed with oxalic acid (12.7 mg in 0.2 ml distilled water), cooled to 5 C and the plasma decanted after centrifugation at 2000 x g for 20 minutes. Both plasma and serum samples were frozen immediately after processing and stored at -20 C until assayed for hormone concentrations. Hematocrit values were determined every 12 hours during the sampling period by centrifugation of whole blood in heparinized microtubules and evaluating the percent of packed cells.

The cannulae were removed after the intensive sampling period. The older gilts were placed in outside dirt lots and the younger gilts remained inside in concrete slotted floor pens. Plasma samples (15 ml) for progesterone determination were obtained by puncture of the venae cavae of all gilts, one week prior to estradiol treatment, at treatment and once a week for three weeks after treatment.

LH was determined in all serum samples by radioimmunoassay as described by Hallford et al. (1975). Plasma estradiol was quantified by radioimmunoassay (Hoagland and Wettemann, 1979) in the plasma samples

obtained after estradiol treatment. Plasma progesterone was analyzed in plasma samples, obtained at weekly intervals, by a radioimmunoassay similar to the one discussed by Hallford et al. (1975). The minor modifications in the radioimmunoassay procedures are described in a subsequent section.

Experiment II: Influence of Estradiol on Serum Luteinizing Hormone Concentrations in Prepubertal Gilts

A 2 x 2 factorial experiment (Table I) was conducted to evaluate the influence of estradiol on serum LH concentration in ten prepubertal gilts at 10 weeks of age and ten gilts at 20 weeks of age. All gilts were cannulated one day prior to treatment. The gilts received estradiol (50 μg in 1 ml corn oil; IM) or corn oil (1 ml) and were placed in individual crates for blood sampling as in the preliminary experiment. Serum (6 ml) and plasma (10 ml) samples and hematocrit determinations were obtained at the same times as for the younger gilts in the preliminary experiment except serum sampling was extended to 40 hours after treatment.

The cannulae were removed after the intensive sampling period and the gilts were managed as in experiment I. Five weekly plasma samples (15 ml) were obtained starting one week prior to treatment, as described for experiment I, to evaluate luteal function. Serum LH, plasma estradiol and plasma progesterone concentrations were determined by radioimmunoassays.

Experiment III: Influence of Estradiol on the GnRH Induced Release of Luteinizing Hormone in Prepubertal Gilts

A 3 x 2 factorial experiment (Table I) was conducted to evaluate the effect of estradiol or corn oil on the induced release of LH from the anterior pituitary by exogenous gonadotropin releasing hormone (GnRH) in the prepubertal gilt. Fifteen 9 week old and fifteen 19 week old gilts were cannulated and placed in individual crates as in the first two experiments.

After serum samples (6 ml) were collected every two hours for 24 hours after estradiol or corn oil treatment, GnRH (25 μg in 1 ml saline) or saline (1 ml) was infused, via the cannulae, and serum samples were obtained at 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00 and 4.00 hours after GnRH. Sampling continued every hour until 40 hours after estradiol or corn oil treatment. Plasma samples (10 ml) were obtained at 0, 2, 12 and 24 hours after the estradiol or corn oil treatment for determination of plasma estradiol concentrations. Hematocrit values were determined on all gilts as in the first two experiments. The cannulae were removed after the intensive sampling period and the gilts were managed as in the first two experiments. Five weekly plasma samples (15 ml) were obtained from all gilts starting one week before treatment, as in experiment I to evaluate luteal function.

Hormone Quantification

Luteinizing Hormone (LH)

Serum LH concentrations were determined by a radioimmunoassay

similar to the method described by Niswender et al. (1970) using a specific antisera (No. 566 supplied by G. D. Niswender) to porcine LH. Dilution duplicates of 200 µl and 300 µl of serum were assayed. Buffer (PBS + .1 percent gel; Appendix Table XVI) was added to serum samples to produce a volume of 500 µl per assay tube. Standard curves were prepared from barrow serum containing 18 ng of LH per ml based on purified porcine LH (LER-786-3). Serum was used as the standard because insufficient purified LH was available and the valid parallel part of the standard curve usually had a greater range when serum was used compared to purified LH. Barrow serum was diluted with buffer so that standard tubes contained 0, .04, .07, .14, .28, .56, 1.12, 2.25, 4.50 and 9.00 ng of LH.

Radioiodinated porcine LH (LER-786-3; ^{125}I-LH) was prepared using a method similar to Niswender et al. (1970) except ^{125}I-LH was used rather than ^{131}I. To increase the binding of ^{125}I-LH to the antisera (No. 566) the semi-purified ^{125}I-LH recovered from the Biogel-60 (Bio-Rad Laboratories, Richmond, Calif.) column after iodination was purified. A glass column (1.75 x 4 cm) was partially plugged with cotton and filled with anion exchange resin (AG 1 x 10 chloride form, Bio-Rad Laboratories, Richmond, Calif.) and equilibrated with 5 ml of PBS (pH 7). Five hundred microliters of porcine serum (containing 4.49 ± 0.15 ng of LH per ml) were applied to the column. The resin was again equilibrated with 5 ml of PBS. Then 500 µl of ^{125}I-LH (approximately .04 µg LH and 1 x 10^8 dpm) was applied to the column, allowed to enter the column bed and eluted with PBS (pH 7). Fractions of 500 µl were collected. Approximately 50 percent of the radioactivity applied to the anion exchange column was eluted in fractions

2, 3 and 4. These fractions were diluted with buffer (PBS + .1 percent gel; Appendix Table XVII) and used in the assays with a range of total binding of 15 to 25%.

Serum samples obtained from a barrow before and after GnRH were quantified at the beginning and end of each assay. Concentrations of LH in the samples averaged 4.49 ± 0.15 ng/ml (mean + SE, N = 40) and 18.13 ± 0.47 ng/ml (mean ± SE, N = 36) and the between and within coefficients of variation calculated for these determinations were 11.1 ± 0.8 percent and 7.7 ± 0.7 percent, respectively. When 2, 4 or 8 ng of LH were added to a serum sample, 107.7 ± 1.1, 117.0 ± 4.6 and 96.4 ± 6.4 percent (means ± SE, N = 4), respectively, were recovered.

Estradiol

Estradiol was quantified by a specific radioimmunoassay similar to that used by Wettemann et al. (1972) and validated in our laboratory by Hoagland and Wettemann (1979) for porcine plasma. A water sample, a barrow sample and the barrow sample with an additional 20 pg of estradiol per ml were included in the estradiol assays. The average estradiol concentrations in the water, barrow and barrow plus estradiol samples were 0.38 ± 0.11, 4.17 ± 0.51 and 27.58 ± 2.56 pg/ml (mean ± SE, n = 15), respectively. An average of 107.7 ± 8.2 percent (mean ± SE, n = 15) of the 20 pg of estradiol added to the plasma sample was recovered. The between and within coefficients of variation calculated using the barrow samples in two assays were 15.3 ± 3.4 percent (mean ± SE, n = 2) and 13.4 ± 4.8 percent (mean ± SE, n = 4), respectively.

Progesterone

Plasma progesterone was quantified by a radioimmunoassay similar to that described by Kittok et al. (1973). A major modification was that a second antibody (sheep anti-rabbit gamma globulin) was used to separate bound and free ^{3}H-progesterone instead of charcoal dextran. The antisera (MSU 74 was supplied by H. D. Hafs at Michigan State University) had a slight cross reaction (2.6 percent) with 17 α OH progesterone (4-PREGNEN-17 α-OL-3,20-DIONE) but there was no cross-reaction with any other major steroid hormone. The following modifications were made in the procedures. An initial incubation (2 hours at room temperature) of the sample or standard with the antisera to progesterone was performed. Then, approximately 2000 dpm of ^{3}H-progesterone (progesterone-1,2,6,7-^{3}H; specific activity 105 Ci/mM) was added to the assay tube which was incubated at 5 C for 24 hours. A 72 hour incubation at 5 C followed the addition of the second antibody. The bound and free hormone was separated by centrifugation (1600 g at 5 C for 15 minutes) and the supernate was carefully decanted into scintillation vials, mixed with protein bound scintillation fluid (Appendix Table XVII) and the radioactivity quantified. Generally each assay contained a barrow sample, a barrow sample with 5 ng of progesterone added, 36 unknowns and three standard curves. The barrow samples without and with 5 ng of progesterone averaged 0.6 ± 0.2 and 6.8 ± 1.3 ng/nl, respectively (mean ± SE, n = 6). Average recovery of the 5 ng of added progesterone was 98.5 ± 1.6 (mean ± SE, n = 6) percent. The between and within assay coefficients of variations calculated using the same samples in two assays were 10.2 ± 1.2 (mean ± SE, n = 2) and 8.6 ± 1.6 (mean + SE, n = 4) percent, respectively.

Statistical Analysis

Serum LH concentrations at each sampling time, average serum LH concentrations over the various periods, area under the LH curve, the number of peaks of serum LH concentration greater than one standard deviation above the mean, plasma progesterone concentrations and plasma estradiol concentrations were described by:

$$Y_{ijk} = \mu + T_i + A_j + TA_{ij} + E_{ijk}$$

where Y_{ijk} is one of the above measurements and where μ is the overall mean, T is treatment, A is age and TA is treatment by age interaction. The components μ, T_i, A_j and TA_{ij} were treated as fixed effects of all records of treatment i and age j. Random error effect E_{ijk} was specific to each observation. Estimated differences between treatment by age means were obtained by method of least squares. Significant differences were determined by least significant difference (LSD).

The hematocrit determinations in experiment II and III were described by:

$$Y_{ijk} = \mu + H_i + A_j + HA_{ij} + E_{ijk}$$

where Y_{ijk} is hematocrit value and where μ is the overall mean, H is sampling time, A is age and HA is sampling time by age interaction. The components μ, H_i, A_j and HA_{ij} were treated as fixed effects of all records of sampling time i and age j. Random error effect E_{ijk} was specific to each observation. Estimated differences between sampling time by age means were obtained by method of least squares. Significant differences were determined by least significant differences.

The LH response to GnRH infusion in experiment III for each treatment by age group was fitted to the polynomial of lowest order that gave an adequate fit and were described by:

$$Y_{ijk} = \mu + X_i + \sum_{k=1}^{\ell} \beta_K H_j^k + E_{ij}$$

where Y_{ij} is LH concentration and where μ is the overall mean, X is the animal, ℓ is the order of the fitted polynominal, β_k is the coefficient in the polynomial and H is the sampling time. The components μ, X_i and H_j were treated as fixed effects of all records of animal i and time j. Random error effect E_{ij} was specific to each observation. Estimated differences between treatment by age group means and coefficients in the polynomial equations were obtained by method of least squares. Significant differences were determined by least significant differences.

CHAPTER IV

RESULTS AND DISCUSSION

Experiment I: Preliminary Experiment to Determine the Effect of Dosage of Estradiol on Serum Luteinizing Hormone Concentrations in Prepubertal Gilts

The cannulae as described by Kreider (1975) remained functional throughout the intensive sampling period in both the 9 week (64 $\pm$ 5.0 day old) and 19 week (135 $\pm$ 5.0 day old) old gilts. Body weights of the young and old gilts on the day of treatment were 14.5 $\pm$ 1.6 and 56.1 $\pm$ 1.6 kg, respectively.

Within each age group, gilts that received the greater dose (200 µg) of estradiol had greater plasma estradiol concentrations at one and two hours after treatment than the gilts receiving the smaller dose (50 µg). However, plasma estradiol concentrations (Appendix Table X) at 4, 6, 8, 12, 24 and 36 hours after treatment were similar for all treatments within age groups. Although not significant ($P < .20$) the younger gilts treated with 200 µg of estradiol had greater plasma estradiol concentrations than the older gilts receiving the 200 µg of estradiol. Plasma estradiol concentrations were significantly greater at one and two hours after treatment compared to pretreatment

concentrations in the 9 and 19 week old gilts treated with 50 μg of estradiol.

Hematocrit values decreased over time for the 9 week old gilts and the greatest reduction occurred during the first 13 hours of sampling. Hematocrit values averaged over treatments were 31.8 ± 0.9, 21.8 ± 0.9, 25.2 ± 0.9 and 23.0 ± 0.9 percent, respectively, for 1, 13, 25 and 37 hours after the onset of sampling.

Plasma progesterone concentrations for all gilts were less than 0.5 ng/ml at one week before, and at one, two and three weeks after estradiol treatment. These minimal progesterone concentrations indicate the lack of functional corpora lutea before and after treatment.

Serum LH concentrations (Appendix Table XI) decreased to less than 1 ng/ml for both treatments and age groups by 6.5 hours after treatment and usually remained less than 1 ng/ml until 24 hours after treatment. Between 29 and 36 hours after treatment, average serum LH concentrations increased to greater than 2 ng/ml for both treatment and both age groups. Average LH concentrations in the 19 week old gilts were greater than 2 ng/ml for three consecutive samples (33.0, 33.5 and 34.0) after treatment with 200 μg of estradiol. These increases in serum LH indicate a positive effect of estradiol on LH release. Thus the sampling schedule was increased to 40 hours after treatment in subsequent experiments to evaluate the stimulatory effect of estradiol on LH secretion.

Experiment II: Influence of Estradiol on Serum Luteinizing Hormone Concentrations in Prepubertal Gilts

The 10 week old and 20 week old prepubertal gilts used in this

experiment were 69.1 ± 2.2 or 139.1 ± 2.1 days of age and weighed 21.4 ± 2.4 or 55.5 ± 2.6 kg, respectively, on the day of treatment. Twenty percent of the 10 week old gilts and 40 percent of the 20 week old gilts had attained puberty by eight months of age. These gilts were subjected to Oklahoma summer ambient temperatures starting at four months of age. Exposure to elevated ambient temperatures could be responsible for the delay in the attainment of puberty.

Plasma Estradiol

Plasma estradiol concentrations are illustrated in Figures 1 and 2 and listed in Appendix Table XII. Prior to estradiol injection, all gilts had similar estradiol concentrations; 4.4 ± 1.8, 5.4 ± 1.8, 6.1 ± 1.6 and 6.4 ± 1.6 pg/ml for 10 week and 20 week control gilts and 10 week and 20 week estradiol treated gilts, respectively. These concentrations are similar to those in prepubertal heifers (Gonzalez-Padilla et al., 1975a) and prepubertal miniature gilts (Elsaesser et al., 1978). But, these estradiol values are less than the concentrations in prepubertal gilts determined with assays in which the lower limit of detection was 10 pg/ml (Foxcroft et al., 1975; Elsaesser and Foxcroft, 1978; Elsaesser and Parvizi, 1979).

Plasma estradiol concentrations attained a maximum by one hour after treatment in both 10 week and 20 week old gilts, which is similar to previous work in 9 to 11 week old prepubertal gilts (Foxcroft et al., 1975). The maximum estradiol concentration for the younger gilts (156 ± 24 pg/ml) was greater ($P < .01$) than the maximum for the older gilts (35 ± 27 pg/ml). This was expected since the young gilts weighed less and had less blood volume in which the injected estradiol

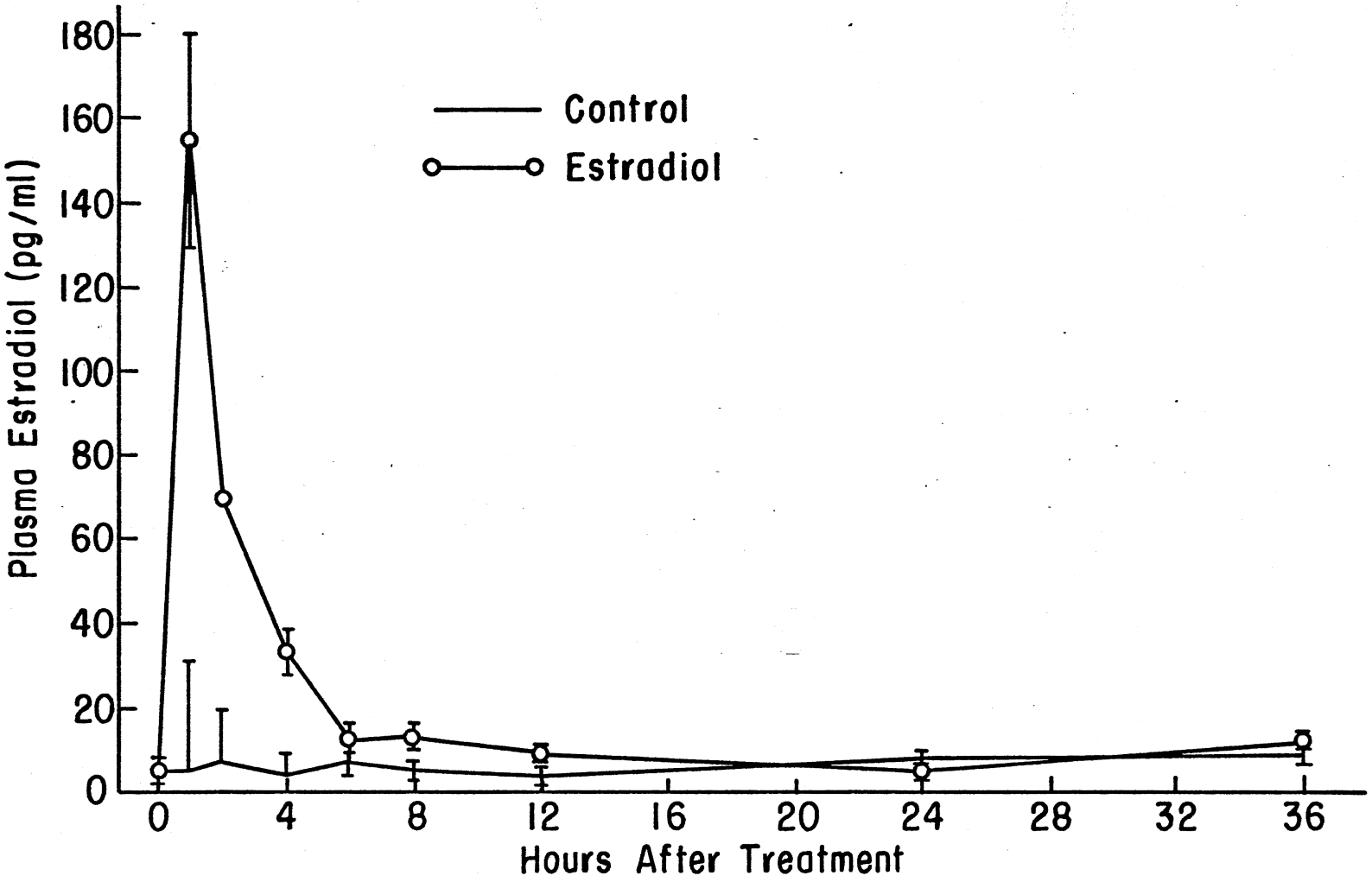

Figure 1. Plasma Estradiol in 10 Week Old Prepubertal Gilts Treated With Corn Oil or Estradiol (50 μg) in Experiment II

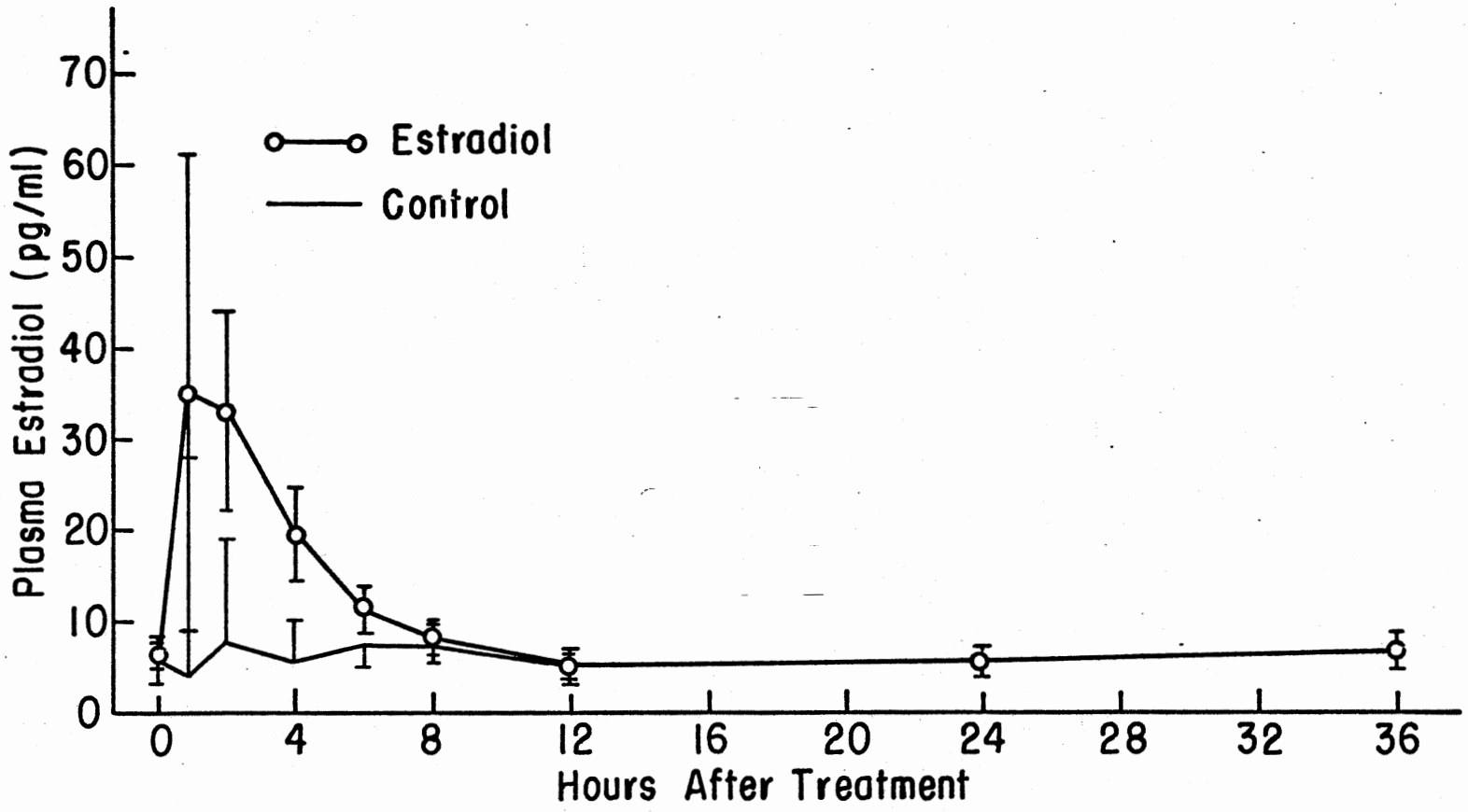

Figure 2. Plasma Estradiol in 20 Week Old Prepubertal Gilts Treated With Corn Oil or Estradiol (50 μg) in Experiment II

was distributed. The disappearance of estradiol from the plasma was more rapid for the younger estradiol treated gilts compared to the older treated gilts. Similar disappearance rates to these in the present experiment have been noted in 9 to 11 week old gilts (Foxcroft et al., 1975). Plasma estradiol concentrations in the younger treated gilts increased to five-fold the concentrations in the older treated gilts by one hour, however, by four hours after treatment concentrations were similar in young and old gilts. Perhaps the greater than physiological plasma estradiol concentrations in the younger gilts increased the clearance of estradiol or perhaps specific and nonspecific receptors located in various tissues in the younger gilts were capable of binding more hormone per kg of body weight than in the older gilts.

The young and old control gilts had similar plasma estradiol concentrations throughout the sampling period and averaged 8.1 ± 2.2 pg/ml. At 24 and 36 hours after treatment, plasma estradiol concentrations returned to pretreatment concentrations and were similar for all four treatment groups.

Hematocrit Values

Older gilts had greater ($P < .01$) hematocrit values throughout the bleeding period (Table II) which is consistant with previous work in swine and other species (Swenson, 1970). For both age groups, hematocrit values decreased ($P < .01$) during the first 13 hours, regardless of treatment. During the next 12 hours of sampling, the hematocrit values plateaued, indicating a replenishing of red blood cells. Then a significant ($P < .05$) decrease in hematocrit values occurred during the last 12 hours of sampling. The decrease in blood

TABLE II

HEMATOCRIT VALUES DURING REPEATED SAMPLING OF PREPUBERTAL GILTS IN EXPERIMENT II[a]

Hours After Treatment	Age	
	10 Week	20 Week
1	28.4 ± 1.0[b] (9)	36.6 ± 0.9[c] (10)
13	23.7 ± 1.9[c] (7)	29.8 ± 1.8[f] (8)
25	23.4 ± 1.0[c] (9)	30.9 ± 1.0[f] (10)
37	19.7 ± 2.0[d] (7)	25.4 ± 1.6[g] (10)

[a]Data are expressed as means ± standard error (percent) with the number of observations in parentheses.

[bcdefg]Means in the same row or column with different superscripts are significantly ($P < .05$) different.

hematocrit with time would be expected due to the frequent blood sampling which removed approximately 400 ml of whole blood from each gilt.

Plasma Progesterone

Average plasma progestrone concentrations at one week before and just prior to treatment were similar and less than 0.5 ng/ml for gilts on all treatments (Table III). Therefore, none of the gilts had functional corpora lutea or other major sources of progesterone prior to treatment. Plasma progesterone concentrations were minimal (< 0.5 ng/ml) during the three weeks after treatment and were not influenced by treatment or age of gilt. Thus, treatment did not initiate corpora luteal development. The lack of ovarian response to the estradiol induced release of endogenous gonadotropins in the present study substantiates the theory that the prepubertal ovary is not capable of responding to endogenous gonadotropins. Perhaps the induced release of gonadotropins was of insufficient magnitude or duration to stimulate ovarian morphological changes. The ovaries of gilts younger than four months of age (Chakraborty et al., 1973) and lambs younger than five months of age (Mayar et al., 1978) did not respond to repeated GnRH induced release of endogenous gonadotropins. Whereas, eight month old anestrous gilts (Wettemann et al., 1973) responded to estradiol valerate by expressing estrus and ovulation.

Serum Luteinizing Hormone

Serum LH concentrations (Figures 3 and 4 and Appendix Table XIII) during 0 to 6 hours after treatment were similar for all four groups.

TABLE III

PLASMA PROGESTERONE CONCENTRATIONS IN PREPUBERTAL GILTS BEFORE AND AFTER CORN OIL OR ESTRADIOL (50 ug) TREATMENT IN EXPERIMENT II[a]

Time in Weeks	Treatment			
	Control		Estradiol	
	10 Week	20 Week	10 Week	20 Week
-1	0.3 ± 0.03 (4)	0.4 ± 0.03 (5)	0.3 ± 0.03 (5)	0.4 ± 0.03 (5)
0	0.4 ± 0.04 (4)	0.4 ± 0.04 (5)	0.4 ± 0.04 (5)	0.4 ± 0.03 (5)
1	0.4 ± 0.04 (4)	0.4 ± 0.04 (5)	0.4 ± 0.04 (5)	0.4 ± 0.04 (5)
2	0.3 ± 0.06 (4)	0.3 ± 0.05 (5)	0.3 ± 0.05 (5)	0.4 ± 0.05 (5)
3	0.3 ± 0.05 (4)	0.3 ± 0.04 (5)	0.4 ± 0.04 (5)	0.3 ± 0.04 (5)

[a]Data are expressed as means ± standard error (ng/ml) with the number of observations in parentheses.

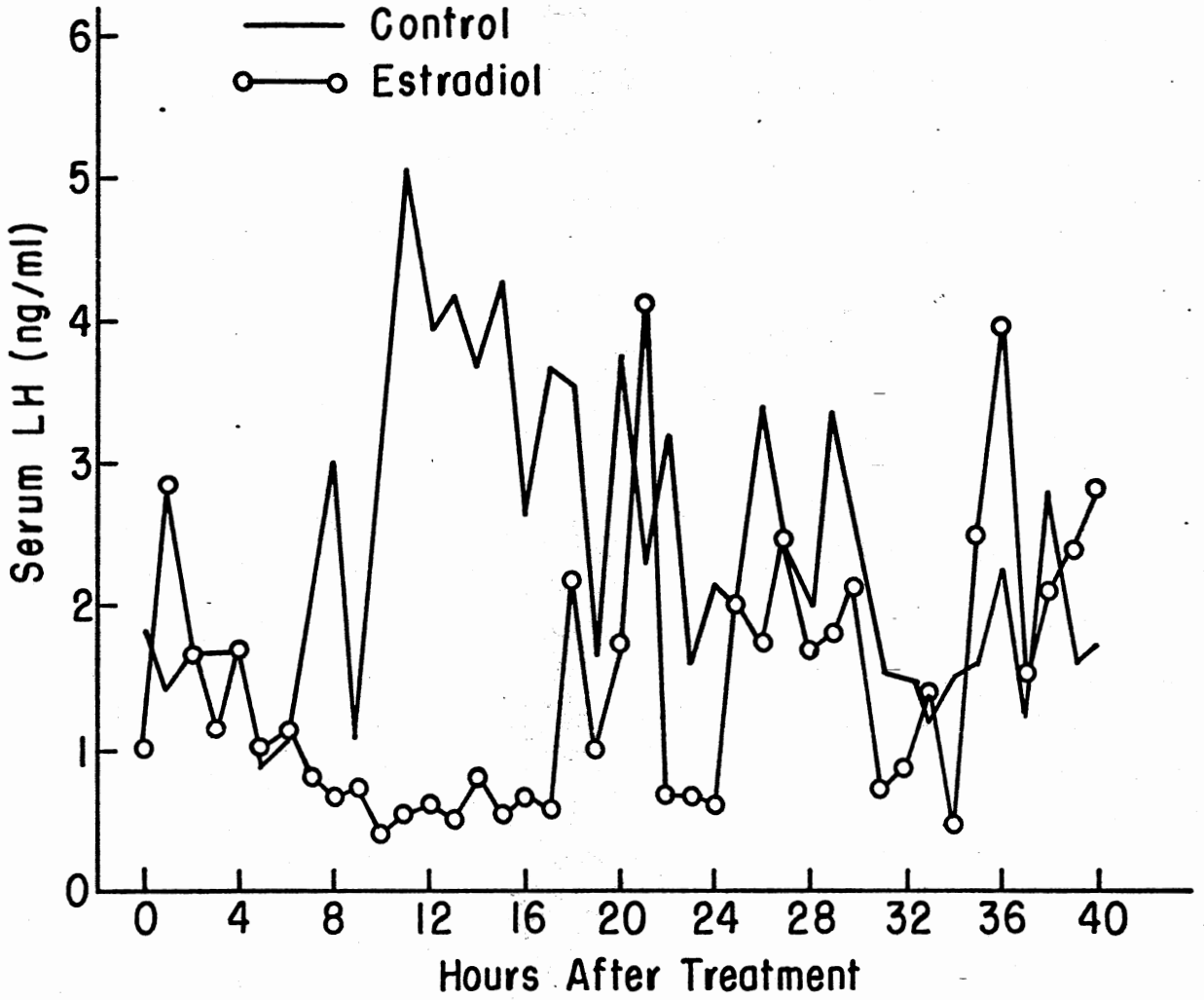

Figure 3. Serum Luteinizing Hormone in 10 Week Old Prepubertal Gilts Treated With Corn Oil or Estradiol (50 µg) in Experiment II

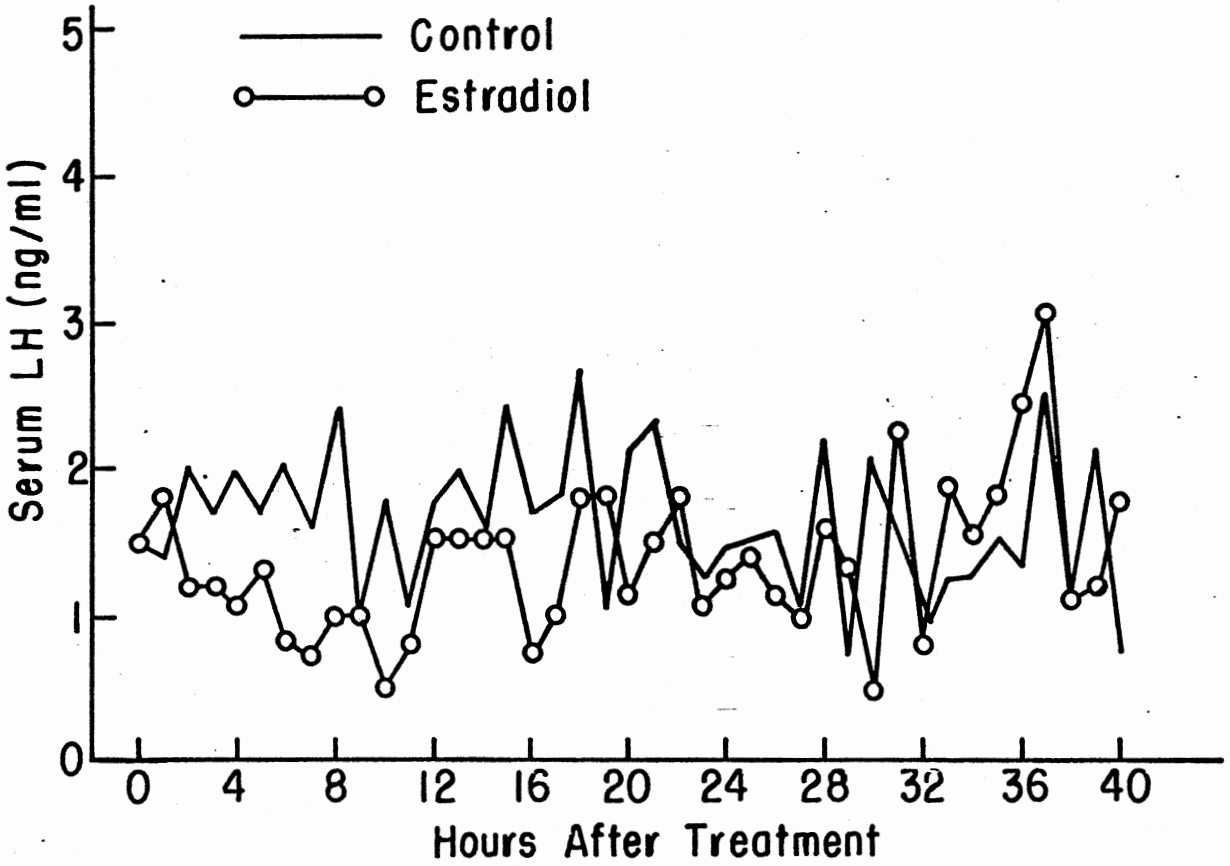

Figure 4. Serum Luteinizing Hormone in 20 Week Old Prepubertal Gilts Treated With Corn Oil or Estradiol (50 µg) in Experiment II

of gilts. Serum LH concentrations were less ($P < .05$) in the 10 week old estradiol treated gilts than in the 10 week old control gilts from 7 to 17 hours after treatment. Estradiol treatment did not alter serum LH concentrations in the older gilts. Actually, the older gilts had similar serum LH concentrations throughout the experiment. Serum LH concentrations during the hours 11, 12, 13 and 17 after treatment were significantly greater in the young control gilts as compared to the old control gilts. During 18 to 40 hours after treatment, serum LH concentrations were similar for all four treatemnt groups.

Luteinizing hormone concentrations averaged over all bleedings were not significantly different across treatment by age groups. Analysis of variance was performed on the average serum LH concentrations at various periods after treatment (Table IV). These analyses added little additional information except that the average serum LH concentration during 10 to 19 hours after treatment were less ($P < .05$) in the estradiol treated gilts as compared to the oil treated gilts. Also during 30 to 40 hours after treatment serum LH concentrations were slightly elevated in the estradiol treated gilts as compared to the control gilts ($P < .20$).

When the areas under the serum LH concentration curves were compared for 1 to 20 hours after treatment the control gilts had greater ($P < .05$) areas than the estradiol treated gilts (Table V). During the second twenty hours (21 to 40 hours after treatment) the areas under the LH curves were similar for all groups of gilts, indicating a lack of positive LH response by forty hours to estradiol administration.

The number of LH peaks (increases in LH > 1 S.D. above the mean; Table VI) during the entire bleeding period were not influenced by age

TABLE IV

SERUM LUTEINIZING HORMONE IN PREPUBERTAL GILTS DURING VARIOUS PERIODS AFTER CORN OIL OR ESTRADIOL (50 μg) TREATMENT IN EXPERIMENT II[a]

Time in Hours	Treatment			
	Control		Estradiol	
	10 Week	20 Week	10 Week	20 Week
0-40	2.4 + 0.7	1.4 ± 0.5	1.7 ± 0.5	1.4 ± 0.5
0-19	2.6 ± 0.4[b]	1.8 ± 0.3[bc]	1.1 ± 0.3[c]	1.2 ± 0.3[c]
20-40	2.2 ± 0.5	1.5 ± 0.4	1.8 ± 0.4	1.5 ± 0.4
0-9	1.7 ± 0.4	1.8 ± 0.3	1.4 ± 0.3	1.1 ± 0.3
10-19	3.7 ± 0.6[b]	1.8 ± 0.5[c]	0.8 ± 0.5[c]	1.4 ± 0.5[c]
20-29	2.6 ± 0.6	1.6 ± 0.5	1.7 ± 0.5	1.3 ± 0.5
30-40	1.8 ± 0.4	1.5 ± 0.4	1.8 ± 0.4	1.7 ± 0.4

[a]Data are expressed as means ± standard error (ng/ml) of four animals in the young control group and five animals in the other three groups.

[bc]Means in a row with different superscripts are significantly ($P < .05$) different.

TABLE V

AREA UNDER THE SERUM LUTEINIZING HORMONE CONCENTRATION CURVE AFTER CORN OIL OR ESTRADIOL (50 μg) TREATMENT OF PREPUBERTAL GILTS IN EXPERIMENT II[a]

Hours After Treatment	Treatments			
	Control		Estradiol	
	10 Week	20 Week	10 Week	20 Week
1-40	83.4 ± 17.2	67.4 ± 15.4	56.1 ± 15.4	54.9 ± 15.4
1-20	44.5 ± 9.2[b]	36.5 ± 8.2[bc]	21.7 ± 8.2[c]	24.1 ± 8.2[c]
21-40	36.2 ± 8.7	28.2 ± 7.8	31.5 ± 7.8	29.2 ± 7.8

[a]Data are expressed as means ± standard error (ng/ml x hr) of four animals in the young control group and five animals in the other three groups.

[bc]Means in the same row with different superscripts are significantly ($P < .05$) different.

TABLE VI

NUMBER OF PEAKS[a] OF SERUM LUTEINIZING HORMONE DURING VARIOUS PERIODS AFTER TREATMENT OF PREPUBERTAL GILTS WITH CORN OIL OR ESTRADIOL (50 μg) IN EXPERIMENT II[b]

Hours After Treatment	Treatments			
	Control		Estradiol	
	10 Week	20 Week	10 Week	20 Week
1-40	7.0 ± 1.2	5.6 ± 1.1	4.4 ± 1.1	4.6 ± 1.1
1-20	4.8 ± 0.9[c]	3.2 ± 0.8[cd]	1.4 ± 0.8[d]	1.4 ± 0.8[d]
21-40	2.2 ± 0.7	2.4 ± 0.6	3.0 ± 0.6	3.2 ± 0.6
1-10	0.8 ± 0.6	1.8 ± 0.5	1.0 ± 0.5	0.2 ± 0.5
11-20	4.0 ± 0.9[c]	1.4 ± 0.8[d]	0.4 ± 0.8[d]	1.2 ± 0.8[d]
21-30	1.5 ± 0.5	1.4 ± 0.5	1.6 ± 0.5	1.0 ± 0.5
31-40	0.8 ± 0.3[c]	1.0 ± 0.3[c]	1.4 ± 0.3[c]	2.2 ± 0.3[d]

[a]Luteinizing hormone concentrations > one standard deviation above the mean.

[b]Data are expressed as means ± standard error of four animals in the young controls and 5 animals in the other three groups.

[cd]Means in the same row with different superscripts are significantly different.

or treatment. Less peaks ($P < .05$) occurred during the first half of the bleeding period (1 to 20 hours after treatment) in the young estradiol treated gilts when compared to the young corn oil treated gilts. Whereas, only a slight reduction in the number of peaks occurred in the old estradiol treated gilts compared to the old control gilts. The number of peaks that occurred during the second half of the bleeding period (21 to 40 hours after treatment) were slightly ($P < .30$) increased in the estradiol treated gilts compared to the control gilts. During the last ten hours (31 to 40 hours after treatment) the old estradiol treated gilts had more ($P < .05$) peaks than the other three groups of gilts.

These data indicate that 10 and 20 week old prepubertal gilts have similar average serum LH concentrations. However, the variability in serum LH concentrations was greater in the 10 week old gilts as compared to the 20 week old gilts. This suggests an age dependent change in the control of LH release.

The inhibition of serum LH concentrations by exogenous estradiol in the younger gilts was more pronounced than in the older gilts and agrees with previous studies (Foxcroft et al., 1975; Pomerantz et al., 1975). However, the initial inhibition by estradiol occurred about four hours later in the present study as compared to others (Foxcroft et al., 1975; Pomerantz et al., 1975). The greater estradiol concentrations in the younger treated gilts compared to the older gilts may be responsible for the difference in serum LH inhibition between the young and old estradiol treated gilts. The hypothalamus and anterior pituitary of the younger gilts were exposed to greater plasma concentrations of estradiol which could saturate the estradiol receptors in these

tissues. Thus in the younger gilts, more receptors may have been occupied which could cause intracellular changes which regulate the amount of LH released from the anterior pituitary into the peripheral circulation. An alternative explanation could be that the hypothalamus and pituitary of younger prepubertal animals may be more sensitive to estradiol than older prepubertal animals (Odell and Swerdloff, 1976). Thus, the same amount of estradiol could have a greater inhibition on LH release in the younger gilts. Inconsistent with this theory, is the observation that the 10 week old control gilts had greater serum LH concentrations than the 20 week old control gilts, yet their estradiol concentrations were similar.

The fluctuations in serum LH concentrations are probably spontaneous prepubertal releases of LH which persists for approximately one hour (Foxcroft et al., 1975; Pomerantz et al., 1975). These increases or peaks in serum LH are probably under the control of GnRH and the variability noted in the serum LH concentrations could indicate incomplete control of the neurons in the hypothalamus that release GnRH (Flerko, 1975). The inhibition of these spontaneous releases of LH in the present study suggests that estradiol inhibits GnRH release and/or the release of LH from the anterior pituitary. The early inhibition of LH release after estradiol treatment in the prepubertal gilt (Pomerantz et al., 1975), adult ovariectomized ewe (Coppings and Malven, 1976) and the adult ovarectomized rat (Vilchez-Martinez et al., 1974) cannot be overcome by the administration of GnRH; whereas, the later hours of inhibition can be readily overcome with exogenous GnRH. Thus, the initial inhibition of serum LH after treatment of young gilts with estradiol may be due to the action of estradiol at the

anterior pituitary and hypothalamus and the later inhibition by estradiol could be at the hypothalamus. Since serum LH was not altered by estradiol treatment of the older gilts, there may be an all or none response of serum LH to estradiol (Beck and Convey, 1977; Swanson and McCarthy, 1978) and the required minimum plasma estradiol concentrations were not achieved in these larger gilts. The inhibitory effects of estradiol on serum LH concentrations may require time for intracellular changes to occur. Plasma estradiol concentrations in the young treated gilts were not inhibited until 4 hours after estradiol treatment. Initially, estradiol binds to specific receptors in the anterior pituitary and hypothalamus where intracellular changes occur after the receptor bound estradiol translocates from the cytoplasm to the nucleus (O'Malley and Means, 1974). These changes control the release of LH from the pituitary either by inhibition of GnRH release from the hypothalamus or inhibition of pituitary sensitivity to GnRH.

The ability of the hypothalamic-pituitary unit to release gonadotropins in response to estradiol could not be fully evaluated in these gilts because the sampling schedule was too short. Previous workers (Elsaesser et al., 1978; Elsaesser and Foxcroft, 1978; Elsaesser and Parvizi, 1979) have indicated a positive response of serum LH concentrations to exogenous estradiol administration. Serum LH concentrations increased significantly above baseline at 60 hours after estradiol treatment in the 60 day old gilts and 50 hours in the 161 day old gilts. The maximum LH concentrations obtained were similar for both ages and occurred earlier in the older gilts as compared to the younger gilts (60 to 80 hours after estradiol treatment, respectively). In the present study, more peaks of LH were noted during

the last ten hours (30 to 40 hours after treatment) of the bleeding period for the estradiol treated gilts. Also, during this same period, the estradiol treated gilts had slightly greater average serum LH concentrations than the control gilts. This could be construed to indicate the beginning of an increase in endogenous LH release. Estradiol could be acting at the preoptic neurons to elicit more GnRH synthesis and release or at the pituitary to increase its sensitivity to GnRH. The responsiveness of the anterior pituitary to exogenous GnRH, as measured by the amount of LH released, increases about the same time after estradiol administration as the positive effect of estradiol on LH release is noted (Coppings and Malven, 1976; Vilchez-Martinez et al., 1974). Thus, the pituitary response to GnRH does change due to estradiol and this enhanced release may explain in part the positive effect of estradiol on LH release.

A positive response to estradiol treatment is present in 20 day old rats (Ronnekleiv et al., 1978) and 60 day old prepubertal gilts (Elsaesser et al., 1978; Elsaesser and Foxcroft, 1978; Elsaesser and Parvizi, 1979). The positive LH response to estradiol is more variable and occurs later at these ages as compared to the response in 40 day old rats and 161 day old gilts. A greater dose of estradiol is required to elicit the positive LH response in the 20 day old rat as in the 40 day old rat. Also the positive LH response can be evoked in 14 day old rats if pretreated with estradiol or progesterone (Puig-Duran and MacKinnon, 1978).

A certain combination of gonadotropins, steroids and age are required to promote the necessary receptor populations or intracellular enzyme concentrations in the hypothalamus and anterior pituitary such

that an increase in plasma estradiol can begin the sequence of events that lead to LH release. The number of estradiol receptors in the anterior pituitary and hypothalamus of prepubertal gilts were similar from 2 to 6 months of age (Diekman and Anderson, 1979). Perhaps subtle alterations of receptors or intracellular enzyme concentrations occur that cannot be determined with the present assay systems. In any event, the positive effects of estradiol on LH release becomes functional prior to attainment of puberty. It appears the next event necessary for attainment of puberty is sufficient estradiol secretion. Estradiol secretion in the prepubertal animal in response to exogenous and endogenous gonadotropins depends on maturational changes (age of the animal) and the amount of gonadotropins. Therefore, the ovary must progress through unknown maturational changes during the prepubertal period that are necessary for further pubertal development. Efforts should be made to determine when the ovary can synthesize and release sufficient quantities of estradiol and what changes in the ovary occur during this maturational period.

Experiment III: Influence of Estradiol on the GnRH Induced Release of Luteinizing Hormone in the Prepubertal Gilt

The 9 week old gilts utilized in this experiment were 63.4 ± 1.0 days of age and the 19 week old gilts were 134.8 ± 1.0 days of age. The younger gilts weighed less ($P < .01$) than the older gilts (17.6 ± 1.7 and 59.8 ± 1.7 kg) on the day of treatment. None of the nine week old gilts and 30 percent of the 19 week old gilts had attained puberty by eight months of age. The gilts in this experiment were subjected to

elevated ambient temperatures similar to the gilts in experiment II. Elevated ambient temperatures and the stress of intensive sampling could have delayed puberty attainment.

Plasma Estradiol

Plasma estradiol concentrations were greater ($P < .01$) in both age groups after treatment of gilts with estradiol (Figures 5 and 6; Appendix Table XIV). Maximum estradiol concentrations occurred at two hours after treatment. The maximum plasma estradiol concentrations after treatment with estradiol were similar to the concentrations achieved in experiment II. As in experiment II 50 μg of estradiol was administered to both the 9 and 19 week old gilts. The younger gilts had less blood volume to dilute the administered estradiol, consequently the younger gilts had greater ($P < .05$) plasma estradiol concentrations than the older estradiol treated gilts. Plasma estradiol concentrations returned to baseline concentrations (6.9 ± 0.8 pg/ml) and were similar for all gilts at 12 and 24 hours after treatment.

Hematocrit Values

Similar to experiment II, the older gilts had greater hematocrit values than the younger gilts. Hematocrit values (Table VII) decreased ($P < .01$) for both age groups during the first 13 hours, regardless of treatment. During the second 12 hours of sampling, the hematocrit values plateaued, indicating a replenishing of red blood cells. Then reminiscent of hematocrit values in experiment II, a significant ($P < .01$) decrease occurred during the last 12 hours of sampling. The decrease in blood hematocrit with time would be expected due to the frequent blood sampling which removed 400 ml of whole blood from each gilt.

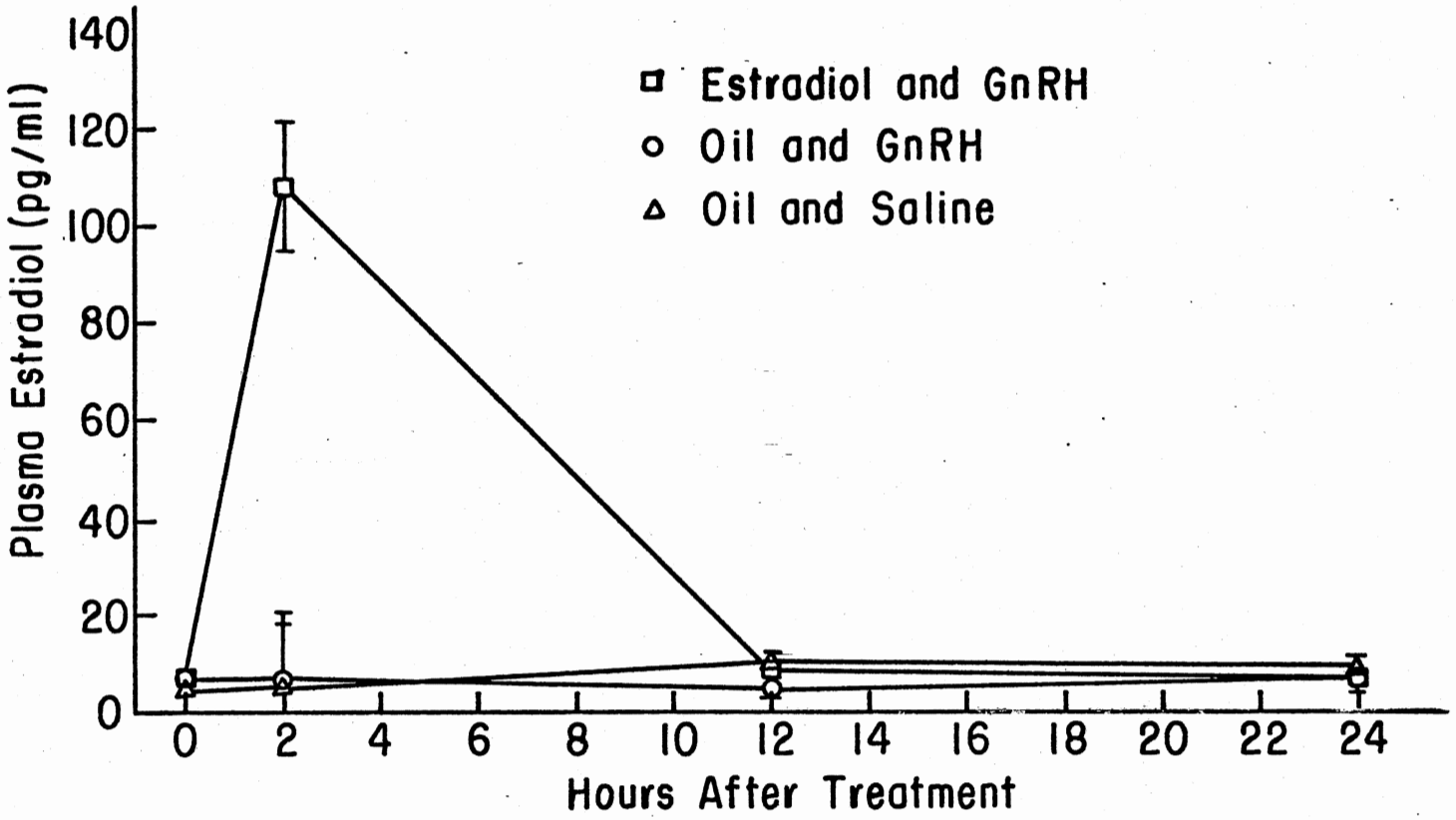

Figure 5. Plasma Estradiol in 9 Week Old Prepubertal Gilts After Treatment in Experiment III

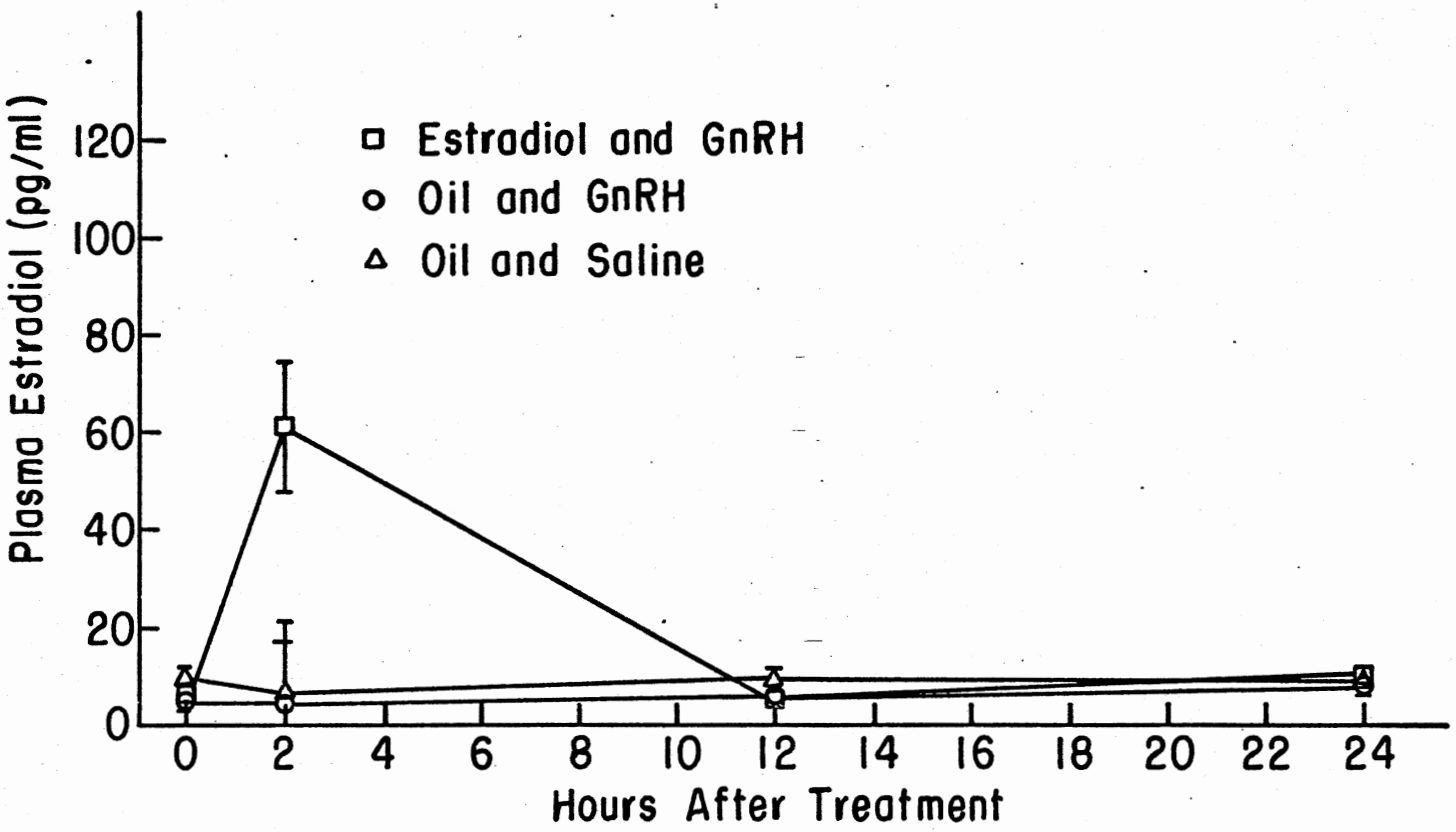

Figure 6. Plasma Estradiol in 19 Week Old Prepubertal Gilts After Treatment in Experiment III

TABLE VII

HEMATOCRIT VALUES DURING REPEATED SAMPLING OF PREPUBERTAL GILTS IN EXPERIMENT III[a]

Hours After Treatment	Ages	
	9 Weeks	19 Weeks
1	28.2 ± 0.5[b] (15)	33.4 ± 0.5[e] (15)
13	22.9 ± 1.5[c] (13)	27.3 ± 1.4[f] (15)
25	23.1 ± 1.2[c] (15)	30.0 ± 1.2[f] (15)
37	21.5 ± 1.3[d] (15)	27.5 ± 1.3[g] (15)

[a]Data are expressed as means ± standard errors (percent) with the number of observations in parentheses.

[bcdefg]Means in the same row or column with different superscripts are significantly ($P < .05$) different.

Plasma Progesterone

Average plasma progesterone concentrations at one week before and just prior to oil or estradiol treatment were similar and less than 0.5 ng/ml for gilts on all treatments (Table VIII). Therefore, as in experiment II, none of the gilts had functional corpora lutea or other sources of progesterone prior to treatment. Average plasma progesterone concentrations for three weeks after treatment were below 0.5 ng/ml and unaffected by treatment.

If any of the gilts possessed tertiary or Graffian follicles on the day of treatment, the GnRH infusion would probably have elicited ovulation. The younger gilts, as in experiment II and nine week old gilts in experiments reported by others (Dziuk and Gehlbach, 1966; Foote, 1972) lacked sufficient ovarian maturation to respond to GnRH or gonadotropin treatments. The lack of response to GnRH injection in the older gilts is perplexing, yet in agreement with previous work (Chakraborty et al., 1973). Four month old gilts usually possess numerous vesicular follicles (3 to 6 mm in diameter) and will respond to exogenous gonadotropin treatment by expressing ovulation and estrus (Dzuik and Gehlbach, 1966; Foote, 1972). Gonadotropin releasing hormone administration stimulates the release of endogenous gonadotropins in prepubertal rats (MacKinnon et al., 1978), lambs (Lee et al., 1976b) and gilts (Chakraborty et al., 1973). Therefore the GnRH infusion in the present study should have stimulated increased circulating concentrations of LH and maybe FSH. Perhaps, the GnRH induced release of endogenous gonadotropins was of insufficient duration or magnitude to stimulate morphological changes of the ovary in the 19 week old gilts.

TABLE VIII

PLASMA PROGESTERONE CONCENTRATIONS IN PREPUBERTAL GILTS BEFORE AND AFTER TREATMENT IN EXPERIMENT III[a]

Time in Weeks	Treatments						
	Oil and Saline		Oil and GnRH		Estradiol and GnRH		
	9 weeks	19 weeks	9 weeks	19 weeks	9 weeks	19 weeks	S.E.
-1	0.4	0.2	0.3	0.2	0.2	0.3	± 0.06
0	0.3	0.3	0.2	0.3	0.2	0.4	± 0.08
1	0.2	0.3	0.3	0.2	0.2	0.3	± 0.06
2	0.2	0.2	0.2	0.2	0.2	0.2	± 0.04
3	0.2	0.2	0.3	0.2	0.2	0.2	± 0.06

[a]Data are expressed as means (ng/ml) with five observations per mean.

Luteinizing Hormone

Prior to oil or estradiol treatment, serum LH concentrations (Figures 7 and 8) were significantly influenced by age of gilt. Immediately before treatment the younger gilts had less ($P < .05$) serum LH concentrations than the older gilts (Appendix Table XV). These differences in serum LH concentrations can probably be attributed to normal fluctuations in serum LH concentrations in prepubertal animals. Inhibition of serum LH concentrations in the estradiol treated gilts occurred at 14 and 16 hours after treatment ($P < .05$) and was more pronounced in the young estradiol treated gilts than the older gilts. Serum LH concentrations during 18 to 24 and 29 to 40 hours after oil or estradiol treatment were similar for all treatment by age groups except for significant, but probably random fluctuations that were not associated with any treatment at 20, 34, and 35 hours after treatment. The LH response to saline or GnRH, during 24 to 28 hours after oil or estradiol treatment, were fitted to polynomial regression equations and were not subjected to analysis of variance at each sampling time.

Since the objectives of experiment III were different than the objectives of experiment II, less frequent blood samples were taken during the first 24 hours after treatment. Inhibition of serum LH concentrations after estradiol treatment were less apparent with the infrequent sampling regimen. Yet, a significant treatment effect was noted which started later (14 versus 7 hours after treatment) and was of a shorter duration (2 versus 11 hours) than in experiment II. Greater plasma estradiol concentrations in the younger gilts as compared to the older gilts can explain in part the more pronounced LH suppression in the younger gilts.

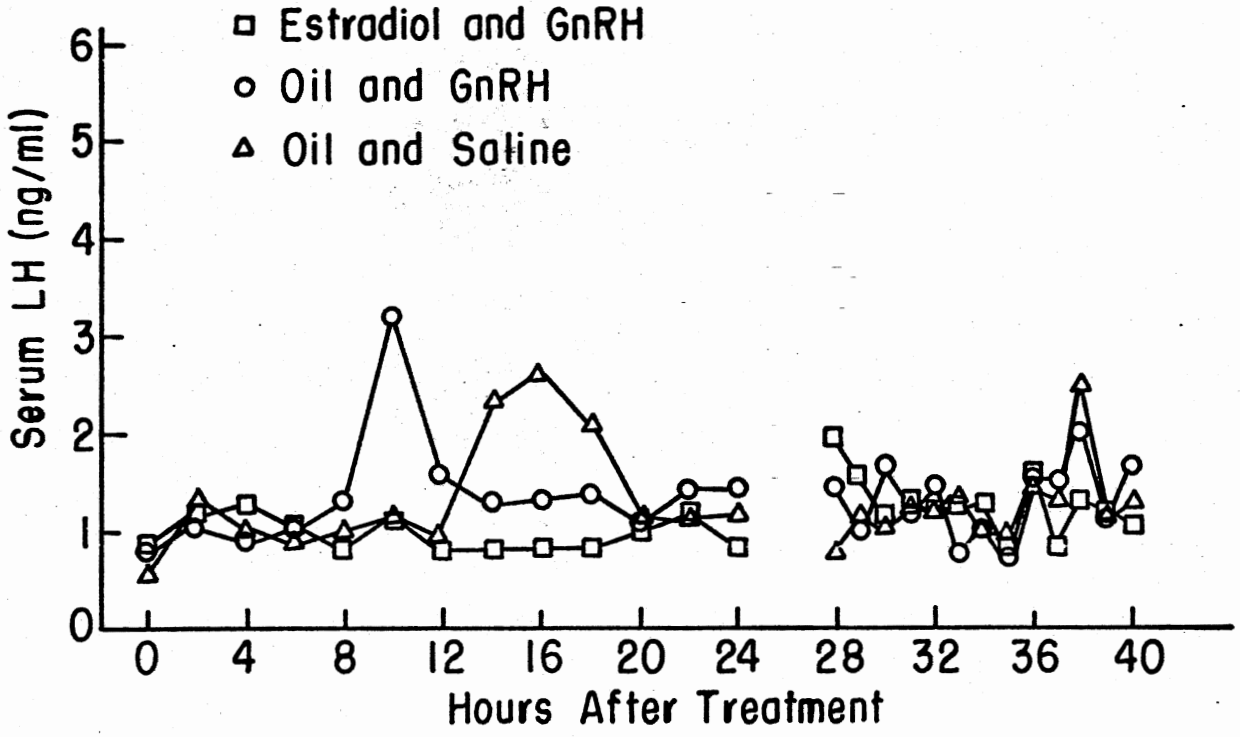

Figure 7. Serum Luteinizing Hormone in 9 Week Old Prepubertal Gilts After Treatment in Experiment III

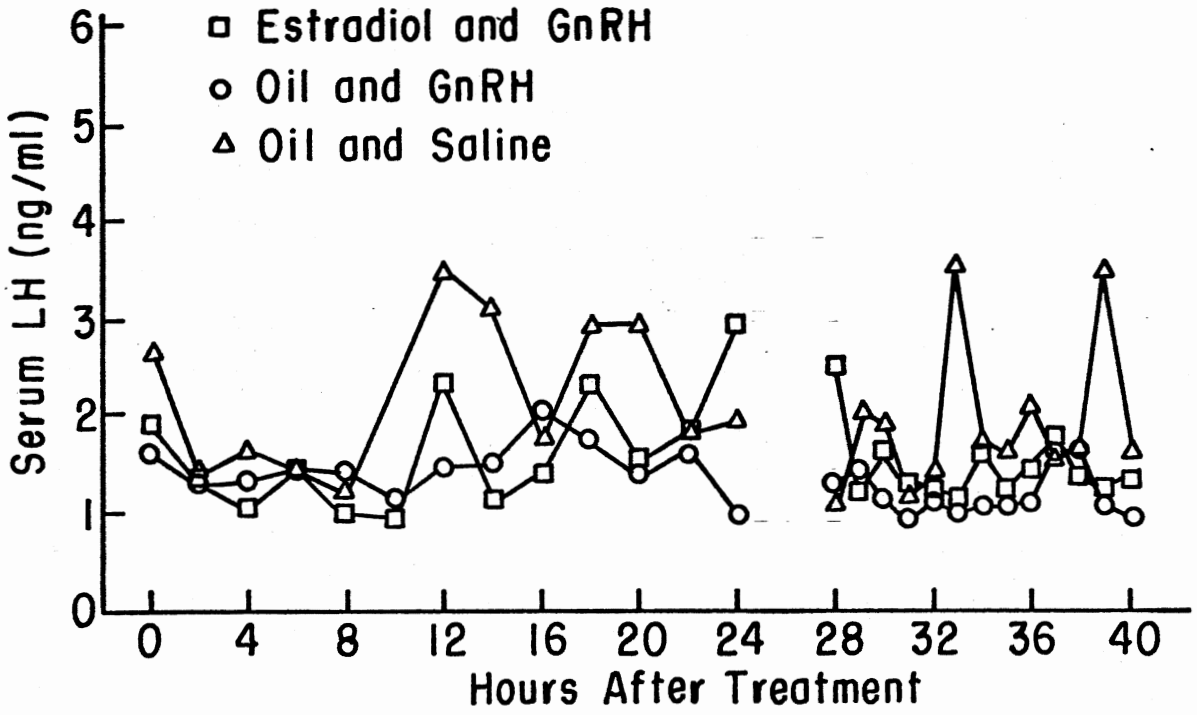

Figure 8. Serum Luteinizing Hormone in 19 Week Old Prepubertal Gilts After Treatment in Experiment III

A positive effect of estradiol on serum LH concentration was not observed in this experiment. This lack of response could be related to the sampling regimen that was short in duration or to GnRH treatment at 24 hours after estradiol. Blood sampling occurred for 40 hours after oil or estradiol treatment and the positive response of serum LH to estradiol has not been noted until 50 to 60 hours after estradiol treatment in prepubertal gilts (Elsaesser et al., 1978; Elsaesser and Foxcroft, 1978; Elsaesser and Pravizi, 1979). Chakraborty et al. (1973) observed that GnRH depletes pituitary stores of LH in prepubertal gilts and that GnRH inhibits the estradiol induced LH release in ovariectomized heifers (Beck and Convey, 1977).

Response to GnRH Challenge

Serum LH and plasma estradiol concentrations were similar for all gilts at 24 hours after estradiol or corn oil administration. Gonadotropin releasing hormone or saline was administered and serum LH concentrations increased significantly in the GnRH treated animals. The responses of serum LH concentrations to GnRH or saline were described by polynomial regression equations (Figure 5). Serum LH concentrations over time in the 9 week and 19 week old oil and saline treated gilts were best described by third order regression equations (Table IX) with similar intercepts and beta values. The four groups that received GnRH had greater serum LH concentrations after GnRH and the responses were best explained with fifth order regression equations. The equations that best fit the LH response in the 9 week oil or estradiol treated gilts had similar intercepts and beta values. Similarly, the polynomial equations that explained the LH response to GnRH in the

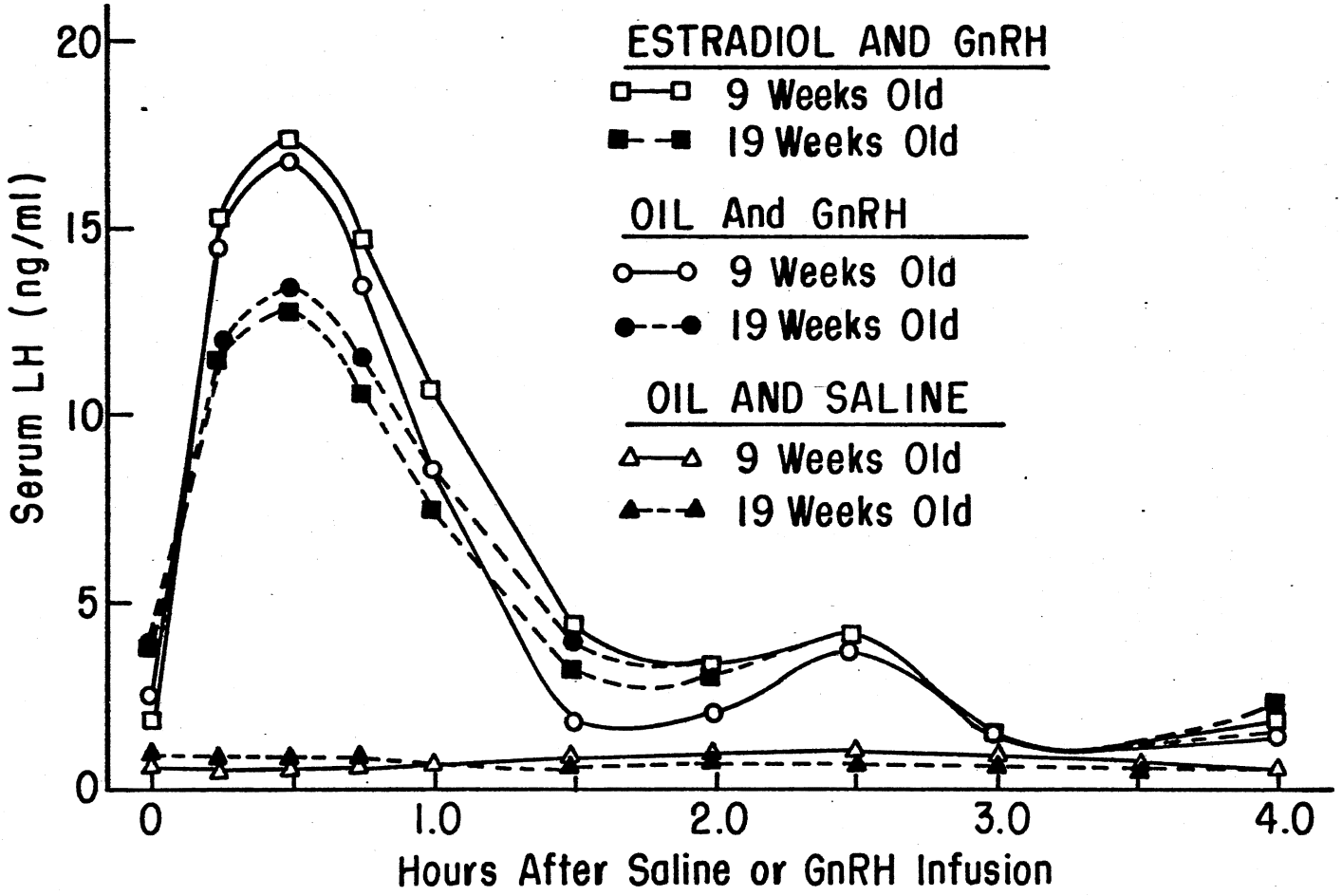

Figure 9. Serum Luteinizing Hormone Response to GnRH Infusion in 9 and 19 Week Old Prepubertal Gilts 24 Hours After Treatment With Oil or Estradiol in Experiment III

TABLE IX

POLYNOMIAL REGRESSION EQUATIONS FOR THE LUTEINIZING HORMONE RESPONSE TO GnRH INFUSION OF PREPUBERTAL GILTS IN EXPERIMENT III

Treatment	Polynomial Equations
Oil and Saline	
9 week [a]	$LH = 1.2 - (0.75)T + (0.76)T^2 - (0.15)T^3$
19 week [a]	$LH = 1.8 - (1.12)T + (0.64)T^2 - (0.10)T^3$
Oil and GnRH	
9 week [b]	$LH = 2.45 + (82.30)T - (147.70)T^2 + (94.40)T^3 - (25.51)T^4 + (2.46)T^5$
19 week [bc]	$LH = 2.10 + (58.34)T - (98.41)T^2 + (61.20)T^3 - (16.38)T^4 + (1.58)T^5$
Estradiol and GnRH	
9 week [b]	$LH = 1.83 + (78.22)T - (130.00)T^2 + (79.54)T^3 - (20.96)T^4 + (1.99)T^5$
19 week [c]	$LH = 3.67 + (51.40)T - (92.02)T^2 + (58.65)T^3 - (15.86)T^4 + (1.53)T^5$

[abc]The regression equations associated with the ages with different superscripts are different ($P < .05$) in at least one beta value.

19 week corn oil or estradiol treated gilts had similar intercepts and beta values. Comparisons made between the fifth order polynomial equations involving age were different ($P < .05$) in one or more beta values except when the differences ($P < .13$) in the response of the 19 week old oil and GnRH treated gilts and the 9 week old estradiol and GnRH treated gilts were compared.

The younger gilts treated with GnRH had greater maximum serum LH concentrations than the older gilts given GnRH. Maximal LH concentrations were attained by gilts of both ages by 30 minutes after GnRH infusion and the duration of the increased serum LH after GnRH occurred for approximately 2.5 hours. This LH response is similar in magnitude and duration to results previously reported (Chakraborty et al., 1973). By three hours after GnRH infusion, serum LH concentration in the GnRH treated gilts decreased to concentrations similar to control gilts.

These data indicate that estradiol pretreatment did not facilitate LH release induced by GnRH at 24 hours after estradiol treatment. The 9 week or 19 week old gilts treated with estradiol had similar maximum LH concentrations and duration of release when compared to gilts of the same age that received corn oil. LH release after GnRH treatment is increased by pretreatment of ovariectomized rats (Vilchez-Martinez et al., 1974), ewes (Coppings and Malven, 1976) and heifers (Beck and Convey, 1977) with estradiol. The LH response to GnRH in ovariectomized heifers (Beck and Convey, 1977) six hours after estradiol treatment was of a longer duration than when GnRH was infused after corn oil. Yet, when GnRH was infused at 12 hours after estradiol treatment the duration of the LH release was greater than when GnRH was infused at 6 hours. The intracellular alterations at the anterior pituitary and/or neurons in

the hypothalamic area which are necessary for the positive response of LH after estradiol treatment may require a period of time to become fully functional.

Greater LH responses after GnRH infusion occur when GnRH is given near the time that estradiol has a positive effect on LH (Beck and Convey, 1977). In the prepubertal gilt, it requires approximately 80 hours to observe the maximum positive response of LH to estradiol administration. Thus, to determine if estradiol will increase the duration of LH release after GnRH administration, the GnRH infusion should be performed at approximately 50 hours after estradiol treatment. At 50 hours after estradiol treatment the necessary intracellular alterations at the anterior pituitary and hypothalamic area to cause the enhanced release of LH should have occurred. In the present study, it was assumed that the positive LH response to estradiol would be evoked by 36 hours and the time to evaluate the LH response to GnRH should be before the estradiol induced release of LH. Therefore, saline or GnRH was infused 24 hours after the pretreatment with oil or estradiol.

The younger gilts received the same dose of GnRH as the older gilts. The blood concentrations of GnRH were probably greater in the younger gilts as compared to the older gilts. Thus, the pituitaries of the young gilts would be subjected to the same number of moles of GnRH but at a greater concentration per ml of blood than the older gilts. If the pituitary GnRH receptors sequester a certain percentage of the GnRH in the blood, the younger gilts should have more GnRH bound at the pituitary. This increase in pituitary bound GnRH may explain the greater LH response to GnRH in the younger gilts as compared to the

older gilts. An alternative explanation for the greater response to GnRH in young gilts and rats has been given by Lee et al. (1976b) and MacKinnon et al. (1978). They suggest a bimodal responsiveness of pituitary sensitivity to GnRH in that intermediate aged prepubertal rats (MacKinnon et al., 1978) and prepubertal rams (Lee et al., 1976b) had a greater LH response to GnRH than did younger and older prepubertal rats and rams. The combination of increased pituitary sensitivity to GnRH and a greater serum GnRH concentration could have been responsible for the greater release of LH after GnRH in the younger gilts.

CHAPTER V

SUMMARY

Three experiments were conducted with 9 to 10 and 19 to 20 week old prepubertal gilts to investigate the effects of estradiol on serum LH concentrations. A second objective was to determine if pretreatment with estradiol influences the GnRH induced LH release.

In the first experiment two groups of 4 gilts averaging 64 ± 2.0 and 135 ± 10.2 days of age and weighing 14.5 ± 1.6 and 56.1 ± 1.6 kg were cannulated and assigned within age groups to receive 50 to 200 μg of estradiol. Within each age group, gilts that received the greater dose (200 μg) of estradiol had greater plasma estradiol concentrations than the gilts receiving the smaller dose (50 μg) at one and two hours after treatment. Since the maximum estradiol concentrations achieved in the gilts receiving 200 μg of estradiol were greater than physiological concentrations in nonpregnant swine, and the influence on serum LH concentrations were similar for both estradiol doses, the 50 μg dose was selected for the subsequent experiments.

In the preliminary experiment serum LH concentrations decreased to less than 1 ng/ml for both treatments and age groups by 6.5 hours after treatment and usually remained less than 1 ng/ml until 24 hours after treatment. Between 29 and 36 hours after treatment, average serum LH concentrations increased to greater than 2 ng/ml for both treatment and both age groups. The sampling schedule was increased to 40 hours

after estradiol treatment in the two subsequent experiments.

In the second experiment, two groups of ten gilts averaging 69.1 ± 2.2 or 139.1 ± 2.1 days of age and weighing 21.4 ± 2.4 or 55.5 ± 2.6 kg, respectively, were cannulated one day prior to random assignment within age groups to the estradiol (50 μg) or the corn oil treatment.

Plasma estradiol concentrations increased significantly ($P < .01$) to a maximum at one hour after estradiol treatment (averaging 4.7 ± 26.8, 4.0 ± 24.0, 155.7 ± 24.0 and 35.3 ± 26.8 pg/mg, respectively, for 10 and 20 week old control and estradiol treated gilts respectively). The 10 week old estradiol treated gilts had greater ($P < .01$) plasma estradiol concentrations than the 20 week old treated gilts. Plasma estradiol concentrations returned to pretreatment values by 12 hours after estradiol treatment.

Hematocrit values decreased as sampling increased with significant ($P < .01$) decreases at 13 and 37 hours after treatment. Treatment had no influence on hematocrit values; whereas, the younger gilts had less ($P < .01$) packed cell volume than the older gilts throughout the sampling period.

Plasma progesterone concentrations were similar and less than 0.5 ng/ml in samples obtained at one week before treatment, on the day of treatment and at one, two and three weeks after treatment. This indicates that the estradiol treatment did not induce ovulation and development of corpora lutea. Inhibition of serum LH concentration was first noted at 7 hours after treatment in the 10 week old estradiol treated gilts and continued until 17 hours after treatment; whereas, serum LH concentrations in 20 week old gilts during this period were reduced but not significantly. Average serum LH concentrations from

18 to 40 hours after treatment were similar for all groups of gilts.

Area under the LH curve for each gilt was similar for all treatment by age groups. The area under the LH curve for the first 20 hours was significantly ($P < .05$) less for the 10 week old estradiol treated gilts when compared to the control gilts of a similar age; whereas, the older gilts had similar areas. The areas under the second half of the LH curve (21 to 40 hours after treatment) were similar for all gilts.

Episodic releases of LH were designated peaks if the LH concentration was greater than one standard deviation above the mean LH concentration calculated for each gilt. Average number of peaks that occurred during the entire bleeding period were similar for all treatments. When the bleeding period was divided into ten hour intervals, estradiol treated gilts had fewer peaks during 11 to 20 hours after treatment than the control gilts. However, during 31 to 40 hours after treatment, the estradiol treated gilts had more ($P < .05$) LH peaks than did the control gilts. Other than the increase in the number of serum LH peaks, there were no substantial indications of a positive response of LH to estradiol treatment.

In the third experiment, two groups of fifteen gilts averaging 63.4 ± 1.0 or 134.8 ± 1.0 days of age and weighing 17.6 ± 1.7 or 59.8 ± 1.7 kg, respectively, were randomly assigned within age group to one of three treatments: oil and saline, oil and 25 μg of GnRH or 50 μg of estradiol and 25 μg of GnRH. The GnRH or saline was infused 24 hours after the intramuscular injection of corn oil or estradiol.

Plasma estradiol increased significantly from 6.03 ± 0.8 pg/ml before treatment to a maximum of 108.2 ± 12.7 and 61.4 ± 12.7 pg/ml

for young and old estradiol treated gilts, respectively. Plasma estradiol concentrations returned to pretreatment values by 12 hours after treatment.

Similar to experiment II, hematocrit values decreased significantly over time, and averaged 30.8 ± 0.8, 25.1 ± 0.8, 26.6 ± 0.8 and 24.5 ± 0.8 percent, respectively, for 1, 13, 24 and 37 hours after treatment. The 19 week old gilts had significantly greater ($P < .05$) hematocrit values as compared to the 9 week old gilts, but treatment did not influence hematocrit values.

Plasma progesterone concentrations (ng/ml) were similar for all gilts and were less than 0.5 ng/ml from one week before until three weeks after treatment. Thus, estradiol and/or GnRH did not induce formation of corpora lutea.

Plasma LH concentrations were significantly reduced at 14 and 16 hours after treatment with estradiol and the reduction in LH was more dramatic in the younger than in the older gilts. Average serum LH concentrations during 18 to 24 and 28 to 40 hours after corn oil or estradiol treatment were similar for all groups of gilts.

Serum LH concentration increased ($P < .01$) after treatment of control or estradiol treated gilts with GnRH. The response of serum LH concentrations to GnRH over time were explained by third order regression equations with similar intercepts and beta values for the 10 and 20 week old oil and saline treated gilts. Whereas, fifth order polynomial equations were required to best fit the data from the other treatment by age groups. Comparisons of the fifth order regression equations indicated that the LH response to GnRH was different ($P < .13$) in the young gilts as compared to the old gilts, irregardless of

estradiol pretreatment. The 10 week old gilts released more LH after the infusion of GnRH than the 20 week old gilts. Estradiol pretreatment did not alter the LH response to GnRH infusion in either age group.

In conclusion, integration of the data in the present experiments indicates that a functional negative estradiol feedback mechanism is present in 10 and 20 week old prepubertal gilts. Positive effects of estradiol on LH release were not evident by 40 hours after treatment. The release of LH from the anterior pituitary after GnRH is greater in 10 week old gilts as compared to 20 week old gilts. Estradiol injection 24 hours before GnRH infusion does not enhance the LH response to GnRH infusion.

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TABLE X

PLASMA ESTRADIOL CONCENTRATIONS IN PREPUBERTAL GILTS BEFORE AND AFTER ESTRADIOL (50 or 200 μg) TREATMENT IN EXPERIMENT I[a]

Hours After Treatment	Treatments			
	50 μg Estradiol		200 μg Estradiol	
	9 Week	19 Week	9 Week	19 Week
0	16.6	11.3	12.4	7.7
1	145.1	137.0	609.3	571.2
2	75.5	44.2	347.6	307.1
4	54.7	54.2	273.7	122.1
6	53.2	20.3	66.8	53.8
8	27.2	10.8	43.9	35.0
12	20.6	4.0	29.4	12.6
24	34.9	11.0	40.7	6.0
36	13.4	2.0	25.2	5.5

[a]Data are expressed as means (pg/ml).

TABLE XI

SERUM LUTEINIZING HORMONE CONCENTRATIONS IN PREPUBERTAL GILTS BEFORE AND AFTER ESTRADIOL (50 or 200 μg) TREATMENT IN EXPERIMENT I[a]

Hours After Treatment	Treatments			
	50 μg Estradiol		200 μg Estradiol	
	9 Week	19 Week	9 Week	19 Week
-2.5		1.1 ± 0.1		1.1 ± 0.1
-2.0	1.4 ± 0.7	2.1 ± 0.7	1.2 ± 0.7	1.0 ± 0.7
-1.5		0.8 ± 1.2		1.1 ± 0.2
-1.0	5.6 ± 1.2	0.8 ± 1.2	0.8 ± 1.2	0.9 ± 1.2
-0.5		1.0 ± 0.1		1.2 ± 0.1
-0.0	4.5 ± 2.0	1.4 ± 2.0	4.5 ± 2.0	1.5 ± 2.0
0.5		0.8 ± 0.4		1.4 ± 0.4
1.0	1.0 ± 0.2	1.0 ± 0.2	1.6 ± 0.2	1.0 ± 0.2
1.5		1.0 ± 0.2		1.2 ± 0.2
2.0	1.2 ± 0.2	1.1 ± 0.3	1.1 ± 0.2	1.2 ± 0.2
2.5		1.0 ± 0.2		0.9 ± 0.2
3.0	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
3.5		1.0 ± 0.1		0.8 ± 0.1
4.0	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
4.5		0.8 ± 0.3		0.6 ± 0.3
5.0	1.2 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	1.2 ± 0.2
5.5		0.8 ± 0.2		0.8 ± 0.2
6.0	0.9 ± 0.2	0.9 ± 0.3	0.6 ± 0.2	1.0 ± 0.2
6.5		1.0 ± 0.2		0.7 ± 0.2
7.0	0.9 ± 0.2		0.7 ± 0.2	0.6 ± 0.2
7.5		0.8 ± 0.2		1.0 ± 0.2
8.0	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
8.5		0.8 ± 0.3		0.9 ± 0.2
9.0	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	1.0 ± 0.1
9.5		0.8 ± 0.2		0.9 ± 0.2
10.0	0.6 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	0.9 ± 0.1

TABLE XI (Continued)

Hours After Treatment	Treatments			
	50 µg Estradiol		200 µg Estradiol	
	9 Week	19 Week	9 Week	19 Week
10.5		0.6		0.9
11.0	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	1.0 ± 0.2
11.5		0.6		1.1
12.0	0.7 ± 0.2	0.5 ± 0.2	0.8 ± 0.2	1.0 ± 0.2
12.5		1.0		1.1
13.0	0.80 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.0 ± 0.2
13.5				0.8
14.0	0.9 ± 0.2		0.6 ± 0.2	0.7 ± 0.2
14.5		0.6		
15.0	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.2
15.5		1.3 ± 0.1		0.8 ± 0.1
16.0	0.8 ± 0.2		0.5 ± 0.2	0.9 ± 0.3
16.5		0.4		1.1
17.0	0.8 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.2 ± 0.1
17.5		0.8 ± 0.3		1.0 ± 0.2
18.0	0.90 ± 0.1		0.6 ± 0.1	1.0 ± 0.1
18.5		0.8 ± 0.1		1.0 ± 0.1
19.0	0.8 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
19.5		1.0 ± 0.1		1.0 ± 0.1
20.0	1.0 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
20.5		0.9 ± 0.1		0.9 ± 0.1
21.0	0.8 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.8 ± 0.1
21.5		1.0 ± 0.2		0.8 ± 0.2
22.0	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
22.5		0.9 ± 0.1		0.8 ± 0.1
23.0	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
23.5				0.7 ± 0.2

TABLE XI (Continued)

Hours After Treatment	Treatments			
	50 µg Estradiol		200 µg Estradiol	
	9 Week	19 Week	9 Week	19 Week
24.0	1.4 ± 0.4	0.6 ± 0.4	0.5 ± 0.4	0.9 ± 0.4
24.5		0.8 ± 0.2		0.8 ± 0.2
25.0	1.2 ± 0.3		0.8 ± 0.3	1.0 ± 0.4
25.5		0.9		1.1
26.0	0.8 ± 0.2		0.8 ± 0.2	0.8 ± 0.2
26.5		1.1		0.9
27.0	1.0 ± 0.2	0.9 ± 0.4	0.6 ± 0.2	0.7 ± 0.4
27.5		0.6 ± 0.3		0.8 ± 0.2
28.0	1.6 ± 0.3	0.9 ± 0.4	0.6 ± 0.3	0.9 ± 0.4
28.5		0.5		1.0
29.0	2.2 ± 1.2	2.90 ± 1.2	0.6 ± 1.2	1.0 ± 1.2
29.5	0.8 ± 1.0	0.8 ± 1.0	2.5 ± 1.0	1.0 ± 1.0
30.0	1.0 ± 0.2	1.2 ± 0.2	0.6 ± 0.2	0.9 ± 0.3
30.5		0.9 ± 0.5		
31.0	0.8 ± 0.2	0.7 ± 0.3	0.8 ± 0.2	0.8 ± 0.2
31.5		1.2 ± 0.5		0.8 ± 0.7
32.0	0.8 ± 1.0	0.8 ± 1.0	2.5 ± 1.0	1.0 ± 1.0
32.5		0.8 ± 0.1		1.0 ± 0.1
33.0	0.6 ± 0.7	0.8 ± 0.7	1.0 ± 0.7	2.4 ± 0.7
33.5		0.7 ± 0.9		2.2 ± 0.9
34.0	0.6 ± 1.1	1.0 ± 1.1	2.8 ± 1.1	2.3 ± 1.1
34.5		0.9 ± 0.1		1.2 ± 0.1
35.0	2.1 ± 0.3	0.8 ± 0.3	0.8 ± 0.2	1.2 ± 0.2
35.5		0.7 ± 0.2		0.8 ± 0.2
36.0	1.4 ± 0.2	0.9 ± 0.2	1.4 ± 0.2	1.2 ± 0.2

[a]Data are expressed as means ± standard error (ng/ml) with one or two observations per period.

TABLE XII

PLASMA ESTRADIOL CONCENTRATIONS IN PREPUBERTAL GILTS BEFORE AND AFTER CORN OIL OR ESTRADIOL (50 μg) TREATMENT IN EXPERIMENT II[a]

Hours After Treatment	Treatments			
	Control		Estradiol	
	10 Week	20 Week	10 Week	20 Week
0	4.4 ± 1.8 (3)	5.4 ± 1.8 (3)	6.1 ± 1.6 (4)	6.4 ± 1.6 (4)
1	4.7 ± 2.68[b](4)	4.0 ± 24.0[b](5)	155.7 ± 24.0[c](5)	35.3 ± 26.8[b](4)
2	7.7 ± 13.0[b](4)	7.5 ± 11.6[b](5)	69.1 ± 11.6[c](5)	33.5 ± 11.6[b](5)
4	4.0 ± 5.3[b](4)	5.3 ± 4.7[b](5)	33.3 ± 5.3[c](4)	19.3 ± 5.3[c](4)
6	6.5 ± 2.6 (4)	7.4 ± 2.6 (4)	12.0 ± 2.3 (5)	10.8 ± 2.6 (4)
8	4.8 ± 2.0[b](4)	7.4 ± 2.0[b](4)	13.1 ± 2.0[c](4)	8.0 ± 1.8[bc](5)
12	3.6 ± 1.6[b](4)	4.8 ± 1.4[b](5)	9.4 ± 1.4[c](5)	5.2 ± 1.4[b](5)
24	7.7 ± 1.4 (4)	5.5 ± 1.3 (5)	4.9 ± 1.3 (5)	5.5 ± 1.3 (5)
36	8.6 ± 2.4 (4)	6.4 ± 2.2 (5)	10.7 ± 2.4 (4)	6.5 ± 2.2 (5)

[a]Data are expressed as means ± standard error (pg/mg) with the number of observations in parentheses.

[bc]Means in the same row with different superscripts are significantly ($P < .05$) different.

TABLE XIII

SERUM LUTEINIZING HORMONE CONCENTRATIONS IN PREPUBERTAL GILTS BEFORE AND AFTER CORN OIL OR ESTRADIOL (50 μg) TREATMENT IN EXPERIMENT II[a]

Hours Before and After Treatment	Treatments			
	Control		Estradiol	
	Young	Old	Young	Old
-2	1.3 ± 0.7 (4)	1.8 ± 0.7 (5)	2.6 ± 0.7 (5)	1.2 ± 0.7 (4)
-1	1.3 ± 0.8 (4)	2.5 ± 0.7 (5)	2.4 ± 0.7 (5)	0.9 ± 0.7 (5)
0	1.9 ± 0.6 (4)	1.5 ± 0.6 (5)	1.0 ± 0.6 (4)	1.5 ± 0.6 (5)
1	1.4 ± 1.1 (4)	1.4 ± 1.0 (5)	2.9 ± 1.0 (5)	1.8 ± 1.0 (5)
2	1.7 ± 0.7 (4)	2.0 ± 0.7 (5)	1.7 ± 0.7 (5)	0.8 ± 0.7 (5)
3	1.7 ± 0.5 (4)	1.7 ± 0.5 (5)	1.2 ± 0.5 (4)	1.2 ± 0.5 (5)
4	1.7 ± 1.0 (4)	2.0 ± 0.8 (5)	1.7 ± 0.8 (5)	1.1 ± 1.0 (4)
5	0.9 ± 0.4 (4)	1.7 ± 0.4 (5)	1.0 ± 0.4 (5)	1.3 ± 0.4 (5)
6	1.1 ± 0.5 (4)	2.1 ± 0.5 (5)	1.2 ± 0.5 (5)	0.8 ± 0.5 (5)
7	2.1 ± 0.4^{b}(4)	1.6 ± 0.4^{b}(5)	0.8 ± 0.4^{c}(5)	0.8 ± 0.4^{c}(4)
8	3.0 ± 0.8^{b}(4)	2.4 ± 0.7^{bc}(5)	0.7 ± 0.7^{c}(5)	1.0 ± 0.7^{c}(5)
9	1.1 ± 0.3 (4)	1.0 ± 0.3 (4)	0.8 ± 0.3 (5)	1.0 ± 0.3 (5)
10	2.9 ± 0.6^{b}(4)	1.8 ± 0.6^{b}(4)	0.4 ± 0.5^{c}(5)	0.5 ± 0.6^{c}(4)
11	5.1 ± 1.0^{b}(4)	1.1 ± 0.9^{c}(5)	0.6 ± 0.9^{c}(5)	0.8 ± 1.0^{c}(4)
12	4.0 ± 0.8^{b}(4)	1.8 ± 0.8^{c}(4)	0.7 ± 0.7^{c}(5)	1.6 ± 0.7^{c}(5)
13	4.2 ± 0.9^{b}(4)	2.0 ± 0.8^{bc}(5)	0.5 ± 0.8^{c}(5)	1.5 ± 0.8^{c}(5)
14	3.7 ± 1.3 (4)	1.7 ± 1.2 (5)	0.9 ± 1.2 (5)	1.6 ± 1.2 (5)
15	4.3 ± 1.0^{b}(3)	2.4 ± 0.8^{b}(5)	0.5 ± 0.8^{c}(5)	1.5 ± 0.8^{c}(5)
16	2.7 ± 0.8 (4)	1.7 ± 0.8 (5)	0.7 ± 0.8 (5)	0.8 ± 0.8 (5)
17	3.7 ± 0.5^{b} (4)	1.8 ± 0.4^{c}(5)	0.6 ± 0.4^{c}(5)	1.0 ± 0.5^{c}(4)
18	3.6 ± 1.3 (4)	2.7 ± 1.2 (5)	2.2 ± 1.2 (5)	1.8 ± 1.2 (5)
19	1.7 ± 0.7 (3)	1.1 ± 0.6 (5)	1.0 ± 0.6 (5)	1.8 ± 0.6 (5)
20	3.8 ± 1.3 (4)	2.1 ± 1.2 (5)	1.8 ± 1.2 (5)	1.1 ± 1.2 (5)
21	2.3 ± 1.6 (4)	2.3 ± 1.6 (4)	4.1 ± 1.4 (5)	1.5 ± 1.6 (4)
22	3.2 ± 1.0 (4)	1.5 ± 0.9 (5)	0.7 ± 0.9 (5)	1.9 ± 0.9 (5)
23	1.6 ± 0.4 (4)	1.3 ± 0.4 (5)	0.7 ± 0.4 (5)	1.1 ± 0.4 (5)

TABLE XIII (Continued)

Hours Before and After Treatment	Treatments			
	Control		Estradiol	
	Young	Old	Young	Old
24	2.2 ± 0.6 (4)	1.5 ± 0.5 (5)	0.6 ± 0.5 (5)	1.3 ± 0.5 (5)
25	2.0 ± 0.6 (4)	1.6 ± 0.5 (5)	2.0 ± 0.5 (5)	1.4 ± 0.5 (5)
26	3.4 ± 1.2 (4)	1.7 ± 1.0 (5)	1.8 ± 1.0 (5)	1.1 ± 1.0 (5)
27	2.3 ± 0.9 (4)	1.1 ± 0.8 (5)	2.5 ± 0.9 (4)	1.0 ± 0.9 (4)
28	2.0 ± 0.7 (4)	2.2 ± 0.6 (5)	1.7 ± 0.6 (5)	1.7 ± 0.6 (5)
29	3.3 ± 0.7[b] (4)	0.8 ± 0.6[c] (5)	1.9 ± 0.6[bc] (5)	1.4 ± 0.6[c] (5)
30	2.5 ± 0.9 (4)	2.1 ± 0.8 (5)	2.2 ± 0.8 (5)	0.5 ± 0.8 (5)
31	1.6 ± 0.5 (4)	1.5 ± 0.5 (5)	0.8 ± 0.5 (5)	2.3 ± 0.5 (5)
32	1.5 ± 0.3 (4)	0.8 ± 0.3 (5)	0.9 ± 0.3 (5)	0.9 ± 0.3 (5)
33	1.2 ± 0.7 (4)	1.3 ± 0.6 (5)	1.4 ± 0.6 (5)	1.9 ± 0.6 (5)
34	1.5 ± 0.4 (4)	1.3 ± 0.4 (4)	0.5 ± 0.4 (4)	1.6 ± 0.4 (5)
35	1.6 ± 0.8 (4)	1.6 ± 0.8 (5)	2.5 ± 0.8 (4)	1.9 ± 0.8 (4)
36	2.3 ± 1.0 (4)	1.3 ± 0.9 (5)	4.0 ± 1.0 (4)	2.5 ± 0.9 (5)
37	1.3 ± 0.8 (4)	2.5 ± 0.7 (5)	1.6 ± 0.8 (4)	3.0 ± 0.7 (5)
38	2.9 ± 0.9 (4)	1.1 ± 0.8 (5)	2.1 ± 0.9 (4)	1.2 ± 0.8 (5)
39	1.6 ± 0.8 (4)	2.2 ± 0.8 (4)	2.4 ± 0.8 (4)	1.2 ± 0.8 (4)
40	1.8 ± 0.9 (3)	0.8 ± 0.7 (5)	2.9 ± 0.8 (4)	1.9 ± 0.7 (5)

[a]Data are expressed as means ± standard error (ng/ml) with the numbers of observations in parentheses.

[bc]Means in the same row with different superscripts are significantly ($P < .05$) different.

TABLE XIV

PLASMA ESTRADIOL CONCENTRATIONS IN PERPUBERTAL GILTS BEFORE AND AFTER TREATMENT IN EXPERIMENT III[a]

Hours After Treatment	Treatments					
	Oil and Saline[b]		Oil and GnRH		Estradiol and GnRH	
	9 week	19 week	9 week	19 week	9 week	19 week
0	4.5 ± 2.0 (5)	9.3 ± 2.0 (5)	6.4 ± 2.0 (5)	3.7 ± 2.0 (5)	7.1 ± 2.0 (5)	5.2 ± 2.0 (5)
2	5.4 ± 12.7[c] (5)	6.7 ± 14.2[c] (4)	7.3 ± 12.7[c] (5)	5.4 ± 12.7[c] (5)	108.2 ± 12.7[d] (5)	61.4 ± 12.7[d] (5)
12	10.0 ± 2.1 (5)	9.3 ± 2.1 (5)	5.8 ± 2.1 (5)	6.0 ± 2.1 (5)	8.1 ± 2.1 (5)	5.4 ± 2.3 (4)
24	8.9 ± 2.4 (5)	9.3 ± 2.4 (5)	6.3 ± 2.4 (5)	8.1 ± 2.4 (5)	6.6 ± 2.4 (5)	9.5 ± 2.4 (5)

[a]Data are expressed as means ± standard errors (pg/ml) with the number of observations in parentheses.

[b]Oil or estradiol (50 ug) was injected (IM) twenty-four hours prior to the infusion of saline or GnRH (25 ug).

[cd] Means in the same row with different superscripts are significantly ($P < .05$) different.

TABLE XV

SERUM LUTEINIZING HORMONE CONCENTRATIONS IN PREPUBERTAL GILTS BEFORE AND AFTER TREATMENT IN EXPERIMENT III[a]

Time in Hours	Treatment					
	Oil and Saline		Oil and GnRH		Estradiol and GnRH	
	9 Week	19 Week	9 Week	19 Week	9 Week	19 Week
-2	1.0 ± 0.6^{b}(5)	2.1 ± 0.6^{bc}(5)	1.4 ± 0.6^{b}(5)	2.2 ± 0.6^{bcd}(5)	1.0 ± 0.6^{bc}(5)	3.1 ± 0.6^{d}(5)
-1	0.9 ± 0.3^{bc}(5)	1.3 ± 0.3^{bcd}(5)	0.7 ± 0.3^{d}(5)	1.9 ± 0.3^{c}(5)	1.7 ± 0.3^{bc}(5)	1.1 ± 0.3^{bcd}(5)
0	0.6 ± 0.4^{b}(5)	2.6 ± 0.4^{c}(5)	0.8 ± 0.4^{bd}(5)	1.6 ± 0.4^{cd}(5)	0.8 ± 0.4^{bd}(5)	1.9 ± 0.4^{c}(5)
2	1.3 ± 0.2 (5)	1.4 ± 0.2 (5)	1.0 ± 0.2 (5)	1.3 ± 0.2 (5)	1.1 ± 0.2 (5)	1.3 ± 0.2 (5)
4	1.0 ± 0.2^{b}(5)	1.6 ± 0.2^{c}(5)	0.9 ± 0.2^{b}(5)	1.4 ± 0.2^{bc}	1.2 ± 0.2^{bc}(5)	1.0 ± 0.2^{b}(5)
6	0.9 ± 0.2 (5)	1.5 ± 0.2 (5)	1.0 ± 0.2 (5)	1.4 ± 0.3 (5)	1.1 ± 0.2 (5)	1.5 ± 0.2 (5)
8	1.0 ± 0.2 (5)	1.2 ± 0.2 (5)	1.3 ± 0.2 (5)	1.4 ± 0.2 (5)	0.8 ± 0.2 (5)	1.0 ± 0.2 (5)
10	1.1 ± 0.6 (5)	2.3 ± 0.6 (5)	3.2 ± 9.6 (5)	1.2 ± 0.6 (5)	1.2 ± 0.6 (5)	1.0 ± 0.6 (5)
12	0.9 ± 0.9^{b}(4)	3.5 ± 0.8^{c}(5)	1.5 ± 0.8^{bc}(5)	1.5 ± 0.9^{bc}(4)	0.8 ± 0.8^{b}(5)	2.3 ± 0.9^{bc}(4)
14	2.3 ± 0.7^{bc}(4)	3.1 ± 0.6^{c}(5)	1.3 ± 0.6^{b}(5)	1.5 ± 0.6^{bc}(5)	0.8 ± 0.6^{b}(5)	1.1 ± 0.6^{b}(5)
16	2.6 ± 0.6^{b}(4)	1.8 ± 0.5 ^{bc}(5)	1.3 ± 0.5^{bc}(5)	2.0 ± 0.5^{bc}(5)	0.8 ± 0.5^{c}(5)	1.4 ± 0.5^{bc}(5)
18	2.1 ± 0.8 (4)	2.9 ± 0.7 (5)	1.3 ± 0.7 (5)	1.7 ± 0.7 (5)	0.8 ± 0.7 (5)	2.4 ± 0.7 (5)
20	1.1 ± 0.4^{b}(5)	3.0 ± 0.4^{c}(5)	1.1 ± 0.4^{b}(5)	1.4 ± 0.4^{b}(5)	1.0 ± 0.4^{b}(5)	1.6 ± 0.4^{b}(5)
22	1.1 ± 0.3 (5)	1.9 ± 0.3 (5)	1.4 ± 0.4 (4)	1.6 ± 0.4 (4)	1.1 ± 0.3 (5)	1.8 ± 0.3 (5)
24	1.1 ± 0.7 (5)	1.9 ± 0.7 (5)	1.4 ± 0.7 (5)	1.0 ± 0.7 (5)	0.8 ± 0.7 (5)	2.9 ± 0.9 (5)

TABLE XV (Continued)

Time in Hours	Treatment					
	Oil and Saline		Oil and GnRH		Estradiol and GnRH	
	9 Week	19 Week	9 Week	19 Week	9 Week	19 Week
24.25	1.1 ± 1.9^{b}(5)	1.3 ± 2.2^{b}(4)	17.6 ± 1.9^{c}(5)	13.9 ± 1.9^{c}(5)	16.7 ± 1.9^{c}(5)	13.2 ± 1.9^{c}(5)
24.50	1.1 ± 2.4^{b}(5)	1.3 ± 2.4^{b}(5)	16.7 ± 2.4^{c}(5)	13.5 ± 2.4^{c}(5)	17.1 ± 2.4^{c}(5)	13.0 ± 2.4^{c}(5)
24.75	0.9 ± 1.7^{b}(5)	1.1 ± 1.7^{c}(5)	11.7 ± 1.9^{c}(4)	10.0 ± 1.7^{c}(5)	12.7 ± 1.7^{c}(5)	10.0 ± 0.7^{c}(5)
25.00	1.2 ± 1.6^{b}(5)	1.5 ± 1.6^{b}(5)	7.2 ± 1.6^{c}(5)	6.8 ± 1.6^{c}(5)	10.5 ± 1.6^{c}(5)	5.8 ± 1.6^{c}(5)
25.50	1.0 ± 1.2^{b}(5)	1.0 ± 1.2^{b}(5)	3.7 ± 1.2^{c}(5)	6.5 ± 1.2^{c}(5)	5.8 ± 1.2^{c}(5)	5.1 ± 1.2^{c}(5)
26.00	1.9 ± 0.6 (5)	1.4 ± 0.6 (5)	2.3 ± 0.6 (5)	3.1 ± 0.6 (5)	3.3 ± 0.6 (5)	2.9 ± 0.6 (5)
26.50	1.6 ± 0.5 (5)	1.6 ± 0.5 (5)	2.5 ± 0.5 (5)	2.1 ± 0.5 (5)	2.6 ± 0.5 (5)	2.7 ± 0.5 (5)
27.00	1.8 ± 0.3 (5)	1.2 ± 0.3 (5)	1.7 ± 0.3 (5)	1.7 ± 0.3 (5)	1.8 ± 0.3 (5)	2.1 ± 0.3 (5)
28.00	0.8 ± 0.3^{b}(5)	1.1 ± 0.3^{bd}(5)	1.4 ± 0.3^{bd}(5)	1.3 ± 0.3^{bd}(5)	1.9 ± 0.3^{cd}(5)	2.5 ± 0.4^{c}(4)
29	1.2 ± 0.4 (5)	2.1 ± 0.4 (5)	1.0 ± 0.4 (5)	1.5 ± 0.4 (5)	1.5 ± 0.4 (5)	1.2 ± 0.4 (5)
30	1.0 ± 0.4 (5)	1.9 ± 0.4 (5)	1.7 ± 0.4 (5)	1.2 ± 0.4 (5)	1.1 ± 0.4 (5)	1.7 ± 0.4 (5)
31	1.0 ± 0.3 (4)	1.1 ± 0.2 (5)	1.1 ± 0.2 (5)	1.0 ± 0.2 (5)	1.3 ± 0.2 (5)	1.3 ± 0.2 (5)
32	1.0 ± 0.3 (5)	1.5 ± 0.3 (5)	1.5 ± 0.3 (5)	1.2 ± 0.3 (5)	1.2 ± 0.3 (5)	1.2 ± 0.3 (5)
33	1.4 ± 1.1 (4)	3.6 ± 1.0 (5)	0.7 ± 1.0 (5)	1.0 ± 1.0 (5)	1.2 ± 1.0 (5)	1.1 ± 1.0 (5)
34	1.0 ± 0.3^{b}(5)	1.7 ± 0.3^{c}(5)	1.0 ± 0.3^{b}(5)	1.1 ± 0.3^{bc}(5)	1.2 ± 0.3^{bc}(5)	1.7 ± 0.3^{bc}(5)
35	1.0 ± 0.3^{bc}(5)	1.6 ± 0.3^{b}(5)	0.7 ± 0.3^{c}(5)	1.1 ± 0.3^{bc}(5)	0.8 ± 0.3^{c}(5)	1.3 ± 0.3^{bc}(5)

TABLE XV (Continued)

Time in Hours	Treatment					
	Oil and Saline		Oil and GnRH		Estradiol and GnRH	
	9 Week	19 Week	9 Week	19 Week	9 Week	19 Week
36	1.4 ± 0.6 (5)	2.1 ± 0.6 (5)	1.5 ± 0.6 (5)	1.1 ± 0.6 (5)	1.5 ± 0.6 (5)	1.5 ± 0.6 (5)
37	1.3 ± 0.6 (5)	1.6 ± 0.5 (5)	1.5 ± 0.5 (5)	1.6 ± 0.5 (5)	0.8 ± 0.5 (5)	1.8 ± 0.5 (5)
38	2.5 ± 0.6 (5)	1.7 ± 0.6 (5)	2.0 ± 0.6 (5)	1.6 ± 0.6 (5)	1.3 ± 0.6 (5)	1.4 ± 0.6 (5)
39	1.0 ± 1.1 (4)	3.5 ± 1.0 (5)	1.1 ± 1.0 (5)	1.1 ± 1.0 (5)	1.2 ± 1.0 (5)	1.3 ± 1.0 (5)
40	1.3 ± 0.6 (4)	1.5 ± 0.5 (5)	1.6 ± 0.5 (5)	1.0 ± 0.5 (5)	1.1 ± 0.5 (5)	1.4 ± 0.5 (5)

[a]Data are expressed as means ± standard errors (ng/ml) with the number of observations in parentheses.

[bc]Means in the same row with different superscripts are significantly ($P < .05$) different.

TABLE XVI

BUFFER SOLUTIONS USED IN RADIOIMMUNOASSAY PROCEDURES

Stock Buffer

A. Monobasic Sodium Phosphate Buffer (0.5 M)

Weigh 69.0 g $NaH_2PO_4 \cdot H_2O$ (monobasic) and dilute to 1,000 ml with glass distilled water. Store at 5 C.

B. Dibasic Sodium Phosphate Buffer (0.5 M)

Weigh 71.0 g anhydrous or 134 g heptahydrate Na_2HPO_4 (dibasic) and dilute to 1,000 ml with glass distilled water. Store at 5 C.

C. Stock Phosphate Buffered Saline (0.05 M)

1. 120 ml of Monobasic Buffer (A)
2. 240 ml of Dibasic Buffer (B)
3. 143 g sodium chloride, NaCL
4. 1.75 g Thimerosol (Merthiolate)
5. Add glass distilled water to a final volume of 3500 ml
6. Check pH; adjust to 7.0, if necessary, using sodium hydroxide or phosphoric acid and store at 5 C

Working Buffers

A. Phosphate Buffered Saline Working Solution (PBS)

Dilute one part PBS Stock with four parts glass distilled water.

B. Phosphate Buffered Saline Plus 0.1% Gelatin (PBS + Gel)

Weigh one gram Knox Gelatin and dilute to 1,000 ml with PBS working solution.

C. Phosphate Buffered Saline Plus Ethylenedinitrilotetra-acetic acid-Disodium Salt (PBS + EDTA) (0.05 M)

1. Weigh 18.61 g disodium EDTA
2. Add about 800 ml PBS, warm and stir until dissolved
3. Adjust pH to 7.0 by adding 5 N NaOH while stirring
4. Adjust volume to 1,000 ml with PBS and store at 5 C

TABLE XVII

PREPARATION OF LIQUID SCINTILLATION FLUIDS

A. Steroid Counting Fluid	
Toluene (Scintillation Grade)	3800 ml
PPO (2,5 diphenyloxazole)	15 g
POPOP (1,4-bis-2-(5-Phenylorazolye)-Benzene)	0.15 g
B. Protein Binding Counting Fluid	
Toluene (Scintillation Grade)	3000 ml
PPO (2,5 diphenyloxazole)	21.7 g

VITA[2]

Thomas Andrew Hoagland

Candidate for the Degree of

Doctor of Philosophy

Thesis: INFLUENCE OF ESTRADIOL ON SERUM LUTEINIZING HORMONE CONCENTRATIONS IN THE PREPUBERTAL GILT

Major Field: Animal Breeding

Biographical:

Personal Data: Born in Boston, Massachusetts, April 8, 1947, the son of Mr. and Mrs. Andrew J. Hoagland; father of two daughters, Elisabeth Ann age 12 and Julie Marie age 10.

Education: Graduated from Braintree High School, Braintree, Massachusetts, in May, 1965; received the Associate of Arts degree from Northeastern University, Boston, Massachusetts, in May, 1970; received the Bachelor of Science degree in Agriculture from the University of Connecticut in May, 1974; received the Masters of Science degree in Agriculture from the University of Connecticut in May, 1976; completed the requirements for the Doctor of Philosophy degree at Oklahoma State University in May, 1980.

Experience: Journeyman machinest and carpenter in Local 629 of the International Brotherhood of Carpenters, Brockton, Massachusetts; Graduate Assistant at the University of Connecticut, 1974-1976 and at Oklahoma State University, 1976-1979.

Organizations: Member of the American Society of Animal Science and Sigma Xi.