ENDOCRINE CHANGES ASSOCIATED WITH EMBRYOGENESIS AND HEAT STRESS IN GILTS

By

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IN GILTS

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CHAPTER I

INTRODUCTION

With an increase in the number of large scale swine confinement systems, farrowing occurs during all months of the year. Reduced litter size and decreased conception rates have been observed during the months of elevated ambient temperature.

It has been demonstrated that exposure of gilts to elevated ambient temperature from 0 to 16 days postbreeding results in lowered conception rates and reduced litter size at 30 days postbreeding. However, the physiological causes of this reduced reproductive performance following heat stress in gilts is unknown.

The reduction in sow reproductive performance could be the result of a direct effect of elevated body temperatures upon the embryo or it could be related to changes in the uterine environment caused by alterations in endocrine function. Modifications in the uterine environment could affect the gametes prior to fertilization, or interfere with the development and implantation of the embryo.

Endocrine parameters associated with early pregnancy and embryogenesis in swine have been studied, however, little information is available on the effects of elevated ambient temperature on endocrine function in gilts. An evaluation of these changes could lead to the development of management or therapeutic practices which would result in improved reproductive performance in swine.

The purposes of this study were to quantitate normal endocrine function in gilts during the first 28 days of pregnancy, and to determine alterations in plasma hormones in gilts exposed to elevated ambient temperatures during 1 to 8 days postbreeding.

CHAPTER II

LITERATURE REVIEW

Estrous Cycle

The porcine estrus cycle can be divided into four phases; proestrus, estrus, metestrus and diestrus. Proestrus is the period of rapid follicular development prior to estrus, when follicles begin to secrete estrogen. Estrogen causes changes in the external genitalia, proliferation of the uterine epithelium and the behavioral expression of estrus or standing heat. Ovulation occurs during the latter part of estrus. Metestrus is the period when the corpora lutea form and begin actively secreting progesterone, which causes increased proliferation of the uterine epithelium. Diestrus is the period between metestrus and proestrus when the corpora lutea regress. If fertilization and implantation occur, the life of the corpora lutea is extended through pregnancy and diestrus does not occur.

Age at First Estrus

Puberty or sexual maturity in gilts is the age when first estrus occurs. Gilts usually exhibit their first estrus between six and eight months of age (Anderson, 1974). Age at puberty, however, can be influenced by level of nutrition, breed, seasons, body weight, inbreeding, management practices, and the presence or absence of males.

Estrous Cycle Length and Duration of Estrus

Gilts are polyestrus, and have an estrous cycle length of about 21 days. The duration of estrus is approximately 2 to 3 days (Asdell, 1964). Small variations in cycle length due to age, breed, and season have been noted (Cole and Cupps, 1972). McKenzie and Miller (1930) reported an average cycle length of 21 to 22 days and the duration of estrus as 40 to 46 hours in Poland China gilts. Recently, Hallford et al. (1975) reported an average cycle length of 19.4 ± .5 days in crossbred (Yorkshire x Hampshire) gilts.

Time of Ovulation

Few studies on the exact time of ovulation in gilts or sows are available. As in most domestic animals, ovulation in the pig occurs during the latter part of estrus, approximately 30 to 40 hours after the onset of heat (Nalbandov, 1964). Signoret et al. (1972) observed that the time of the onset and the duration of ovulation in gilts was decreased by mating. Ovulation in unmated animals began at 38.0 hours and ended at 41.8 hours after the beginning of estrus, while in mated gilts, ovulation began at 34.1 hours and ended by 35.0 hours after the start of estrus.

Endocrine Functions During the Estrous Cycle

Progesterone. Plasma progesterone concentrations in swine decrease to 1 ng/ml or less at or near the time of estrus. Concentrations of progesterone then increase rapidly, beginning on day 3 or 4

(day 0 is the onset of estrus), and reach a maximum of about 30 ng/ml on days 10 to 14 of the cycle. From maximum concentrations near day 14, plasma progesterone declines precipitously to concentrations similar to those observed at estrus by day 18 of the cycle, and remain low through the estrus period (Tillson et al., 1970; Henricks et al., 1972; Stabenfeldt et al., 1969). Progesterone quantities at related times in ovarian venous effluent and in luteal tissue follow similar changes to those in peripheral plasma (Gomes et al., 1965; Mausada et al., 1967). The excretion of total metabolites of progesterone in the urine of sows during the estrous cycle undergo changes similar to those which occur in plasma progesterone concentrations (Tillson et al., 1970).

Estrogens. Concentrations of total estrogens in the plasma of gilts fluctuate around 20 pg/ml during the estrous cycle until about the time plasma progesterone begins to decline (Henricks et al., 1972). Estrogen concentrations then increase rapidly and reach maximum amounts of 40 to 60 pg/ml about two days prior to estrus. Usually, plasma estrogen during proestrus is approximately two fold greater than during the remainder of the cycle. Shearer et al. (1972) reported that plasma estradiol-17β concentrations vary during the cycle from 5 to 35 pg/ml and increase to a maximum of 85 pg/ml on the day prior to the onset of estrus. However, Hallford et al. (1975), observed lower estradiol concentrations when quantified by radioimmunoassay. These increases in plasma estrogens prior to and during early estrus are in agreement with increases observed in urinary excretion of estrogens (Raeside, 1963; Liptrap and Raeside, 1968).

Luteinizing Hormone (LH). LH concentration in the blood serum of pigs remains relatively constant, about 1 ng/ml, throughout the cycle until it increases to maximum concentrations of 2 to 4 ng/ml at or near the time of estrus. Henricks et al. (1972) observed a slight increase in LH concentration which coincided with the rapid decrease in plasma progesterone during the proestrus period. With frequent sampling near the time of estrus, Niswender et al. (1970) observed that highest serum concentrations of LH occurred at the onset of estrus. This suggests a possible physiological relationship between the LH peak and the behavioral manifestations of estrus.

Follicle Stimulating Hormone (FSH). Using a recently developed specific radioimmunoassay for porcine FSH, Rayford et al. (1974) observed concentrations of 6 to 8 ng/ml of FSH in porcine serum on the day prior to behavioral estrus and maximum concentrations of about 10 ng/ml on days 2 and 3 after estrus. In nonpregnant gilts, pituitary FSH concentrations determined by bioassay were low at estrus and remained low until day 4 (Parlow et al., 1964). FSH increased in the pituitary on day 10 and day 18, and then decreased to low concentrations prior to the next estrus.

Corticoids. Limited information is available on plasma corticoid concentrations during the estrous cycles of gilts. Brown et al. (1974) observed elevated plasma cortisol concentrations of 32 ng/ml at estrus, compared to 11.6 ng/ml on day 7 and 7.1 ng/ml on day 14. However, the increases in cortisol at estrus may have been attributable to increased stress and handling during this period rather than to alteration in adrenal function due to the stage of the estrous cycle. Plasma

corticosterone concentrations averaged 10 to 95 percent of the cortisol concentration but did not vary significantly during days 0, 7 or 14 of the estrous cycle.

Pregnancy

Pregnancy begins with fertilization and ends in either abortion or parturition. During this reproductive stage, endocrine alterations occur which support normal fetal growth and development.

Fertilization

Fertilization normally occurrs in the ampullary region of the oviduct. Optimum conception rates in sows are achieved when breeding takes place on the second day of estrus (Hancock and Hovell, 1962). With breedings at this time, sperm are usually present and fertile when ovulation occurs.

Implantation

An embryo is considered to be implanted when it is firmly attached to the endometrium and is no longer free floating within the lumen of the uterine horn.

Macdonald et al. (1967) have demonstrated that LH can initiate implantation in rats. Luteinizing hormone may initiate implantation through increased estrogens, since Yoshinaga and Host (1961) have shown that estrogen causes implantation in lactating rats. Based on these studies, LH and estrogen may play a role in implantation in gilts, however, detailed studies of endocrine changes at the time of implantation have not been reported in swine.

Porcine ova enter the uterus at 3 to 4 days after estrus, usually in the 4-cell stage (Anderson, 1974). The blastocyst has usually formed by about day 7 and the zona pellucida surrounding the blastocyst has been shed. Then the cells of the trophoblast are in contact with the uterine epithelium. The trophoblast starts to proliferate rapidly, endoderm appears, and the blastocyst changes from a small spherical mass to a long, thread-like tube of several feet in length by day 11 to 13 of pregnancy. Intrauterine spacing is complete by this time, and implantation occurs between days 14 and 18. Up to and including the period of implantation, the embryos depend upon absorption of uterine secretions to provide nutrient requirements. Length of gestation in the sow averages approximately 114 days.

Factors Affecting Maintenance of Corpora Lutea

Functional corpora lutea are necessary for the maintenance of pregnancy in the sow throughout gestation and destruction of the corpora lutea at any time during gestation will result in the termination of pregnancy within hours (Anderson, 1966).

The uterus plays a major role in the determination of the life span of the corpora lutea in swine, since hysterectomy of cycling gilts before days 14 to 16 after estrus results in the persistence of the CL for many months (Spies et al., 1960; Anderson et al., 1961; Niel and Day, 1964). Hysterectomy after day 16 of the cycle results in ovulation, estrus and maintenance of the new corpora lutea, indicating that the luteolytic factor present in the uterus had already acted by day 16. Anderson et al. (1966) determined that the presence of

seven-eighths of the non-gravid uterine horn at 35 days after breeding reduced conception or interferred with pregnancy in the opposite horn in 23 of 27 gilts. Decreasing portions of a non-gravid horn led to increasing percentages of gilts unilaterally pregnant at 35 days after breeding. Sixty-one percent of a group of gilts with one-fourth of a non-gravid uterus present remained pregnant in the opposite horn at day 35, however, unilateral luteal regression on the side of the non-gravid horn occurred in 80 percent of these gilts. This suggests both a systemic and local luteolytic effect of the non-gravid uterus in swine.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) may be, or at least mimics, the action of the luteolytic factor produced by the non-gravid uterus. Hallford et al. (1975) have shown that injection of $PGF_{2\alpha}$ on day 12 caused a rapid decrease in plasma progesterone concentrations and significantly decreased the length of the estrous cycle in gilts. Diehl et al. (1974) observed that $PGF_{2\alpha}$ administered to gilts during late gestation caused a rapid decline in plasma progesterone and induced farrowing within 28.9 hours.

Ovarian steroids may be involved in the control of the luteolytic function of the uterus, since continuous daily injections of estrone or estradiol into cycling gilts beginning on day 11 of the cycle resulted in maintenance of the corpora lutea for 23 days after the first injection (Gardner et al., 1963).

Endocrine Function During Pregnancy

Progesterone. During early pregnancy, plasma progesterone in gilts is similar to concentrations during the luteal phase of the

estrous cycle in non-pregnant gilts. Maximum progesterone concentrations of about 30 ng/ml occur near day 14 and then decline gradually to approximately 15 to 20 ng/ml by day 20 (Tillson and Erb, 1967; Stabenfelt et al., 1969; Guthrie et al., 1972; Robertson and King, 1974; Shearer et al., 1972). Plasma progesterone remains at about 10 to 15 ng/ml throughout gestation, until a few hours prior to parturition, when it rapidly declines to low concentrations similar to those observed at estrus (Ash and Heap, 1975; Shearer et al., 1972; Robertson and King, 1974).

Although plasma progesterone is similar in cycling and pregnant gilts during the first 14 days after estrus, Tillson et al. (1970) observed that urinary excretion of metabolites of progesterone was significantly higher in pregnant than in non-pregnant sows as early as four days postbreeding. This indicates that the presence of embryos in the uterus as early as day 4 may alter progesterone metabolism.

Estrogens. Total unconjugated plasma estrogens vary from 10 to 28 pg/ml through day 24 of pregnancy (Guthrie et al., 1972). Similarly, concentrations of estradiol observed by Shearer et al. (1972) varied from 5 to 30 pg/ml in gilts on days 24 to 38 of pregnancy. Robertson and King (1974) observed estrone sulfate concentrations greater than 3 ng/ml plasma between days 23 and day 30 of pregnancy, while plasma estrone and estradiol levels were less than 15 pg/ml. Lunass (1962) reported that the time of maximum estrone excretion in the urine of sows occurs 24 to 27 days after breeding. Urinary estrone sulfate then decreased to low quantities around day 50 before a second increase which was concurrent with the increase in estrone and estradiol just before parturition. At or near parturition, the concentration

of estradiol approaches about 400 pg/ml, while estrone increases to about 2.5 ng/ml or higher. The plasma concentration of both estradiol and estrone decrease rapidly within a few hours after farrowing (Robertson and King, 1974; Ash and Heap, 1975). Shearer et al. (1972) also observed a postpartum decline in plasma estradiol, and a transient increase in estradiol on about the sixth day after parturition. Increased estradiol on day 6 might be related to the anovulatory postpartum heat sometimes observed in sows.

Corticoids. Only limited information is available on plasma corticoids during gestation in swine. Ash and Heap (1975) observed average concentrations of about 33 ng/ml during late pregnancy with no consistent increases at the time of parturition. However, Mololcwu and Wagner (1973) and Killian et al. (1973) found increased plasma corticoids in sows on the day of parturition.

Luteinizing Hormone (LH). Conflicting data exists on plasma LH concentrations in gilts during early pregnancy. Tillson et al. (1970) observed greater plasma LH concentrations in open gilts than in pregnant gilts, while Guthrie et al. (1972) observed greater LH in pregnant than in open gilts. The data presented by Guthrie et al. (1972) may have included some seasonal or between assay variation since comparisons were between groups of gilts from two different experiments conducted at different times. Concentration of LH during middle and late pregnancy and at parturition have not been reported.

Effects of Heat Stress During Pregnancy

It is generally accepted that hot environments tend to have a detrimental effect upon reproductive performance in most domestic animals, at least during certain periods of the reproduction cycle. Increased embryonic mortality and decreased conception rates after exposure to elevated ambient temperature have been reported in rats (Fernandez-Cano, 1958a) cattle (Stott and Williams, 1962; Dunlap and Vincent, 1971), sheep (Dutt, 1963; Alliston et al., 1961) and swine (Tompkins et al., 1967; Edwards et al., 1968; Omtvedt et al., 1971; Warnick et al., 1965). During the summer, sows and gilts in lots with sprinklers and shades had larger litters than those in lots with only shades (Whatley et al., 1957).

The periods during which elevated ambient temperature has a detrimental effect upon conception rates and litter size in gilts have been examined. Warnick et al. (1965) found that exposure of gilts to 32.2 C during the estrous cycle prior to breeding had no effect upon their subsequent reproductive performance. However, exposure to the same temperature during days 3 to 25 postbreeding caused a reduction in the number of live embryos compared to gilts maintained at 15.6 C. Edwards et al. (1968) found that litter size and conception rates were reduced when gilts were exposed to 38.9 C during days 1 to 15 postbreeding, compared to control gilts maintained at 23.4 C. However, reproductive performance in gilts exposed to elevated ambient temperatures during the previous estrous cycle or during days 15 to 30 postbreeding was not significantly influenced. Tompkins et al. (1967) similarly observed

that thermal stress during days 1 to 5 postbreeding caused a reduction in the number of viable embryos when compared to gilts heat stressed during 20 to 25 days postbreeding or control gilts. When gilts were heat stressed 0 to 8 days or 8 to 16 days postbreeding, they had a significant reduction in the number of viable embryos at 30 days postbreeding (Omtvedt et al., 1971).

When gilts were exposed to elevated ambient temperatures during days 102 to 110 postbreeding, they had significantly more stillborn pigs at birth than controls (Omtvedt, 1971). But, reproductive performance of gilts was not affected by thermal stress during 53 to 60 days postbreeding. Heitman et al. (1951) also concluded that exposure of sows to elevated ambient temperature at approximately 85 days of pregnancy would result in death of the sow before it would cause death or abortion of the litter.

From these studies it appears that the period when elevated ambient temperature has the most detrimental effect upon embryo survival is during days 0 to 16 of pregnancy. From days 20 to 25 of pregnancy, until near the time of parturition, gilts appear to be relatively resistant to any effects of heat stress upon reproductive performance.

Elevated ambient temperature may affect the embryo directly through increased body temperatures, or indirectly, by changes in the uterine environment caused by altered endocrine function. Fernandez-Cano (1958a) observed that increased body temperature in rats during early pregnancy induced embryonic degeneration and this effect could be eliminated by adrenalectomy prior to heat stress (Fernandez-Cano, 1958b). Adrenalectomy did not increase the number of viable embryos at 13 days after breeding in rabbits that were heat stressed during early pregnancy

(Howarth, 1969), however, embryonic loss occurred before fertilization in adrenalectomized rabbits and after fertilization in intact rabbits. Embryonic mortality induced by heat stress in ewes may be reduced by adrenalectomy, (Tilton et al., 1972). Adrenal hypersecretion may cause embryonic mortality since embryonic survival in ewes was reduced by injections of hydrocortisone acetate (Howarth and Hawks, 1968). Other alterations in endocrine function may be involved in reduced embryonic survival since exposure of cows to elevated ambient temperature resulted in reduced plasma LH (Madan and Johnson, 1973) and heat stress of heifers caused increased plasma concentrations of progesterone (Mills et al., 1972).

CHAPTER III

MATERIALS AND METHODS

Procedure

The Hampshire x Yorkshire crossbred gilts that were used in this study were 7 to 10 months of age. The gilts were observed for estrus each morning, using a teaser boar, and all gilts had at least one estrous cycle before treatments were initiated.

Experiment I

This experiment was conducted to characterize normal plasma concentrations of progesterone, estradiol, and LH in gilts during the first 28 days of pregnancy or during the estrous cycle after a non-fertile mating. A second objective was to develop a cannulation technique so that blood samples could be collected at regular intervals without stress to the animals.

Gilts were started on the experiment between December, 1973, and May, 1974. After one normal estrous cycle, 13 gilts were cannulated 6 to 12 days prior to estrus. Gilts were anesthetized with sodium thiopental (Pentothal; Abbot Laboratories) and inverted into a V-shaped trough for cannulation. A 12-gauge thinwall needle about 10 cm in length was inserted about 2 cm anterior to and 3 cm lateral to the tip of the sternum (either the left or right side were used). The needle

tip was angled slightly posterior and medially and inserted until it penetrated the anterior vena cava. Penetration of the anterior vena cava was determined by the free flow of blood when the plunger of the 50 ml syringe attached to the needle was withdrawn. Upon penetration of the anterior vena cava, the syringe was removed from the needle and silastic tubing (Dow Corning; 1.14 mm ID, 2.16 mm OD, 120 cm length) was quickly inserted through the needle until about 40 cm was in the vein. When the needle was withdrawn, about 30 cm of the cannula remained in the vessel. Using a trocar (8 ga. stainless steel, 30 cm), the remainder of the cannula was placed under the skin and it was exteriorized at the top of the back as shown in Figure 1. After it was noted that cannulae in some animals came out of the vein due to animal movement and blood pressure against the cannula, a 2 cm collar of larger diameter silastic tubing (1.98 mm, ID; 3.17 mm, OD) was placed around the cannula at the point of insertion and was sutured to the connective tissue below the skin. The collar was secured on the cannula by spray adhesive (Hollister Silicone Medical Adhesive; Dow Corning Type-B). A leather pouch about 9 cm square was sutured to the skin at the top of the back to store the loose end of the cannula. During cannulation, the tubing was flushed with 3 percent sterile sodium citrate. After the cannula was secured, a knot was tied in the exposed end until sampling started. The knot was cut from the cannula and a syringe with a blunted 17 ga needle was inserted to obtain a sample.

Following cannulation, gilts were placed in either metabolism type crates or individual pens until estrus. They received 300,000 units of penn-strepp antibiotic (Merck Chemical Division) for three days after cannulation. Gilts were observed for estrus with a boar

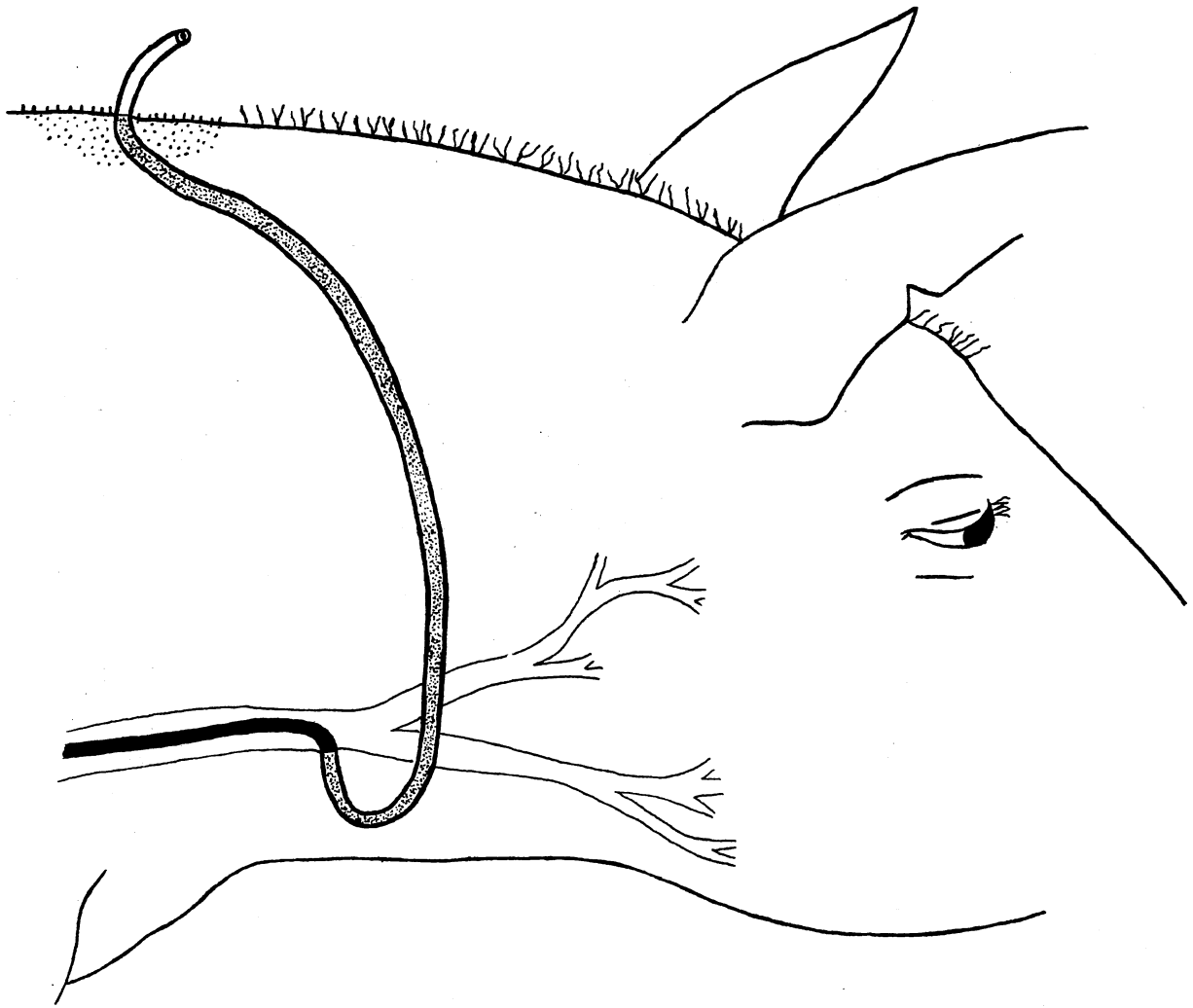

Figure 1. Diagram of Indwelling Cannula in the Procine Anterior Vena Cava

each morning and allowed to mate on the first day of standing heat (day 0) and were artificially inseminated on day 1. A blood sample was collected immediately after breeding on day 0 and each morning thereafter through day 28 of pregnancy, or until cannulae were no longer functional. Animals were confined in individual crates during the experiment and received 12 hrs of artificial lighting (8 am to 8 pm) each day. Embryo and corpora lutea numbers were determined at slaughter on day 33.0 $\pm$ 5.0 ($\bar{X} \pm$ SD). Blood samples of about 25 ml were removed via cannulae and placed in 50 ml plastic centrifuge tubes containing 31.7 g of oxalic acid. Samples were centrifuged at 2700 x g for 15 minutes at 4 C within one hour of collection. Plasma was stored at -10 C until hormones were quantified by radioimmunoassays.

Experiment II

Experiment II was conducted to determine the effects of exposure of gilts to elevated ambient temperatures during days 1 to 8 postbreeding on conception rates, number of live embryos, and endocrine function. Gilts were started on this experiment from July 2, 1974, through December 26, 1974; the number of gilts on the experiment was evenly distributed throughout this period.

Twenty-four gilts were observed for estrus and cannulated as in Experiment I. Gilts were bred naturally on the first day of estrus (day 0) and were artificially inseminated on day 1. A blood sample was collected immediately after breeding on day 0 and another was obtained at 8 pm. Following the second bleeding on day 0, gilts were randomly allotted to either cool or hot environmental chambers. The cool chamber was maintained continuously at 23 C. The hot chamber was

maintained at 35 C from 8 am to 8 pm, and at 32 C from 8 pm to 8 am. Both chambers received 12 hrs of artificial light (8 am to 8 pm) and relative humidity was maintained at 50 percent. Each chamber contained two crates (60 x 150 cm) and gilts were usually confined in pairs in the chambers.

During the eight days (days 1 to 8) of confinement in the environmental chambers, gilts were bled daily at 8 am and 8 pm, while consuming feed. Gilts were fed twice daily and water was provided at chamber temperature ad libitum by a nipple waterer. At 8 pm on day 8, gilts were removed from the environmental chamber and returned to the swine barn. They were maintained in crates or individual pens and blood samples were taken once daily through day 22. Ambient temperature varied from about 7 to 27 C. Blood samples were collected and processed as in Experiment I and were stored at -10 C until assayed for progesterone, estradiol, LH and total corticoids. Gilts were slaughtered at 31.5 $\pm$ 4.9 (Mean $\pm$ SD) days postbreeding and conception rates, corpora lutea number and embryo number were determined.

Plasma Hormone Quantification

Progesterone

Plasma progesterone was quantified by a radioimmunoassay similar to that described by Kittok et al. (1973). The cross reactivity of the antibody (#869, generously provided by Dr. G. D. Niswender, Department of Physiology and Biophysics, Colorado State University, Fort Collins) has been described previously (Niswender, 1973). The specificity of the assay in our laboratory for porcine plasma has been

determined by comparison of results obtained by isolation of progesterone on Sephadex LH-20 columns and those obtained on the same sample without column chromotography (Hallford et al., 1975). Progesterone concentrations obtained for steer plasma and steer plasma plus 5 ng/ml progesterone, included as internal standards in this series of assays averaged 0.4 ± .1 and 5.0 ± .2 ng/ml, respectively (mean ± SE, n = 23).

Estradiol

Estradiol was determined by a specific radioimmunoassay. Antisera prepared against 6β-succinyl-estradiol conjugated to bovine serum albumin (antisera #244) was supplied by Dr. G. D. Niswender. The procedure used was similar to that used by Wettemann et al. (1972) as modified by Hafs et al. (1974) for estradiol in bovine plasma. Triplicate 1 ml aliquots of plasma samples were pipetted into 16 x 85 mm disposable culture tubes. As an estimate of procedural losses, approximately 3000 dpm of ^{3}H-2,4,6,7 estradiol (111 Ci/mmole, purified on a Sephadex LH-20 [Pharmecia Fine Chemicals, Incorporated] column, Swanson et al., 1972) was added to one of each set of triplicate tubes. The plasma samples were vortexed gently and allowed to equilibrate for 30 minutes at room temperature. All three samples were then extracted by vortexing for 1.5 minutes with 4 ml of nanograde benzene. The extract from the tube with ^{3}H-estradiol was transferred with a disposable Pasteur pipette to a scintillation vial and the solvent was evaporated. Steroid scintillation fluid (Appendix Table VI) was added and radioactivity was quantified in a Packard Tri-Carb liquid scintillation spectrometer to estimate procedural losses for each plasma sample. Extracts from the two remaining tubes for each sample were pipetted into 12 x 75 mm disposable

culture tubes and evaporated to dryness with nitrogen. A water blank and a reference plasma sample included in each assay served as checks on solvent purity and assay variability. Standard estradiol was prepared in redistilled ethanol so that 100 ul contained 0, 1, 2, 4, 6, 10, 20, 40, 60 and 100 pg. Two standard curves were included in each rack of 48 tubes.

After evaporation of the solvent from samples and standards, 200 ul of antisera (1:100,000 dilution in phosphate buffered saline plus 0.1 percent gelatin, Appendix Table VII) was added to each tube. Tubes were then gently vortexed and incubated at room temperature for 30 minutes. Approximately 30,000 dpm of ^{3}H-2,4,6,7-estradiol (in 100 ul PBS plus 0.1 percent gelatin) was added to each tube, and tubes were vortexed and allowed to incubate three to four hours at 4 C.

Following incubation, the tubes were placed in an ice water bath for 10 minutes, then 1 ml of dextran-coated charcoal solution (2.5 g activated neutral Norit and .25 g dextran T-150 per liter of glass distilled water) was added to each tube. The charcoal solution was added to all 48 tubes in an assay rack within two minutes. Tubes were vortexed immediately, allowed to incubate 10 minutes in the ice water bath, and then centrifuges at 1600 g at 4 C for 10 minutes. A 500 ul aliquant of the supernate from each sample was diluted with 10 ml of aqueous scintillation fluid (Appendix Table VIII) and radioactivity was quantified. The concentration of estradiol in samples was calculated by interpolation between two points on the standard curve and corrections were made for procedural losses.

To determine the specificity of the antibody for estradiol the cross-reactivity with various other steriods was determined. An

antibody dilution of 1:100,000 gave optimum percent binding (19 percent) and sensitivity (5 percent reduction in binding with 1 pg estradiol). Estriol did not cross-react, while 320 pg estrone was equivalent to about 10 pg estradiol and 200 pg estradiol benzoate was equivalent to 80 pg estradiol. Progesterone (6.4 ng), testosterone (10 ng), cortisol (10 ng) and corticosterone (10 ng) showed little or no cross-reactivity in the assay.

To validate the assay for porcine plasma, estradiol in benzene extracts from plasma samples was isolated on Sephadex LH-20 columns using benzene:methanol (95:5; V:V; Chenault et al., 1975). Columns were prepared by placing a glass fiber filter disk at the bottom of a 2.5 ml glass syringe and pouring a Sephadex LH-20 slurry (in benzene:methanol) into the syringe until the final height reached the 2 ml mark on the syringe. A second filter disk was placed on the top of the LH-20, and the columns were rinsed with approximately 50 ml of solvent before use. The elution pattern was determined by using tritium labelled estrone (~100 Ci/mmole) and estradiol (117 Ci/mmole). A typical elution pattern is illustrated in Figure 2. The estradiol values obtained for 13 plasma samples after chromotography were compared to those obtained when benzene extracts of the same samples were assayed. Estradiol in the extracts (10.4 $\pm$ 1.9 pg/ml) was not significantly different from estradiol after column chromotography (9.9 $\pm$ 1.8 pg/ml). The correlation between the estradiol values determined by the two methods was .94. The between-assay coefficient of variation on 13 determinations of the same sample was 10.2 percent (20.3 $\pm$ 2.1 pg/ml; mean $\pm$ SD).

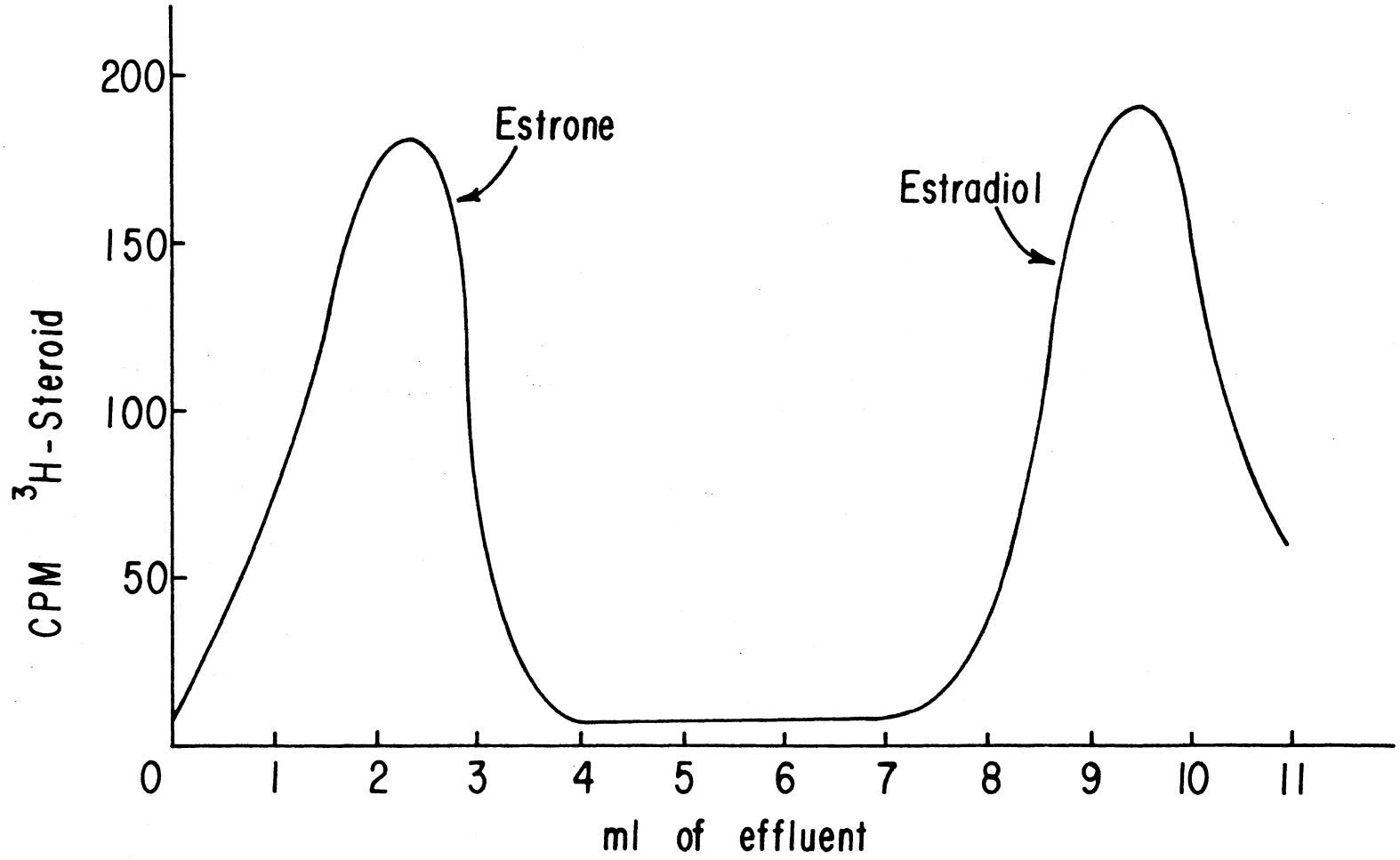

Figure 2. Typical Elution Pattern for Estrone and Estradiol from a Sephadex LH-20 Column

Luteinizing Hormone (LH)

Plasma LH concentrations were determined by radioimmunoassay similar to the method described by Niswender et al. (1970) using a specific antiserum to porcine LH. The procedure for radioiodination of purified porcine LH (LER-786-3) was similar to that of Niswender et al. (1969), except ^{125}I was used rather than ^{131}I. The specificity and sensitivity of this assay in our laboratory has been reported (Hallford et al., 1975). Ovine LH (NIH-LH-S_{18}) was used as a standard since the quantity of porcine LH was limited. Inclusion of a barrow standard in each assay yielded an average of 4.6 $\pm$ .9 ng/ml (mean $\pm$ SD, n = 5) for a between-assay coefficient of variation of 19 percent.

Corticoids

Plasma corticoids were determined by a competitive protein binding assay similar to that described by Murphy (1967) and Smith et al. (1972). Since little information was available on the identity of the major corticoids in swine, cortisol and total corticoids were determined on 15 samples representing pigs from various physiological states. Cortisol was isolated on Sephadex LH-20 using the same solvent system and columns that were described for estradiol. A typical elution pattern for corticosterone and cortisol is shown in Figure 3. Cortisol and total corticoid concentration for various types of pigs are listed in Table I. The overall correlation between total corticoids and cortisol in each sample was 0.98 (n = 15). Since the concentration of cortisol was approximately equal to the total corticoid concentration in all types of pigs, total corticoids rather than individual corticoids were

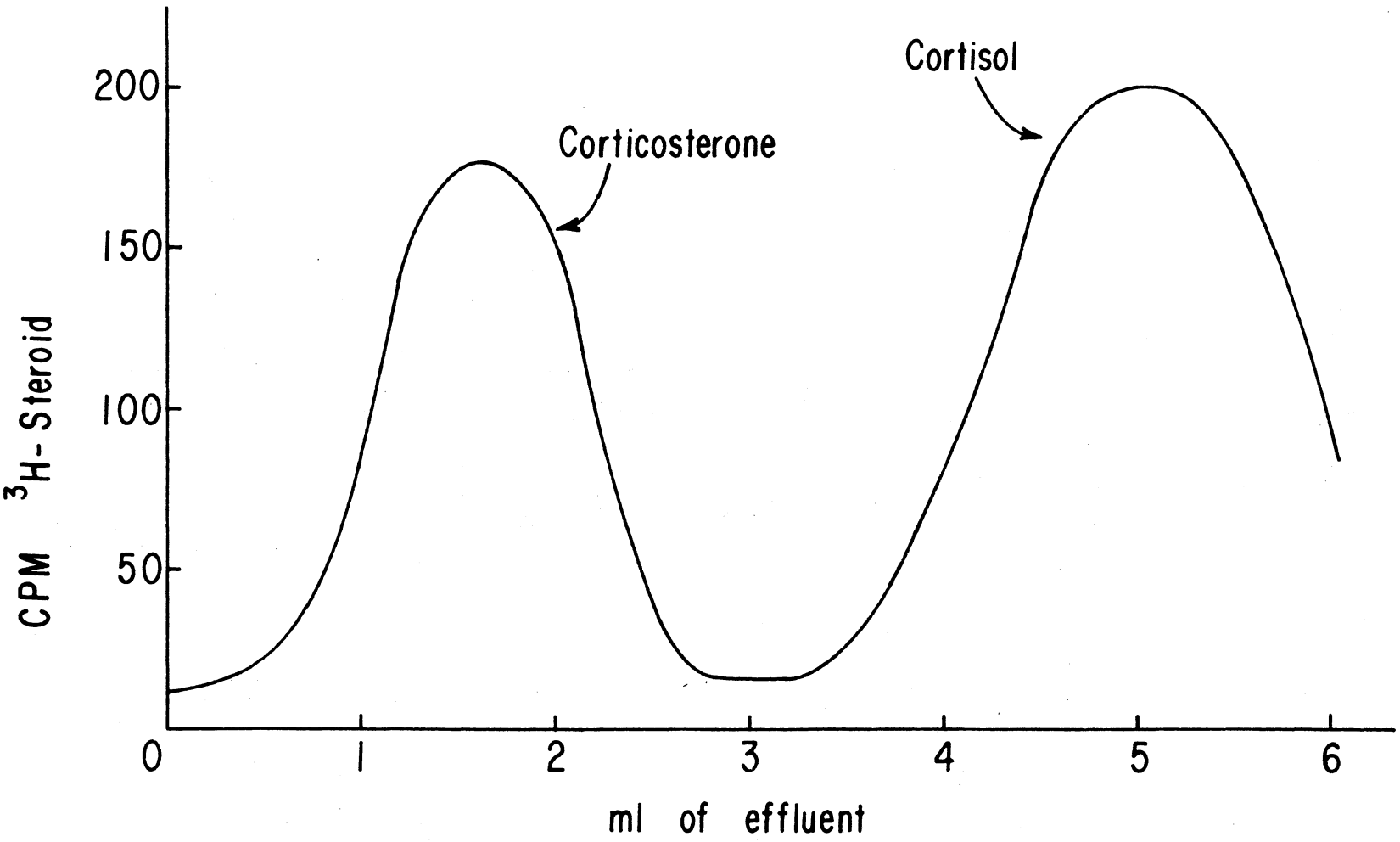

Figure 3. Typical Elution Pattern for Corticosterone and Cortisol from a Sephadex LH-20 Column

TABLE I

CORTISOL AND TOTAL CORTICOIDS IN PLASMA SAMPLES FROM VARIOUS TYPES OF PIGS

Item	No.	Cortisol (ng/ml)[a] (Mean + SE)	Total Corticoids (ng/ml) (Mean + SE)
Barrows	2	54.4 ± 8.6[b]	52.9 ± 3.6
Boars	3	28.5 ± 12.4	29.9 ± 12.0
Gilts	10	31.1 ± 5.3	32.0 ± 5.0
All Animals	15	33.7 ± 4.7	34.4 ± 4.3

[a]Isolated by column chromotography.

[b]Mean ± SE.

determined on samples collected from gilts during confinement in the environmental chambers. Corticoids were not quantified on samples collected after removal of gilts from the environmental chambers because the environment was not controlled post-treatment, so the values would probably have little meaning.

Statistical Analyses

Endocrine data from Experiment I and endocrine data, respiration rates and rectal temperatures from Experiment II were analyzed by least squares (Harvey, 1960) using a split plot design as described by Gill and Hafs (1971) for repeated measurements on the same animal. Table II illustrates the analysis of variance for progesterone in Experiment I, and Table III depicts the analysis of variance for progesterone in Experiment II. In plotting regression curves for each variable, polynomial regression coefficients for effects of days (period) were fitted to the highest significant degree only, up to degree 5. Tests between least squares means were done by student's "t" test using the sub-plot error as the error for testing main effects within sub-plots (Snedecor and Cochran, 1967).

In Experiment I, open gilts were compared to pregnant gilts through day 18, since blood samples were available only through day 18 in most of the open gilts. In addition, this would be near the time of return to estrus in open gilts and further comparisons would have little meaning. Data from the pregnant gilts was analyzed separately through day 28 of pregnancy to obtain the least squares means and the best fit response curves for endocrine variables.

Since blood samples for pregnant gilts in Experiment II were

TABLE II

ANALYSIS OF VARIANCE FOR PLASMA PROGESTERONE IN PREGNANT AND NON-PREGNANT GILTS DURING DAYS 0 THROUGH 18 AFTER ESTRUS

Source	df	MS
Pregnancy	1	5330.55
Gilts within Pregnancy	11	651.73
Period	18	1110.94
Linear	1	12174.22
Quadratic	1	6732.66
Cubic	1	525.86
Quardic	1	135.47
Quintic	1	21.79
Remainder	13	31.30
Pregnancy x Period	18	133.96
Error	160	40.01

TABLE III

ANALYSIS OF VARIANCE FOR PLASMA PROGESTERONE IN GILTS DURING CHAMBER CONFINEMENT

Source	df	MS
Treatment	1	229.50
Pregnancy	1	17.73
Treatment x Pregnancy	1	6.40
Gilts Within Trt. x Pregnancy	20	234.73
Period	15	761.85
Linear	1	10887.17
Quadratic	1	190.29
Cubic	1	85.33
Quardic	1	0.01
Quintic	1	21.41
Remainder	10	24.35
Treatment x Period	15	23.09
Pregnancy x Period	15	31.65
Treatment x Pregnancy x Period	15	9.89
Error	296	18.27

available only through day 13 in some gilts (due to non-patent cannulae), but samples were available in open gilts through day 22 after estrus, two separate analyses were performed. The first analysis was determined for all animals through day 13, and the second analysis was done for days 0 to 22 after estrus with non-pregnant gilts only. Since removal from the environmental chambers on day 8 represented a considerable change in environmental conditions, analysis in both groups was carried out separately for treatment (days 1 to 8) and post-treatment (days 9 to 13 or 9 to 22) periods.

Average daily feed and water intake during chamber confinement in Experiment II was analyzed by analysis of variance, using a completely randomized design.

CHAPTER IV

RESULTS AND DISCUSSION

Experiment I: Endocrine Function During Early Pregnancy

Reproductive Performance

Estrous cycle lengths for all gilts prior to cannulation averaged 19.6 ± .5 days (Mean ± S.E.). Ten of the 13 gilts bred were pregnant at slaughter. The three gilts not pregnant at slaughter exhibited swollen vulvas with mucus discharge and nervousness on days 19, 21 and 23, after estrus. Reproductive tracts of these three gilts recovered at slaughter (33 days postbreeding) were normal in appearance and ovaries contained functional corpora lutea, indicating the gilts had continued to cycle normally. Pregnant gilts averaged 13.2 ± 1.0 corpora lutea and 11.6 ± 1.1 embryos at slaughter (Table IV), which compares favorably with values of 12.6 and 10.3, respectively, reported by Johnson et al. (1975).

Endocrine Response

Progesterone. Least squares regression for plasma progesterone in open and pregnant gilts is depicted in Figure 4 and Appendix Table VIII. Split plot analysis of variance (Appendix Table IX) indicated that pregnant gilts had significantly greater ($P < .025$) progesterone

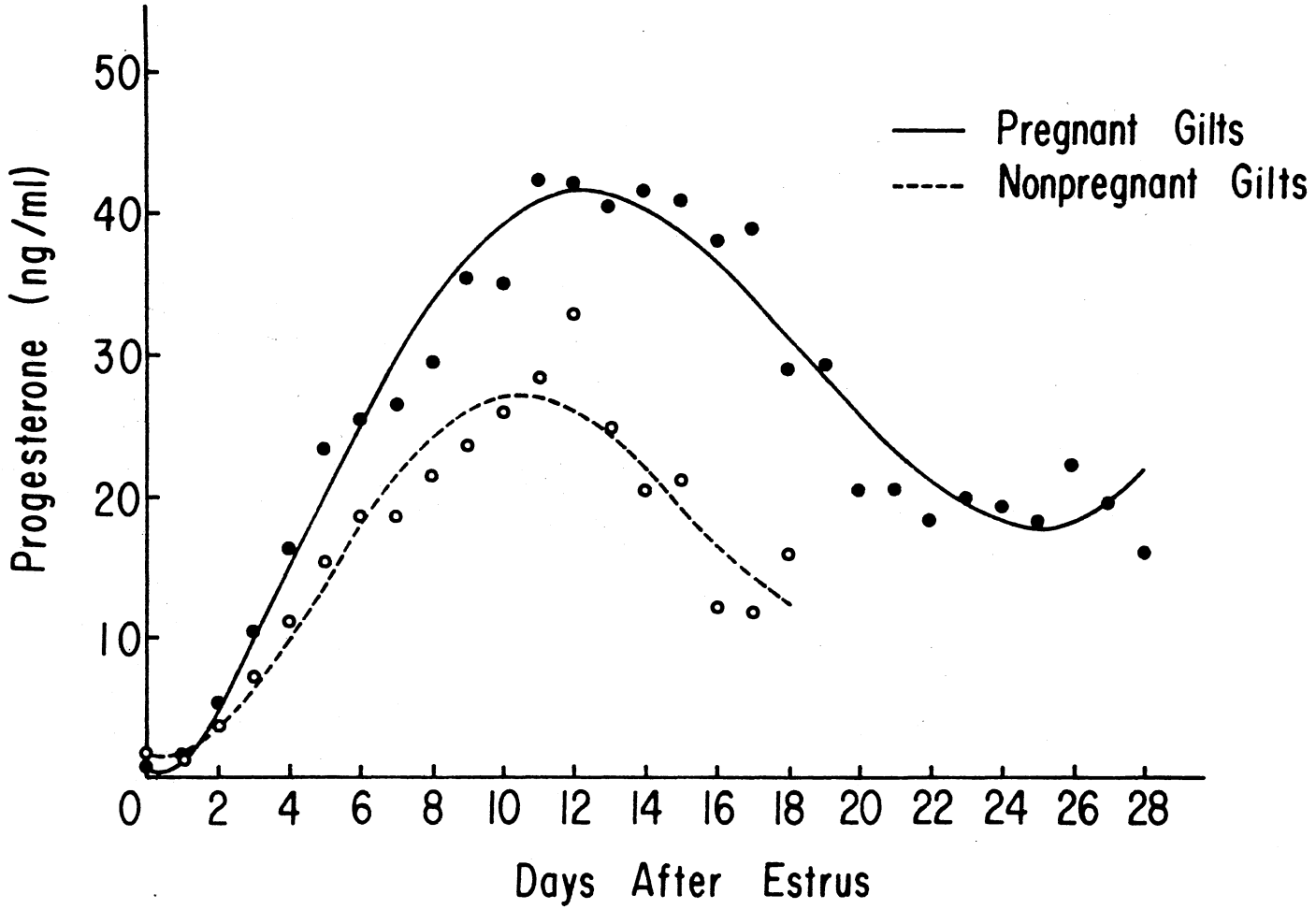

Figure 4. Plasma Progesterone in Pregnant and Non-Pregnant Gilts

concentrations during days 0 to 18 postbreeding than open gilts. A significant ($P < .005$) pregnancy by period interaction indicated that the difference between pregnant and open gilts was not the same on all days. Pregnant gilts had greater ($P < .05$) progesterone concentrations than open gilts by day 5 postbreeding (23.5 $\pm$ 2.7 versus 15.4 $\pm$ 5.1 ng/ml, respectively).

TABLE IV

CONCEPTION RATE, CORPORA LUTEA NUMBERS AND EMBRYO NUMBERS[a] FOR GILTS IN EXPERIMENT I

Gilts (No.)	Pregnant (No.)	Corpora Lutea (No.)	Embryos (No.)
13	10	13.2 $\pm$ 1.0	11.6 $\pm$ 1.1

[a]Mean $\pm$ S.E.

Plasma progesterone in pregnant gilts increased rapidly from concentrations of less than 1 ng/ml on day 0 (first day of estrus) to a maximum of about 41 ng/ml on day 12; then decreased sharply to approximately 21 ng/ml by day 22. Plasma progesterone remained about 20 ng/ml through day 28 of gestation. Progesterone in open gilts was about 2 ng/ml on day 0, decreased to about 1 ng/ml on day 1, then increased sharply to a maximum of about 27 ng/ml on day 11. Following the maximum on day 11, progesterone concentration decreased to about 12 ng/ml

by day 18 after estrus. One gilt was estrus on day 19, and this gilt had a progesterone concentration of 1.7 ng/ml on the day of estrus. The other two non-pregnant gilts showed signs of estrus on days 21 and 23 (not included in the least squares analysis) and had progesterone concentrations of 2.0 and 1.2 ng/ml, respectively. These progesterone concentrations indicate that luteal regression occurred in the three open gilts after cannulation.

These progesterone concentrations during the estrous cycle and early pregnancy (Figure 4) are in agreement with previous values reported (Henricks et al., 1972; Guthrie et al., 1972; Stabenfeldt et al., 1969; Tillson et al., 1970). Other researchers have not reported a significant difference in plasma progesterone concentrations between pregnant and open gilts as early as day 5 postbreeding, however, Tillson et al. (1970) has reported significantly higher metabolites of progesterone in the urine of pregnant versus open gilts as early as day 4 postbreeding.

Estradiol. Least squares means and regression for plasma estradiol in pregnant and open gilts are presented in Figure 5 and Appendix Table X. Estradiol concentrations in open and pregnant gilts were not significantly different during days 0 to 18 (Appendix Table IX). Estradiol in pregnant gilts decreased from 13 pg/ml on day 0 to about 5 pg/ml on day 4 postbreeding, then increased to 9 pg/ml on day 13. Estradiol remained fairly constant through day 22, and then increased through day 28 to about 13 pg/ml. Estradiol in non-pregnant gilts decreased rapidly from 15 pg/ml on the first day of estrus to about 4 pg/ml on day 8 postbreeding then increased to 19 pg/ml on day 18 after estrus. This

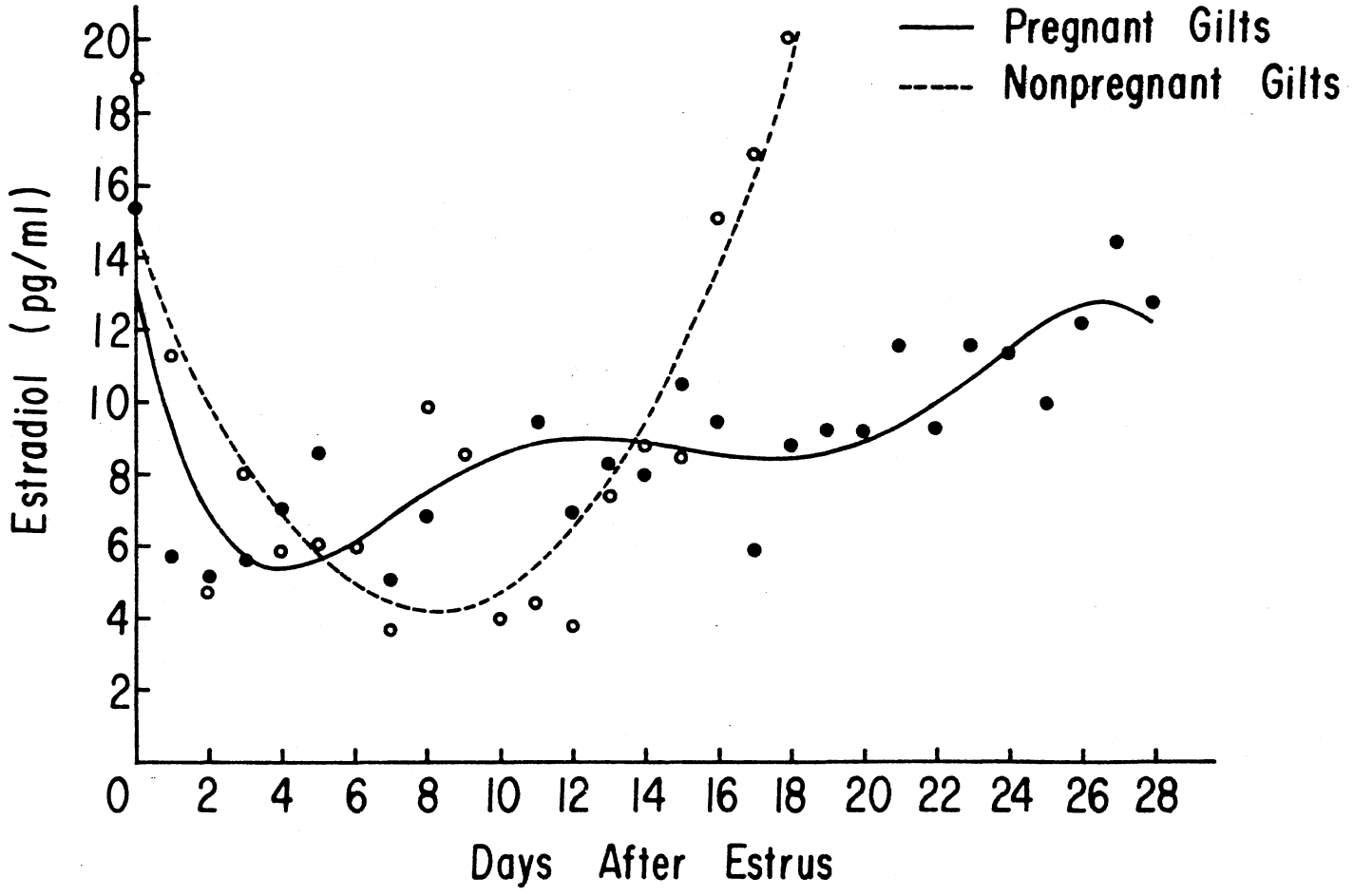

Figure 5. Plasma Estradiol in Pregnant and Non-Pregnant Gilts

increase in estradiol on day 18 was associated with the onset of proestrus.

The increase in estradiol which occurred at about day 13 in pregnant gilts may be associated with placental growth. A rise in estradiol near this time was observed in all eight pregnant gilts from which blood samples were collected. Estrogens have been shown to cause implantation in lactating rats (Yoshinaga and Host, 1961) and it has been suggested that elevated estradiol in cows around day 40 of gestation may be involved in implantation (Wettemann and Hafs, 1973). Estradiol concentrations in this study are similar to, although lower than, those reported for total estrogens by Guthrie et al. (1972) and Henricks et al. (1972). However, Shearer et al. (1972) reported concentrations of 5 to 85 pg/ml of estradiol during the estrous cycle, and concentrations of 10 to 20 pg/ml at day 24 of gestation. Robertson and King (1974) using an assay sensitivity of only 15 pg/ml, were unable to detect estradiol before day 80 of gestation. The rise in plasma estradiol in open gilts on day 18 is in agreement with the increase in estrogens near estrus observed by other workers (Henricks et al., 1972; and Shearer et al., 1972).

Luteinizing Hormone (LH). The curvilinear response of LH in open and pregnant gilts is plotted in Figure 6 and least squares means are presented in Appendix Table XI. Least squares regression curves for open and pregnant gilts were almost identical. LH in both groups decreased rapidly from a maximum concentration of 6 to 7 ng/ml on day 0 to 2 to 3 ng/ml by day 3 and remained about that concentration through day 18 in open gilts and through day 28 in pregnant gilts. Analysis of variance for days 0 to 18 (Appendix Table IX) indicated that mean

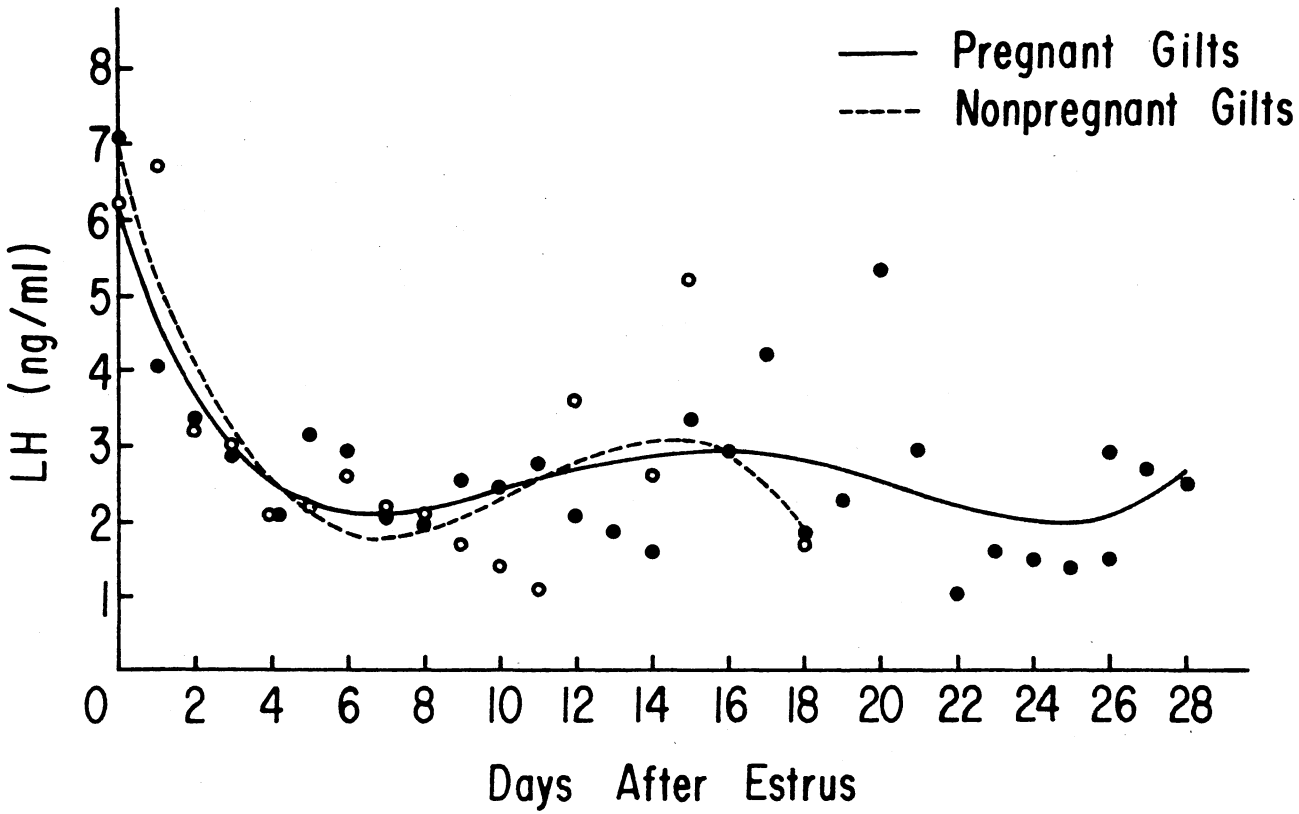

Figure 6. Plasma Luteinizing Hormone in Pregnant and Non-pregnant Gilts

plasma LH was not significantly different in pregnant versus open gilts (2.8 ± .3 versus 2.90 ± .4 ng/ml, respectively). In contrast, Guthrie et al. (1972) reported higher concentration of LH in pregnant gilts than in open gilts, while Tillson et al. (1970) observed higher LH in open gilts than pregnant gilts.

No increases in plasma LH through day 18 were observed in open gilts. This may have been due to the fact that the three gilts did not show signs of estrus until days 19, 21 and 23; and the preovulatory LH surge occurs at or very near the onset of estrus (Niswender et al., 1970; Tillson et al., 1970; Henricks et al., 1972).

Endocrine Relationships. There was a significant ($P < .05$) negative correlation of residuals of -.19 between progesterone and LH in all gilts from days 0 to 18. However, the correlations between estradiol and progesterone ($r = .13$) and between progesterone and estradiol ($r = -.15$) were not significant ($P > .05$).

In pregnant gilts during days 0 to 22 of gestation, there was a significant ($P < .05$) simple correlation of -.21 between progesterone and LH and a non-significant positive correlation of .14 between estradiol and LH. These correlations are consistent with the concepts that progesterone exerts negative feedback and estradiol exerts positive feedback on LH.

For the three open gilts during days 0 to 18 after estrus, there were significant simple correlations of -.41 ($P < .01$) between progesterone and estrogen and -.35 ($P < .05$) between progesterone and LH. There was also a significant ($P < .05$) positive relationship ($r = .32$) between estradiol and LH.

Guthrie et al. (1972) also observed a significant negative correlation between progesterone and estrogen, and a non-significant positive correlation between estrogens and LH. However, in contrast to this study, they did not report a relationship between LH and progesterone.

Experiment II: Endocrine Function in Gilts Exposed to Elevated Ambient Temperature During 1 to 8 Days Postbreeding

Reproductive Performance

Reproductive performance of gilts after exposure to control or elevated ambient temperature is summarized in Table V. Estrous cycle length in all gilts prior to cannulation was 20.0 ± .3 days (mean ± S.E.). Three of 12 control gilts, and only 1 of 12 heat stressed gilts, conceived. The three pregnant control gilts had an average of 13.7 ± .3 corpora lutea and 11.3 ± 1.2 embryos at about 30 days of pregnancy, and the pregnant heat stressed gilt had 13 corpora lutea and 8 embryos. Poor conception rates in both groups may have been due to boar fertility problems rather than to cannulation and daily bleeding or chamber confinement, since 77 percent conception was obtained in the cannulated gilts in Experiment I. In a preliminary trial using the same chambers, it was determined that chamber confinement of gilts did not influence conception (Appendix Table XII).

Feed and Water Consumption

During chamber confinement, water consumption was slightly greater in heat stressed gilts than in control gilts, although the difference

was not significant (5.0 ± .3 versus 4.5 ± .3 liters/day, respectively). However, feed intake in heat stressed gilts was depressed ($P < .005$) compared to controls (1.56 ± .06 versus .86 ± .06 kg/day, respectively).

TABLE V

CONCEPTION RATE AND NUMBERS OF CORPORA LUTEA AND EMBRYOS IN GILTS EXPOSED TO ELEVATED AMBIENT TEMPERATURE

Criteria	Treatment	
	Control	Heat Stressed
Gilts (No.)	12	12
Gilts Pregnant (No.)	3	1
Corpora Lutea (No.)	13.7 ± .3[a]	13.0
Embryos (No.)	11.3 ± 1.2	8.0

[a]Mean ± S.E.

Respiratory Rates and Rectal Temperature

There was a significant ($P < .10$) effect of pregnancy upon respiration (Appendix Table XIII), however, this was probably due to the one pregnant heat stressed gilt having a much higher respiration rate (123.4 respirations/minute) than either control (28.2 respirations/minute) or non-pregnant heat stressed gilts (92.7 respirations/minute)

and, therefore, has little meaning.

Respiratory rates at 8 AM and 8 PM in heat stressed and control gilts during the treatment period are plotted in Figure 7 and in Appendix Table XIV. The respiratory rate of 30.9 ± 3.0 at 8 PM in control gilts was significantly higher ($P < .10$) than the 25.5 ± 3.0 respirations/minute observed for control gilts at 8 AM, suggesting that there was diurnal variation in respiratory rates due to feeding and other activities during the day. As expected, the respiratory rate in heat stressed gilts at 8 PM (127.4 ± 4.0 respirations/minute) was significantly higher ($P < .001$) than the 8 AM respiratory rate (88.0 ± 4.0 respirations/minute), probably due to changes in chamber temperatures as well as the diurnal effect observed in control gilts.

Respiratory rates within treatment each day were similar during confinement, although they did tend to be slightly higher in heat stressed gilts at 8 PM during the last half of confinement than during the first half (Figure 7).

Rectal temperatures during confinement to the environmental chambers are depicted in Figure 8 and Appendix Table XV. Mean rectal temperatures for control gilts at 8 PM were greater ($P < .025$) than rectal temperatures at 8 AM (39.64 ± .07 versus 39.44 ± .07, respectively) indicating activity of eating and other factors caused increased rectal temperatures. Rectal temperatures at 8 AM in heat stressed gilts were greater ($P < .001$) than 8 PM rectal temperatures in control gilts (39.64 ± .07 versus 39.99 ± .09 C), and 8 PM rectal temperatures in heat stressed gilts were greater ($P < .001$) than 8 AM rectal temperatures in heat stressed gilts (40.78 ± .09 versus 39.99 ± .09 C, respectively). A quadratic response curve best described changes in

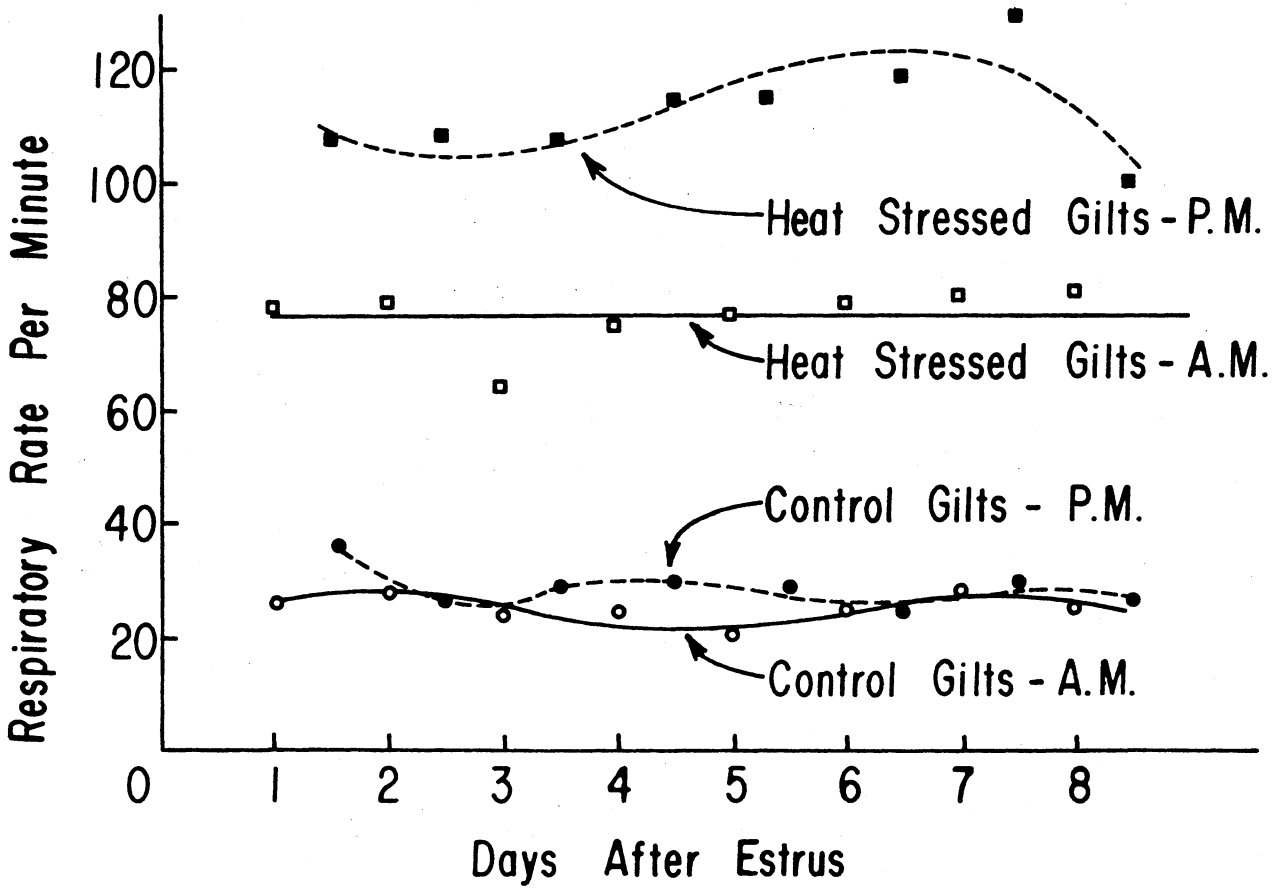

Figure 7. Respiration Rates of Gilts During Exposure to Control or Elevated Ambient Temperature

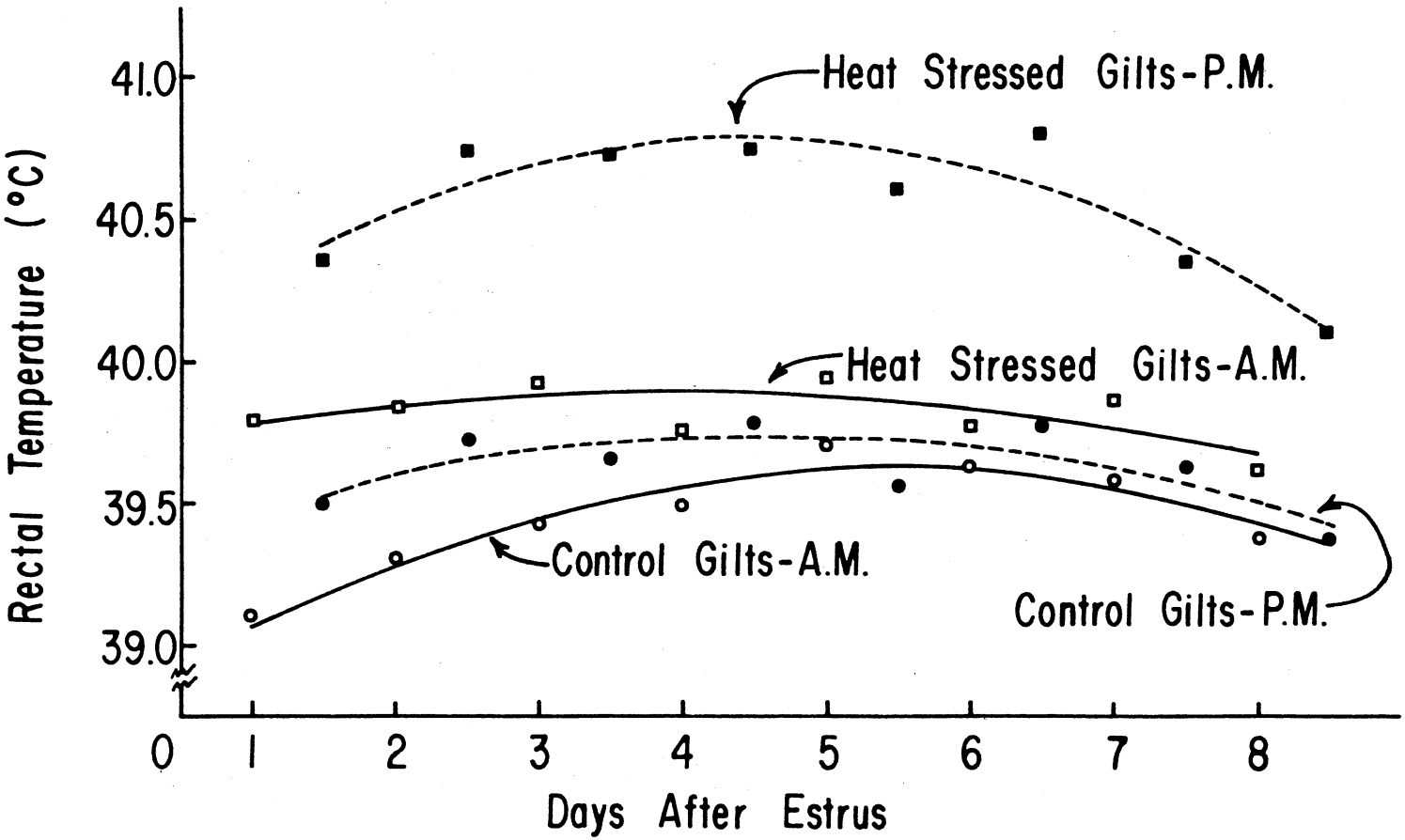

Figure 8. Rectal Temperatures of Gilts During Exposure to Control or Elevated Ambient Temperature

rectal temperatures during confinement. Rectal temperatures tended to increase slightly in all gilts for the first four days of confinement then slowly decreased (Figure 8). The decrease in rectal temperatures in heat stressed gilts at 8 PM during the last half of the confinement period may be related to the increased respiratory rate in these gilts during the same period (Figure 7). This ability of gilts to compensate to exposure to heat stress by reducing rectal temperature has been reported by Edwards et al. (1971). There was an overall correlation of .45 ($P < .05$) between respiratory rate and rectal temperature.

Endocrine Response

Since animals were not actually placed on treatment until after 8 PM on day 0 (first day of estrus) variables for blood samples collected on day 0 were analyzed by a completely randomized design to determine if any differences in endocrine parameters existed between the two groups prior to the application of treatments. Progesterone, estradiol, LH and corticoids were not significantly different between groups (Appendix Table XVI).

Progesterone. Plasma progesterone in all gilts during and after chamber confinement is presented in Figure 9 and Appendix Table XVII. Plasma progesterone during and after treatment was not significantly affected by treatment (Appendix Tables XVIII and XIX). Progesterone concentration was 13.3 $\pm$ 1.0 ng/ml in control gilts during confinement and 28.0 $\pm$ 2.1 ng/ml after confinement compared to 15.6 $\pm$ 2.3 and 35.1 $\pm$ 4.7 ng/ml, respectively, for heat stressed gilts. Plasma progesterone in control gilts increased from about 1 ng/ml on day 1 to about

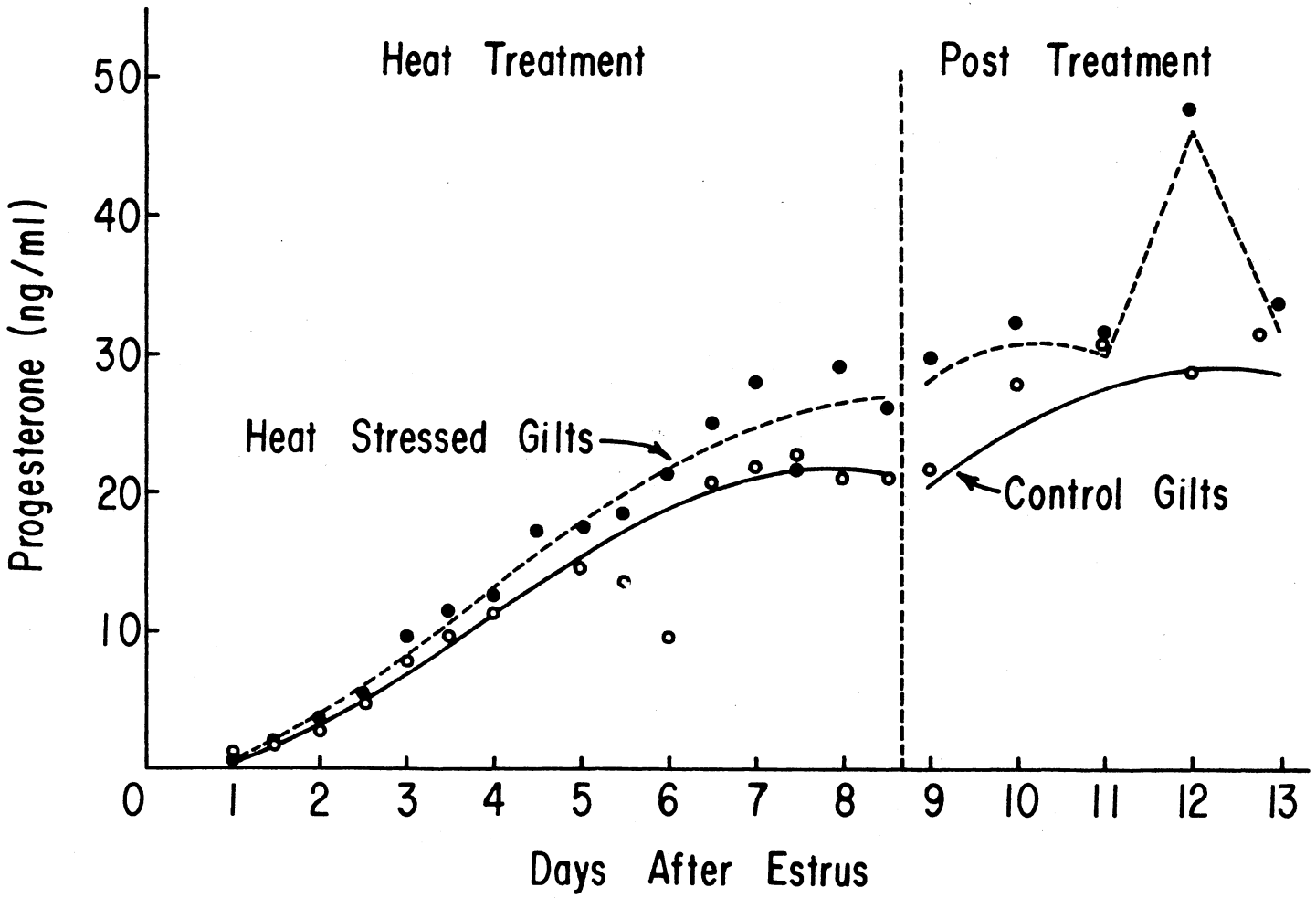

Figure 9. Plasma Progesterone in Gilts During and After Exposure to Control or Elevated Ambient Temperature

21 ng/ml during confinement in the environmental chambers, and continued to increase after removal from the chambers to about 29 ng/ml on day 13 after estrus. Progesterone in heat stressed gilts increased from about 1 ng/ml on day 0 to about 27 ng/ml on day 8. After heat stressed gilts were removed from the chambers, progesterone continued to increase to about 32 ng/ml of day 13 after estrus. The elevation in plasma progesterone on day 12 in stressed gilts (Figure 9) was greatly influenced by the one pregnant gilt with a progesterone concentration of 59 ng/ml on that day.

There was a significant ($P < .05$, Appendix Table XVIII) pregnancy x period interaction for progesterone in gilts during days 1 through 8. This indicated that differences between pregnant and open gilts were not the same for all periods, therefore, a separate analysis was performed including only open gilts on days 1 through 8 and 9 through 22 (Appendix Tables XV and XVI). Plasma progesterone concentrations of 15.5 $\pm$ 1.2 ng/ml for stressed gilts on days 1 to 8 was higher than the mean of 12.7 $\pm$ 1.3 ng/ml in control gilts, but differences were not significant ($P > .10$). Least squares means for progesterone in stressed gilts on days 8 to 13 after estrus were significantly greater ($P < .05$) than those for control gilts. Plasma progesterone in control gilts increased from about 1 ng/ml on day 1 to about 25 ng/ml on day 12, then decreased sharply to about 2 ng/ml on days 21 to 22 after estrus (Figure 10 and Appendix Table XIX). Plasma progesterone in open heat stressed gilts increased from about 2 ng/ml on day 1 to a maximum of about 34 ng/ml on day 11, then decreased to approximately 3 ng/ml on day 20 to 21.

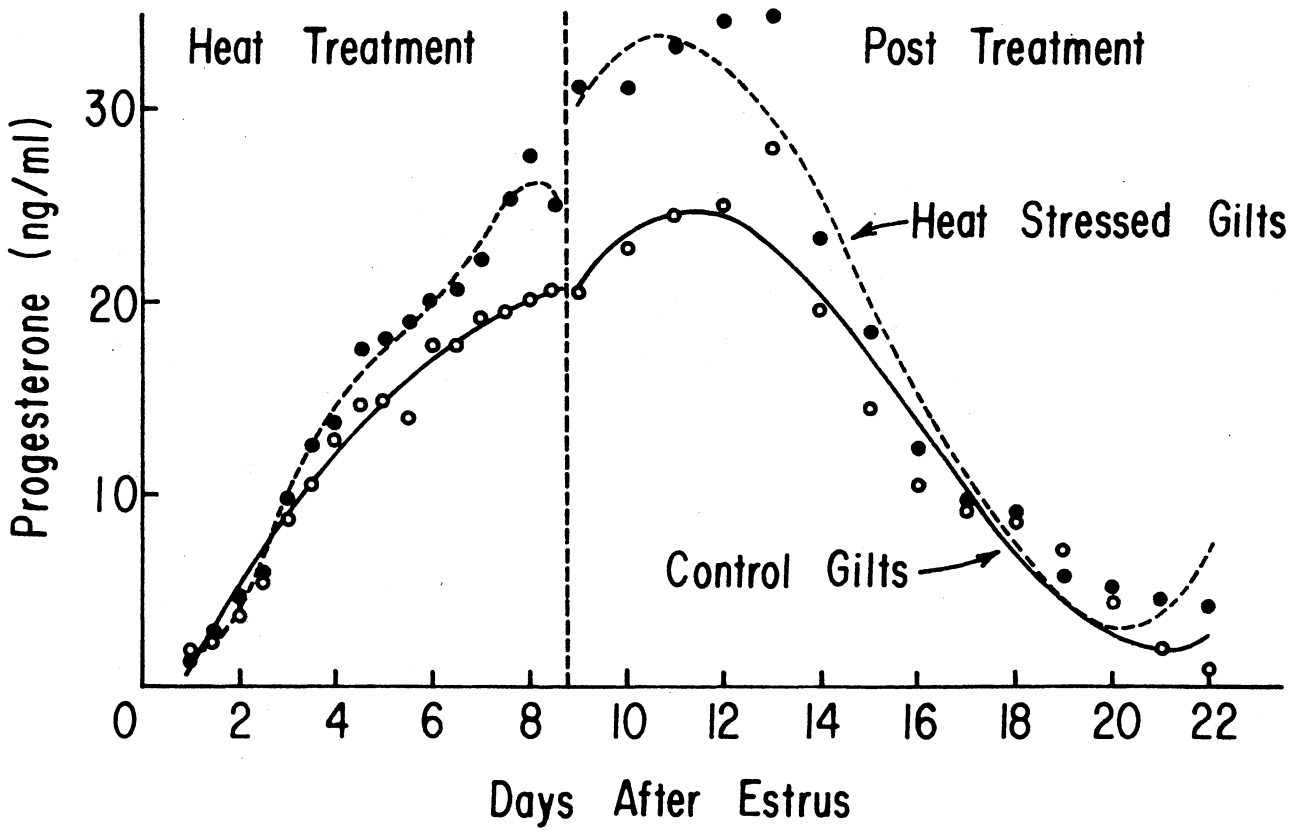

Figure 10. Plasma Progesterone in Non-pregnant Gilts During and After Exposure to Control or Elevated Ambient Temperature

Increased plasma progesterone concentrations in heat stressed gilts could arise from progesterone secretion by the adrenal cortex. Marple et al. (1972) observed that pigs exposed to environmental temperatures that fluctuated between 21.1 C to 32.2 C had a two-fold increase in plasma ACTH levels, while plasma corticoids were slightly reduced or unchanged. Thus, elevated ambient temperature may act directly upon the adrenal cortex and prevent the production of glucocorticoids, and increased concentrations of plasma ACTH could lead to the release of progesterone from the adrenal cortex. Administration of exogenous progesterone at 150 to 200 mg/day beginning on day 4 to 8 postbreeding has been shown to have a detrimental effect upon corpora lutea and embryonic survival in swine (Sammelwitz et al., 1956; Spies et al., 1959). Mills et al. (1972) have also observed elevated plasma progestins in heifers exposed to elevated temperatures during the first three days of pregnancy.

Estradiol. Plasma estradiol in gilts during and after exposure to elevated ambient temperature is depicted in Figure 11 and Appendix Table XXII. Plasma estradiol was significantly greater ($P < .10$) in control gilts than in heat stressed gilts during the treatment period, but was similar in both treatments after gilts were removed from the chambers (Appendix Tables XVIII and XIX). During chamber confinement, plasma estradiol was 6.2 $\pm$ .4 and 4.5 $\pm$ .9 pg/ml for control and heat stressed gilts, respectively, and plasma estradiol after treatment was 6.5 $\pm$ .9 and 6.3 $\pm$ 1.1 pg/ml for control and stressed gilts, respectively. Plasma estradiol in heat stressed gilts decreased sharply from 7.6 ng/ml on day 1 and fluctuated between 4.2 and 6.4 pg through day 8, and increased to about 6.3 pg/ml during the post-treatment period.

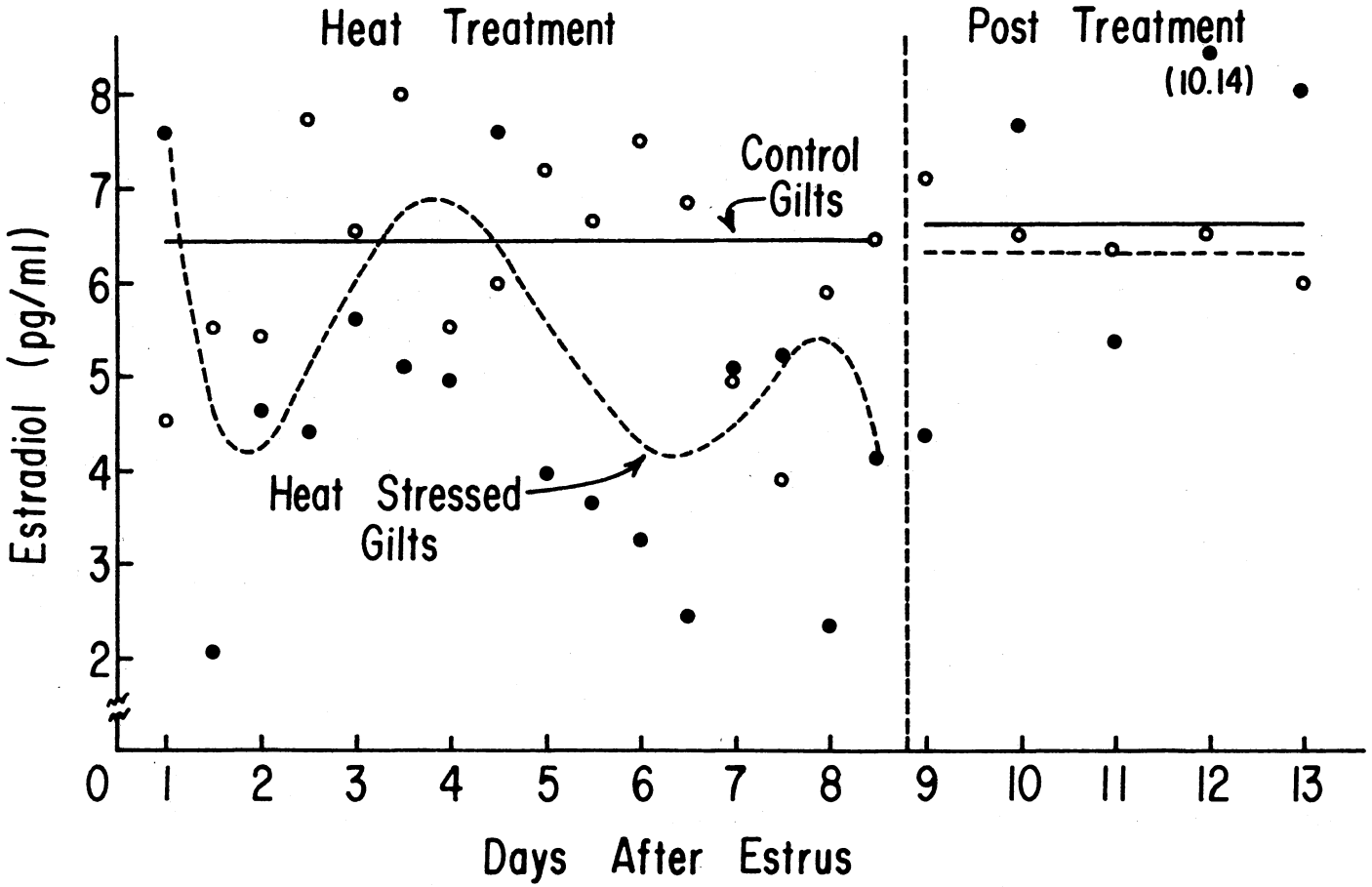

Figure 11. Plasma Estradiol in Gilts During and After Exposure to Control or Elevated Ambient Temperature

Plasma estradiol in open control and heat stressed gilts is plotted in Figure 12. Estradiol concentrations during the post-treatment period in control gilts increased gradually from about 6 pg/ml on day 9 to 9 pg/ml on day 15 and then increased dramatically from about 10 pg/ml on day 20 to 19 pg/ml on day 22. Estradiol in heat stressed gilts during the post-treatment period increased linearly from about 4 pg/ml on day 9 to about 16 pg/ml on day 22 after estrus.

Decreased concentrations of estradiol observed in gilts during the treatment period could play a role in causing reduced reproductive performance. Yoshinaga and Host (1961) have demonstrated that estrogen is necessary for implantation in lactating rats. Similar to these results, Gwazdauskas (1974) observed that exposure of cows to elevated ambient temperature during estrus results in a reduction in plasma estradiol concentrations.

Luteinizing Hormone (LH). Plasma LH concentrations were similar in control and heat stressed gilts when analysis was performed on all gilts or with only open gilts, Appendix Tables XVIII, XIX, XX and XXI. LH concentrations in all gilts are depicted in Figure 13, and Appendix Table XXIII. LH was greater in control gilts than heat stressed gilts on day 1. This greater concentration was probably due to the detection of some of the increases in LH which cause ovulation. No increases in LH occurred through day 22 after estrus in open gilts (Figure 14). Since gilts were not checked for estrus it is not known whether estrus and ovulation occurred after a normal cycle length; however, all reproductive tracts recovered at slaughter about 30 days after breeding appeared to be normal and contained functional corpora lutea. Probably

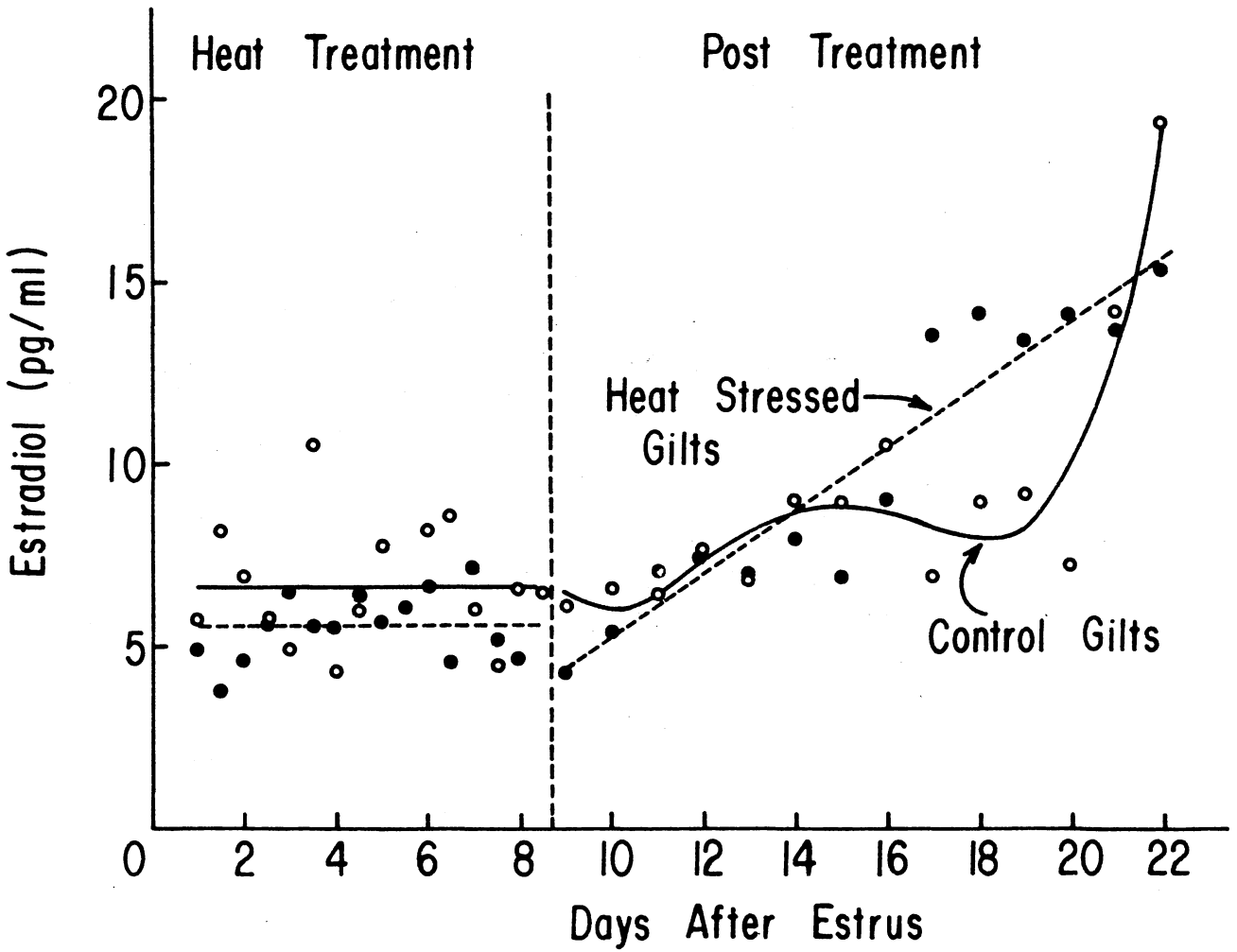

Figure 12. Plasma Estradiol in Non-pregnant Gilts During and After Exposure to Control or Elevated Ambient Temperature

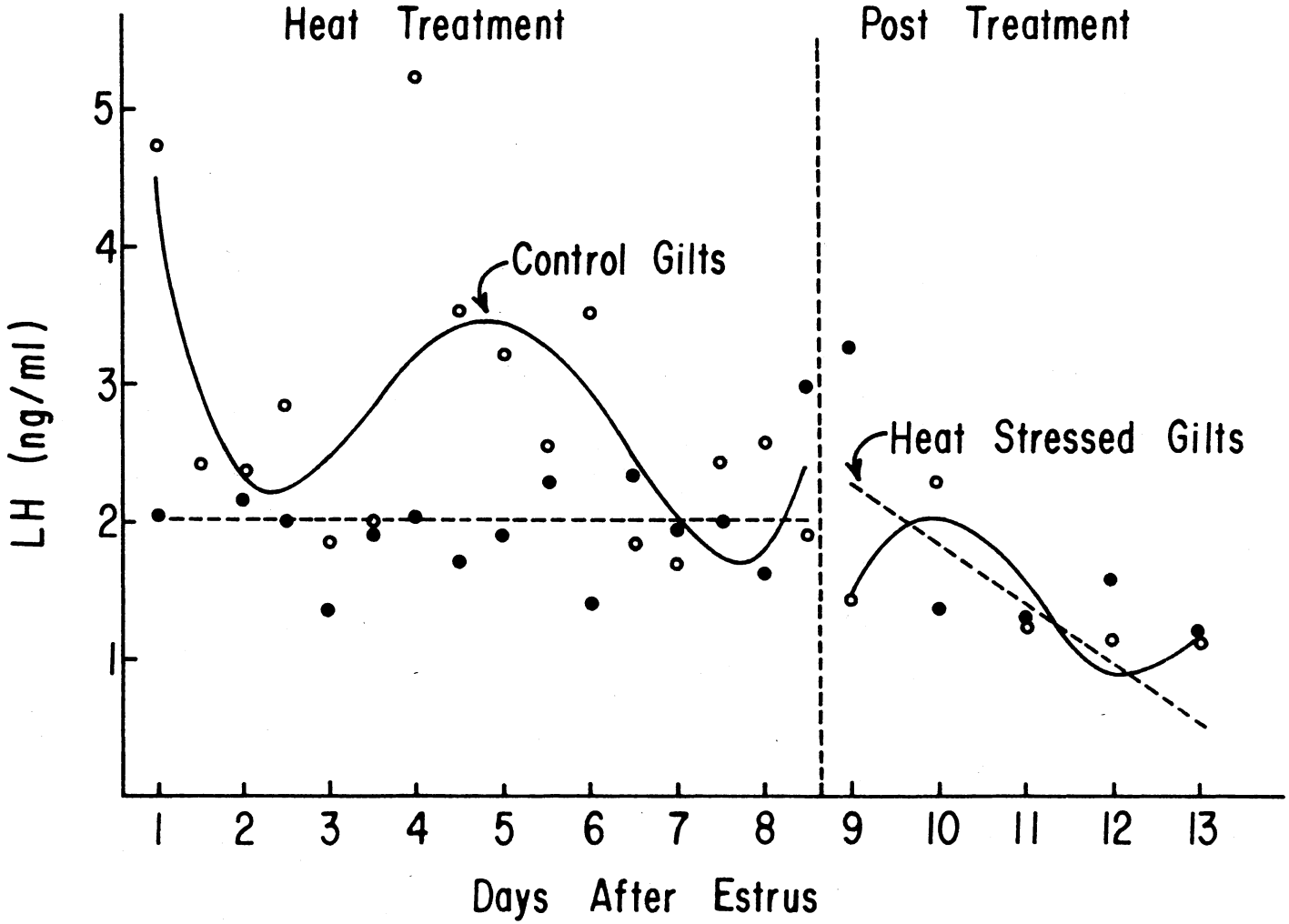

Figure 13. Plasma LH in Gilts During and After Exposure to Control or Elevated Ambient Temperature

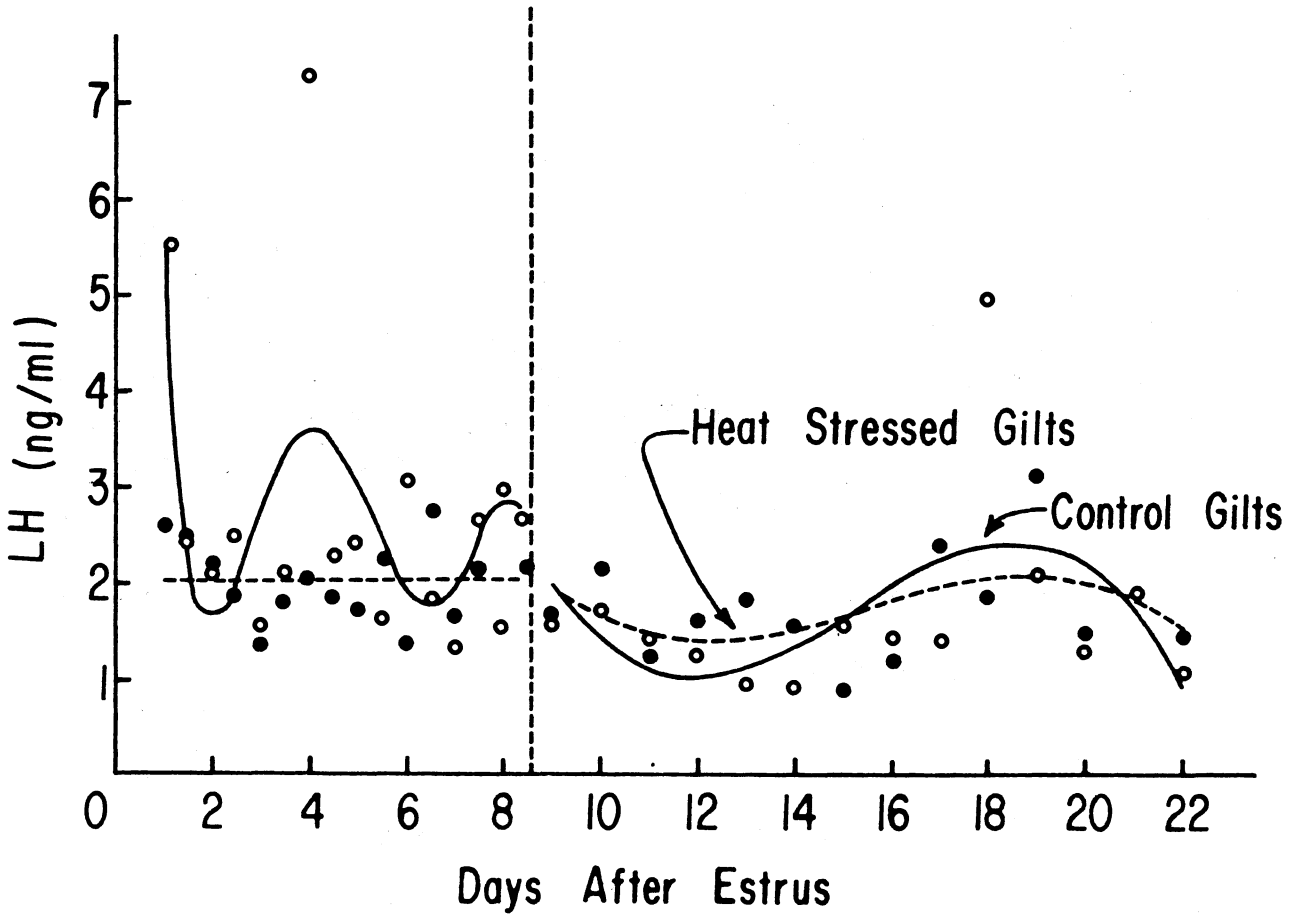

Figure 14. Plasma LH in Non-pregnant Gilts During and After Exposure to Control or Elevated Ambient Temperature

once daily bleeding was not sufficient to detect ovulatory surges of LH which only exist for about 8 hrs (Niswender et al., 1970).

Corticoids. Plasma concentrations of corticoids (Figure 15, Appendix Table XXIII) were not significantly affected by exposure of gilts to elevated ambient temperatures (Appendix Tables XVIII and XX). Corticoid concentrations in control and heat stressed gilts during chamber confinement averaged 24.6 $\pm$ 2.8 and 23.5 $\pm$ 4.7 ng/ml, respectively. Mean concentrations of plasma corticoids for different periods ranged from 10 to 43 ng/ml and there was significant ($P < .005$) variation between gilts within treatment x pregnancy (Appendix Table XVIII). Therefore, very large treatment differences would have to have existed to be detected. Much variation between cows within treatment period has also been observed when cows were exposed to elevated ambient temperature (Gwazdauskas, 1974).

Endocrine Relationships. There were no significant correlations between plasma hormone concentrations during confinement. There was, however, a significant ($P < .05$) correlation among residuals of .17 for rectal temperature and plasma corticoids.

There were no significant correlations between plasma hormone concentrations during the post-treatment period in all gilts, however, there was a significant ($P < .05$) correlation among residuals of -.18 for progesterone and estrogen in open gilts. A similar relationship between progesterone and estrogen was observed in open gilts in Experiment I.

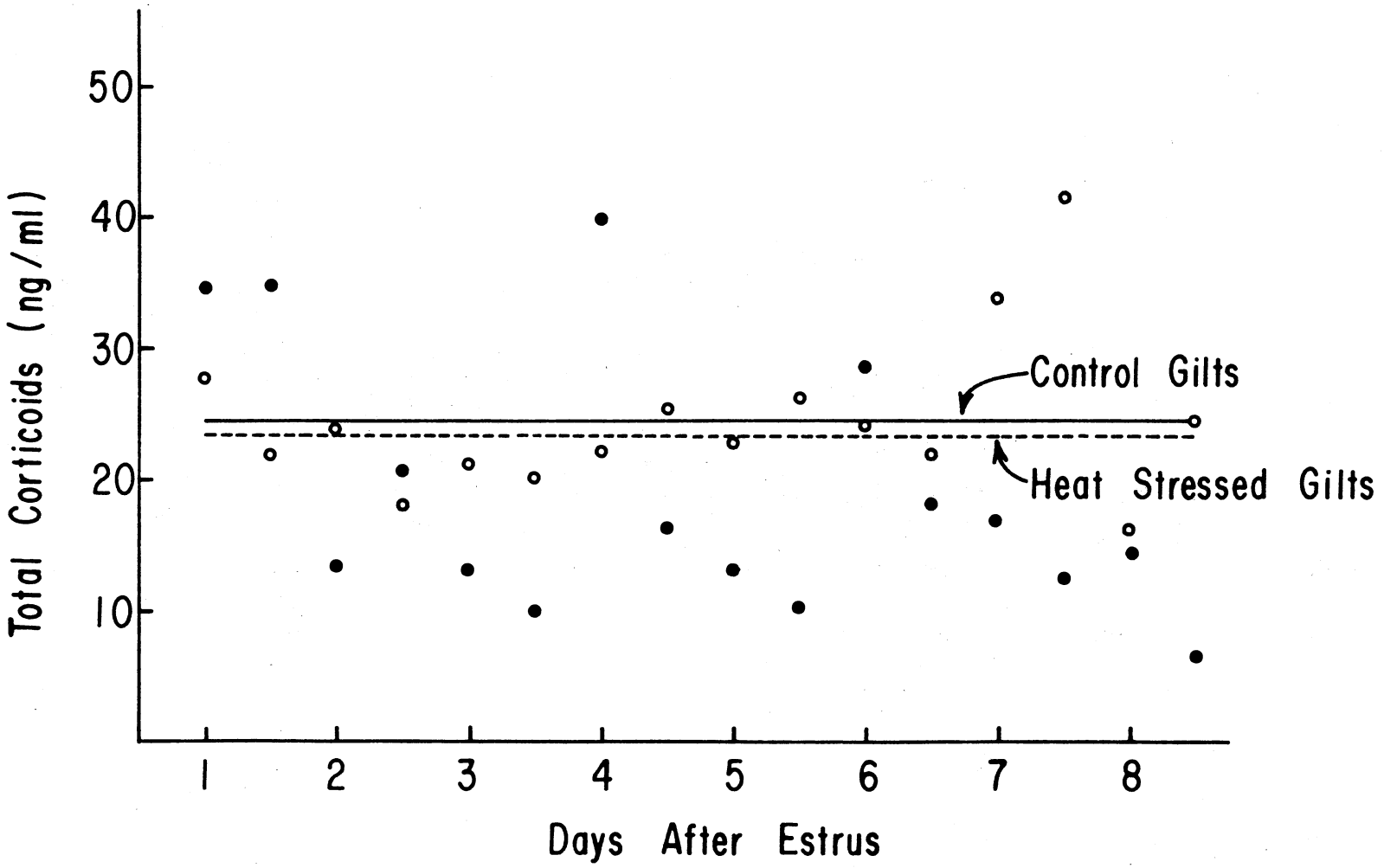

Figure 15. Plasma Corticoids in Gilts During Exposure to Control or Elevated Ambient Temperature

Diurnal Variations. Plasma concentrations of progesterone, corticoids, estradiol and LH were not significantly different between the 8 AM and 8 PM samples (Appendix Table XIII). Thus, under these conditions, it appears that diurnal variations in these hormones do not exist.

CHAPTER V

SUMMARY

Thirteen crossbred gilts were used to study endocrine changes associated with embryogenesis in gilts. Ten of the 13 gilts were pregnant at slaughter at 33.0 ± 5.0 days after estrus. Pregnant gilts had 13.2 ± 1.0 CL and 11.6 ± 1.1 embryos.

Plasma progesterone in pregnant gilts increased from less than 1 ng/ml at estrus (day 0) to a maximum of 41 ng/ml on day 12, then decreased to about 20 ng/ml on day 22 and remained about this concentration through day 28. Plasma progesterone in open gilts at estrus was 2 ng/ml and increased to a maximum of 27 ng/ml on day 11, before decreasing to 12 ng/ml on day 18. Plasma progesterone was significantly ($P < .025$) greater in pregnant than in open gilts during days 0 to 18 (28.0 ± 2.2 versus 16.5 ± 3.7 ng/ml), and was significantly greater ($P < .05$) in pregnant gilts as early as day 5 (23.5 ± 2.7 versus 15.4 ± 5.1 ng/ml).

Plasma estradiol was not significantly different in pregnant and open gilts during days 0 to 18 (7.6 ± 0.9 versus 9.1 ± 1.1 pg/ml). Estradiol in pregnant gilts decreased from 13 pg/ml on day 0 to about 5 pg/ml on day 4 then increased to 9 pg/ml on day 13. Estradiol remained relatively constant through day 22 then increased to about 13 pg/ml by day 28 of gestation. Estradiol in open gilts decreased from 15 pg/ml on day 0 to about 4 pg/ml on day 8, then increased to

19 pg/ml on day 18 after estrus.

Plasma LH in pregnant and open gilts was similar between days 0 to 18 after estrus (2.8 ± .3 versus 2.9 ± .4 ng/ml, respectively). LH in both groups decreased rapidly from a maximum of 6 to 7 ng/ml on the first day of estrus to 2 to 3 ng/ml on day 3, and remained near this concentration through day 18 in open gilts and through day 28 in pregnant gilts.

In the pregnant gilts, there was a negative correlation ($P < .05$) of -.21 between progesterone and LH. In open gilts there were significant ($P < .05$) simple correlations of -.41 between progesterone and estrogen, -.35 between progesterone and LH and .32 between estradiol and LH.

The effects of elevated ambient temperature during days 1 to 8 postbreeding on endocrine response were studied in 24 gilts. Three of 12 control gilts and only 1 of 12 heat stressed gilts conceived. Control gilts had 13.7 ± .3 CL and 11.3 ± 1.2 embryos compared to 13 CL and 8 embryos in stressed gilts at slaughter on day 31.5 ± 4.9.

Respiratory rates and rectal temperatures were significantly lower in control than in heat stressed gilts (28.2 ± 2 versus 107.7 ± 3 breaths/minute and 39.53 ± .05 versus 40.39 ± .08 C, respectively). In addition, 8 PM respiration rates and rectal temperatures were significantly greater than 8 AM respiration rates and rectal temperatures (56.8 ± 3 versus 79.1 ± 3 breaths/minute and 39.72 ± .07 versus 40.21 ± .07 C, respectively).

Water consumption during treatment was not significantly different between control and heat stressed gilts (4.5 ± .3 versus 5.0 ± .3 liters/day), but feed consumption was reduced ($P < .005$) in heat

stressed versus control gilts (.86 ± .06 versus 1.54 ± .06 kg/day).

Plasma progesterone in all gilts was not significantly different between control and heat stressed gilts either during or after chamber confinement (12.7 ± 1.3 versus 15.5 ± 1.2 ng/ml and 28.0 ± 2.1 versus 35.1 ± 4.7 ng/ml, respectively). However, plasma progesterone was greater ($P < .05$) in heat stressed than in control gilts during days 8 to 13 after estrus in non-pregnant gilts. Maximum progesterone concentrations in non-pregnant control and non-pregnant heat stressed gilts of 24.5 and 34.0 ng/ml were attained on day 12 after estrus.

Plasma estradiol was significantly ($P < .10$) greater in control than in heat stressed gilts during chamber confinement (6.2 ± .9 versus 4.5 ± .9 pg/ml, respectively) but was similar post-treatment (6.4 ± .9 versus 6.3 ± 1.1 pg/ml). Plasma estradiol in control gilts varied between about 5.0 and 7.5 pg/ml during and after the treatment period. Plasma estradiol in heat stressed gilts decreased sharply from 7.6 pg/ml during the treatment period to about 5.5 pg/ml then increased to about 6.3 pg/ml during the post-treatment period.

Plasma LH and plasma corticoids were not significantly influenced by elevated ambient temperature. Mean plasma LH concentrations during and after chamber confinement were 2.8 ± .4 and 1.4 ± .2 ng/ml, respectively, in control gilts and 2.0 ± .4 and 1.7 ± .4 ng/ml, respectively, in heat stressed gilts. Plasma corticoids during chamber confinement averaged 24.6 ± 2.8 and 23.5 ± 4.7 ng/ml in control and heat stressed gilts, respectively.

There were no significant correlations between hormones during the treatment period, but rectal temperatures were correlated with plasma corticoids ($r = .17$, $P < .05$). Plasma concentrations of

progesterone, LH, estradiol and corticoids were similar in the samples collected at 8 AM and 8 PM each day.

This study indicates that exposure of gilts to elevated ambient temperatures during the first 8 days after breeding results in decreased plasma concentrations of estradiol and increased progesterone. This altered endocrine function may be related to the reduced reproductive performance observed when gilts are subjected to heat stress during early pregnancy.

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APPENDIXES

TABLE VI

PREPARATION OF LIQUID SCINTILLATION FLUIDS

A. Steroid Counting Fluid	
Toluene (Scintillation Grade)	3800 ml
PPO	15 g
POPOP	0.15 g
B. Protein Binding Counting Fluid	
Toluene (Scintillation Grade)	3000 ml
PPO	21.7 g

TABLE VII

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 andydrous or 134 g heptahydrate Ha_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 Buffer

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 VIII

PLASMA PROGESTERONE IN GILTS DURING THE ESTROUS CYCLE AND DURING EARLY PREGNANCY

Day	No.	Open Gilts	No.	Pregnant Gilts
0	3	1.6 ± 5.1[a]	10	1.1 ± 2.7
1	3	1.3 ± 5.1	10	2.0 ± 2.7
2	3	3.6 ± 5.1	10	5.3 ± 2.7
3	3	7.1 ± 5.1	10	10.6 ± 2.7
4	3	11.0 ± 5.1	9	16.6 ± 2.9
5	3	15.4 ± 5.1	10	23.5 ± 2.7
6	3	18.4 ± 5.1	10	25.5 ± 2.7
7	3	18.1 ± 5.1	10	26.6 ± 2.7
8	3	21.3 ± 5.1	9	29.7 ± 2.9
9	3	23.5 ± 5.1	8	35.6 ± 3.0
10	3	25.8 ± 5.1	8	39.2 ± 3.0
11	3	28.3 ± 5.1	8	42.6 ± 3.0
12	3	32.8 ± 5.1	7	42.3 ± 3.3
13	3	24.8 ± 5.1	6	40.7 ± 3.5
14	3	20.2 ± 5.1	5	42.2 ± 3.9
15	3	21.1 ± 5.1	6	41.1 ± 3.5
16	3	12.1 ± 5.1	6	38.2 ± 3.5
17	3	11.7 ± 5.1	5	39.4 ± 3.9
18	3	15.9 ± 5.1	5	29.3 ± 3.9
19	3		5	29.4 ± 3.9
20			5	20.4 ± 3.9
21			5	20.4 ± 3.9
22			5	18.3 ± 3.9
23			5	19.7 ± 3.9
24			5	19.2 ± 3.9
25			5	18.2 ± 3.9
26			5	22.2 ± 3.9
27			5	19.6 ± 3.9
28			5	16.0 ± 3.9

[a]Least Squares Mean ± S.E.

TABLE IX

ANALYSIS OF VARIANCE OF PLASMA HORMONES DURING DAYS 0 TO 18 OF THE ESTROUS CYCLE OR PREGNANCY

Source	df	Progesterone		Estradiol		LH	
		MS	F	MS	F	MS	F
Pregnancy	1	5330.55	8.18[a]	92.92	1.43	0.04	0.004
Gilts	11	651.73	16.29[b]	64.95	1.65	10.04	1.94[a]
Period	18	1110.94	27.77[b]	94.55	2.40[b]	13.55	2.62[b]
Linear	1	12174.22	304.28	63.98	1.63	59.31	11.48
Quadic	1	6732.66	168.28	943.26	23.97	96.72	18.73
Cubic	1	525.86	13.14	65.59	1.67	53.63	10.38
Quardic	1	135.47	3.39	244.73	6.22	0.44	0.09
Quintic	1	21.79	.55	153.13	3.89	11.80	2.29
Residual	13	31.30	.78	17.79	.45	1.69	0.33
Preg. x Period	18	133.96	3.35[b]	44.53	1.13	3.95	0.76
Error	160	40.01		39.35		5.17	

[a] ($P < .05$)

[b] ($P < .005$)

TABLE X

PLASMA ESTRADIOL (PG/ML) IN GILTS DURING THE ESTROUS CYCLE OR EARLY PREGNANCY

Day	No.	Open Gilts	No.	Pregnant Gilts
0	3	19.0 ± 3.7[a]	10	15.0 ± 2.0
1	3	11.4 ± 3.7	10	5.4 ± 2.0
2	3	4.7 ± 3.7	10	4.8 ± 2.0
3	3	8.0 ± 3.7	10	5.3 ± 2.0
4	3	5.8 ± 3.7	9	6.7 ± 2.1
5	3	6.0 ± 3.7	10	8.3 ± 2.0
6	3	6.0 ± 3.7	10	5.6 ± 2.0
7	3	3.7 ± 3.7	10	4.8 ± 2.0
8	3	9.9 ± 3.7	9	6.5 ± 2.1
9	3	8.5 ± 3.7	8	8.0 ± 2.3
10	3	4.1 ± 3.7	8	8.8 ± 2.3
11	3	4.4 ± 3.7	8	9.2 ± 2.3
12	3	3.8 ± 3.7	7	6.6 ± 2.4
13	3	7.4 ± 3.7	6	8.0 ± 2.6
14	3	8.8 ± 3.7	5	7.8 ± 2.9
15	3	8.5 ± 3.7	6	10.2 ± 2.6
16	3	15.1 ± 3.7	6	9.2 ± 2.6
17	3	16.9 ± 3.7	5	5.5 ± 2.9
18	3	20.4 ± 3.7	5	8.4 ± 2.9
19			5	9.2 ± 2.9
20			5	9.2 ± 2.9
21			5	11.6 ± 2.9
22			5	9.3 ± 2.9
23			5	11.6 ± 2.9
24			5	11.4 ± 2.9
25			5	10.0 ± 2.9
26			5	12.2 ± 2.9
27			5	14.5 ± 2.9
28			5	12.8 ± 2.9

[a]Least squares Mean ± S.E.

TABLE XI

PLASMA LH (NG/ML) IN GILTS DURING THE ESTROUS CYCLE OR EARLY PREGNANCY

Day	No.	Open Gilts	No.	Pregnant Gilts
0	3	6.2 ± 1.4	10	7.0 ± 0.7
1	3	6.7 ± 1.4	10	4.0 ± 0.7
2	3	3.2 ± 1.4	10	3.3 ± 0.7
3	3	3.0 ± 1.4	10	2.8 ± 0.7
4	3	2.1 ± 1.4	9	2.0 ± 0.8
5	3	2.2 ± 1.4	10	3.2 ± 0.7
6	3	2.6 ± 1.4	10	3.0 ± 0.7
7	3	2.2 ± 1.4	10	2.0 ± 0.7
8	3	2.1 ± 1.4	9	1.9 ± 0.8
9	3	1.7 ± 1.4	8	2.5 ± 0.8
10	3	1.4 ± 1.4	8	2.4 ± 0.8
11	3	1.1 ± 1.4	8	2.7 ± 0.8
12	3	3.6 ± 1.4	7	2.0 ± 0.9
13	3	2.6 ± 1.4	6	1.8 ± 1.0
14	3	5.2 ± 1.4	5	1.7 ± 1.1
15	3	1.7 ± 1.4	6	3.3 ± 1.0
16	3	3.1 ± 1.4	6	2.9 ± 1.0
17	3	2.6 ± 1.4	5	4.2 ± 1.1
18	3	1.6 ± 1.4	5	1.8 ± 1.1
19			5	2.3 ± 1.1
20			5	5.3 ± 1.1
21			5	2.9 ± 1.1
22			5	1.0 ± 1.1
23			5	1.6 ± 1.1
24			5	1.5 ± 1.1
25			5	1.4 ± 1.1
26			5	2.9 ± 1.1
27			5	2.7 ± 1.1
28			5	2.5 ± 1.1

[a]Least squares Mean ± S.E.

TABLE XII

CONCEPTION RATES AND NUMBERS OF CORPORA LUTEA AND EMBRYOS FOR GILTS CONFINED TO ENVIRONMENTAL CHAMBERS[a]

Gilt #	Trt.	Sires #	Date Bred	#C.L.	#Embryo
6-5	Chamber	23-5 (14-7)	2/19/75	13	9
86-1	Chamber	Blacky (14-7)	2/21/75	10	6
80-5	Chamber	13-6 (14-7)	2/26/75	10	10
50-4	Chamber	No Notch (14-7)	2/25/75	Returned to Estrus 3/2/75	
$\bar{X}$	Chamber			11	8.3[b]
50-6	Outside	13-6 (14-7)	2/21/75	14	13
6-3	Outside	No Notch (14-7)	2/21/75	Returned to Estrus 3/18/75	
28-5	Outside	13-6 (14-7)	2/25/75	19	13
28-4	O tside	13-6 (14-7)	3/ 7/75	18	16
$\bar{X}$	Outside			17.0	14.0[c]

[a]Gilts were managed, inseminated and confined to control environmental chambers as described for Experiment II.

[b]75.5% of Corpora Lutea No.

[c]82.4% of Corpora Lutea No.

TABLE XIII

ANALYSIS OF VARIANCE OF RESPIRATION RATES, RECTAL TEMPERATURE AND PLASMA HORMONE CONCENTRATIONS FOR 8 AM AND 8 PM OBSERVATIONS

Source	df	Resp.		Temp.		Prog.		Estradiol		LH		Corticoids	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Treatment	1	263351.49	71.73[b]	97.47	17.07[b]	222.51	.95	120.19	3.28[c]	23.78	1.45	1208.78	1.01
Pregnancy	1	13449.20	3.66[c]	7.51	1.31	19.54	.08	100.13	2.74	.21	.01	1137.42	.95
Trt. x Preg.	1	7394.87	2.01	8.62	1.51	5.27	.02	13.69	.37	.05	.003	1115.59	.93
Gilts within TP	20	3671.39	7.35[b]	5.71	5.46[b]	235.30	2.96[b]	36.60	2.01[b]	16.35	2.12[b]	1197.33	3.12[b]
Time[a]	1	27677.77	55.44[b]	43.57	41.69[b]	40.59	.51	.06	.003	1.21	.16	232.08	.60
Time x Preg.	1	594.69	1.19	0.25	.24	2.51	.03	2.64	.14	2.29	.30	110.28	.29
Time x Trt.	1	26164.60	52.41[b]	25.86	24.75[b]	.003	.00	14.19	.78	37.30	4.83	88.06	.23
Error	352	499.20		1.05		79.39		18.21		7.73		383.61	

[a]Time is AM versus PM over periods.

[b]($P < .005$)

[c]($P < .10$)

TABLE XIV

RESPIRATION RATES AT 8 AM AND 8 PM DURING EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Day	Control Gilts No.	Control Gilts 8 AM	Control Gilts 8 PM	Heat Stressed Gilts No.	Heat Stressed Gilts 8 AM	Heat Stressed Gilts 8 PM
1	12	26.5 ± 3[a]	35.7 ± 3	12	78.1 ± 7	107.7 ± 9
2	12	28.7 ± 3	26.8 ± 3	12	78.5 ± 7	108.3 ± 9
3	12	24.1 ± 3	28.6 ± 3	12	64.0 ± 7	107.5 ± 9
4	12	24.8 ± 3	29.4 ± 3	12	74.9 ± 7	114.4 ± 9
5	12	21.0 ± 3	28.9 ± 3	12	76.7 ± 7	115.1 ± 9
6	12	23.9 ± 3	24.7 ± 3	12	79.0 ± 7	119.2 ± 9
7	12	27.8 ± 3	30.1 ± 3	12	80.2 ± 7	129.3 ± 9
8	12	25.3 ± 3	26.7 ± 3	12	81.1 ± 7	100.1 ± 9

[a]Least Squares Mean ± S.E.

TABLE XV

RECTAL TEMPERATURES AT 8 AM AND 8 PM DURING EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Day	Control Gilts No.	Control Gilts 8 AM	Control Gilts 8 PM	Heat Stressed Gilts No.	Heat Stressed Gilts 8 AM	Heat Stressed Gilts 8 PM
1	12	39.10 ± 0.14[a]	39.50 ± 0.15	12	39.78 ± 0.14	40.36 ± 0.17
2	12	39.31 ± 0.14	39.73 ± 0.15	12	39.84 ± 0.14	40.74 ± 0.17
3	12	39.43 ± 0.14	39.66 ± 0.15	12	39.66 ± 0.14	40.73 ± 0.17
4	12	39.50 ± 0.14	39.79 ± 0.15	12	39.77 ± 0.14	40.75 ± 0.17
5	12	39.71 ± 0.14	39.57 ± 0.15	12	39.96 ± 0.14	40.61 ± 0.17
6	12	39.63 ± 0.14	39.78 ± 0.15	12	39.77 ± 0.14	40.81 ± 0.17
7	12	39.59 ± 0.14	39.64 ± 0.15	12	39.87 ± 0.14	40.36 ± 0.17
8	12	39.38 ± 0.14	39.38 ± 0.15	12	39.63 ± 0.14	40.12 ± 0.17

[a]Least Squares Mean ± S.E.

TABLE XVI

PLASMA HORMONE CONCENTRATIONS IN CONTROL AND HEAT STRESSED GILTS PRIOR TO TREATMENT

Treatment Group	No.	Progesterone	Estradiol	LH	Corticoids
Control	12	1.0 ± 0.2	18.1 ± 3.7	17.4 ± 5.1	30.4 ± 5.7
Heat Stressed	12	1.1 ± 0.2	14.9 ± 3.7	17.7 ± 5.1	39.5 ± 5.7

[a]Mean ± S.E.

TABLE XVII

PLASMA PROGESTERONE (NG/ML) IN GILTS DURING AND AFTER EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Day After Estrus	All Gilts		Open Gilts	
	Control	Heat Stressed	Control	Heat Stressed
1[b]	1.2 ± 1.6 (12)[a]	0.9 ± 3.4 (12)	1.8 ± 1.9 (9)	1.7 ± 1.8 (11)
	1.6 ± 1.6 (12)	2.1 ± 3.4 (12)	2.5 ± 1.9 (9)	3.0 ± 1.8 (11)
2	2.8 ± 1.6 (12)	3.7 ± 3.4 (12)	3.8 ± 1.9 (9)	4.8 ± 1.8 (11)
	4.7 ± 1.6 (12)	5.5 ± 3.4 (12)	5.4 ± 1.9 (9)	6.1 ± 1.8 (11)
3	7.8 ± 1.6 (12)	9.7 ± 3.4 (11)	8.8 ± 1.9 (9)	9.9 ± 1.8 (10)
	9.7 ± 1.6 (12)	11.5 ± 3.4 (12)	10.6 ± 1.9 (9)	12.6 ± 1.8 (11)
4	11.3 ± 1.6 (11)	12.6 ± 3.4 (12)	12.7 ± 2.1 (8)	13.7 ± 1.8 (11)
	16.8 ± 1.6 (12)	17.0 ± 3.4 (12)	14.7 ± 1.9 (9)	17.6 ± 1.8 (11)
5	14.5 ± 1.6 (12)	17.5 ± 3.4 (12)	14.7 ± 1.9 (9)	18.1 ± 1.8 (11)
	13.6 ± 1.6 (12)	17.6 ± 3.4 (12)	14.0 ± 1.9 (9)	19.0 ± 1.8 (11)
6	19.5 ± 1.6 (12)	21.3 ± 3.4 (12)	17.7 ± 1.9 (9)	20.0 ± 1.8 (11)
	20.9 ± 1.6 (12)	25.1 ± 3.4 (12)	17.7 ± 1.9 (9)	20.6 ± 1.8 (11)
7	21.9 ± 1.6 (12	28.0 ± 3.4 (11)	19.2 ± 1.9 (9)	22.2 ± 1.8 (11)
	22.7 ± 1.6 (12)	21.7 ± 3.4 (12)	19.5 ± 1.9 (9)	25.4 ± 1.8 (11)
8	21.0 ± 1.6 (12)	29.2 ± 3.4 (12)	20.1 ± 1.9 (9)	27.8 ± 1.8 (11)
	21.9 ± 1.6 (12)	26.2 ± 3.4 (11)	20.5 ± 1.9 (9)	25.0 ± 1.8 (11)
9	21.7 ± 2.6 (12)	29.8 ± 6.0 (9)	20.9 ± 3.2 (9)	30.5 ± 3.6 (8)
10	27.8 ± 2.6 (12)	32.4 ± 6.0 (9)	32.1 ± 3.2 (9)	30.7 ± 3.4 (8)
11	30.4 ± 2.6 (12)	31.7 ± 6.0 (10)	24.7 ± 3.2 (9)	32.6 ± 3.2 (9)
12	28.8 ± 2.6 (12)	47.8 ± 6.0 (9)	25.3 ± 3.2 (9)	34.1 ± 3.4 (8)
13	31.4 ± 2.6 (12)	33.6 ± 6.0 (9)	28.3 ± 3.2 (9)	34.5 ± 3.4 (8)
14			19.9 ± 3.2 (9)	22.8 ± 3.2 (9)
15			14.8 ± 3.4 (9)	17.9 ± 3.6 (7)
16			10.8 ± 3.6 (7)	11.7 ± 3.9 (6)
17			9.4 ± 3.4 (8)	9.3 ± 3.6 (7)
18			8.7 ± 3.6 (7)	8.4 ± 3.6 (7)
19			7.4 ± 3.6 (7)	5.3 ± 3.6 (7)
20			4.7 ± 3.6 (7)	4.6 ± 3.6 (7)
21			2.3 ± 4.9 (4)	4.1 ± 3.9 (6)
22			1.2 ± 4.3 (5)	3.7 ± 4.3 (5)

[a]Mean ± S.E., number in parentheses = number of gilts.

[b]During chamber confinement (days 1 to 8) samples were twice daily at 8 AM and 8 PM.

TABLE XVIII

ANALYSIS OF VARIANCE OF RESPIRATION RATES, RECTAL TEMPERATURES AND PLASMA HORMONE CONCENTRATIONS DURING EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Source	df	Resp. MS	Resp. F	Temp. MS	Temp. F	Prog. MS	Prog. F	Estrogen MS	Estrogen F	LH MS	LH F	Corticoids MS	Corticoids F
Treatment	1	262867.3	71.64[d]	97.17	17.05[d]	229.50	0.98	118.32	3.14[a]	24.69	1.54	1227.56	1.02
Pregnancy	1	13544.6	3.69[a]	7.55	1.32	17.73	0.07	100.25	2.66	0.15	0.01	1119.03	0.93
Trt. x Preg.	1	7469.6	2.04	8.71	1.53	6.40	0.03	14.33	0.38	0.02	0.001	1097.50	0.91
Gilts with TP	20	3669.4	6.79[d]	5.70	5.47[d]	234.73	12.84[d]	37.67	1.98[c]	16.05	1.99[c]	1204.38	3.06[d]
Period	15					761.85	41.69[d]	7.27	0.38	3.12	0.39	330.12	0.84
Linear	1					10887.17	595.82	11.32	0.60	4.77	0.59	372.92	0.95
Quadratic	1					190.29	10.41	16.43	0.87	0.57	0.07	72.46	0.18
Cubic	1					85.33	4.67	7.56	0.40	1.85	0.23	1649.22	4.19
Quardic	1					0.01	0.00	17.22	0.90	17.26	2.14	7.07	0.02
Quintic	1					21.41	1.17	7.73	0.41	0.90	0.11	138.73	0.35
Remainder	10					24.35	1.33	4.87	0.26	2.14	0.27	271.15	0.69
Trt. x Period	15					23.09	1.26	19.99	1.05	10.64	1.32	533.54	1.36
Preg. x Period	15					31.65	1.73[b]	13.81	0.73	3.77	0.47	277.06	0.58
Trt. x Preg. x Period	15					9.89	0.54	8.30	0.44	0.30	0.04	159.59	0.41
Error	296					18.27		18.97		8.06		393.51	

[a] ($P < .10$)

[b] ($P < .05$)

[c] ($P < .025$)

[d] ($P < .005$)

TABLE XIX

ANALYSIS OF VARIANCE OF PLASMA HORMONE CONCENTRATIONS AFTER EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Source	df	Progesterone		Estradiol		LH	
		MS	F	MS	F	MS	F
Treatment	1	638.34	2.30[a]	5.78	0.20	1.11	.62
Pregnancy	1	481.95	1.74	9.50	0.34	1.62	.90
Trt. x Preg.	1	27.14	0.10	22.49	0.79	.99	.55
Gilts within TP	19	277.27	6.77[b]	28.30	1.67	1.80	1.13
Period	4	207.57	5.04	11.36	.67	2.42	1.51
Linear	1	469.98	11.42	15.48	.92	8.14	5.09
Quadratic	1	139.68	3.40	1.56	.09	1.14	.71
Cubic	1	94.44	2.30	1.16	.07	.06	.04
Quardic	1	126.16	3.07	27.26	1.61	.36	.22
Trt. x Period	4	26.41	.64	5.60	0.33	1.22	.76
Preg. x Period	4	55.86	1.35	2.88	0.17	.38	.24
Trt. x Preg. x Period	4	100.04	2.42	14.67	0.87	3.14	1.96
Error	67	41.14		16.91		1.60	

[a] ($P = .16$)

[b] ($P < .005$)

TABLE XX

ANALYSIS OF VARIANCE OF PLASMA HORMONE CONCENTRATIONS IN NON-PREGNANT GILTS DURING EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Source	df	Progesterone		Estradiol		LH		Corticoids	
		MS	F	MS	F	MS	F	MS	F
Treatment	1	585.58	2.34	93.79	2.30	44.24	2.48	7.62	.01
Gilts	18	250.40	12.95[b]	40.82	2.14[b]	17.84	2.04[a]	1180.70	3.08[b]
Period	15	1080.73	55.91[b]	14.43	.76	13.19	1.51	346.14	.90
Linear	1	15625.91	808.41	5.46	.29	12.93	1.48	664.70	1.73
Quadratic	1	367.42	19.01	18.56	.97	10.01	1.15	47.00	1.22
Cubic	1	6.13	0.32	1.07	.06	4.34	.50	152.90	.40
Quardic	1	30.59	1.58	4.85	.25	37.27	4.27	35.82	.09
Quintic	1	98.31	5.09	20.05	1.05	19.10	2.19	1126.82	2.94
Residual	10	8.26	0.43	16.64	.87	11.42	1.31	316.49	.82
Trt. x Period	15	23.00	1.19	21.46	1.12	10.92	1.25	450.55	1.17
Error	256	19.33		19.12		8.74		383.92	

[a] ($P < .025$)

[b] ($P < .005$)

TABLE XXI

ANALYSIS OF VARIANCE OF PLASMA HORMONE CONCENTRATIONS IN NON-PREGNANT GILTS AFTER EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Source	df	Progesterone		Estradiol		LH	
		MS	F	MS	F	MS	F
Treatment	1	599.04	1.53	28.47	.22	.23	0.04
Gilts	17	392.16	6.11[b]	128.58	3.47[b]	6.54	2.22[a]
Period	13	1527.73	23.80[b]	126.92	3.43[b]	5.37	1.83
Linear	1	16789.42	261.53	1405.62	37.96	4.48	1.52
Quadratic	1	204.12	3.18	69.71	1.88	0.00	0.00
Cubic	1	1813.51	28.25	36.60	0.99	22.71	7.72
Quardic	1	231.84	3.61	11.30	0.31	4.71	1.60
Quintic	1	207.74	3.24	48.57	1.31	7.84	2.67
Residual	8	76.74	1.20	9.76	.26	3.77	1.28
Trt. x Period	13	53.46	0.83	37.92	1.02	3.80	1.29
Error	164	64.20	37.03	37.03		2.94	

[a] ($P < .025$)

[b] ($P < .005$)

TABLE XXII

PLASMA ESTRADIOL (PG/ML) IN GILTS DURING AND AFTER EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Day After Estrus	All Gilts		Open Gilts	
	Control	Heat Stressed	Control	Heat Stressed
1[b]	4.5 ± 1.2 (12)[a]	7.6 ± 1.9 (12)	5.8 ± 1.5 (9)	4.8 ± 1.4 (11)
	5.5 ± 1.2 (12)	2.0 ± 1.9 (12)	8.2 ± 1.5 (9)	3.8 ± 1.4 (11)
2	5.5 ± 1.2 (12)	4.7 ± 1.9 (12)	6.9 ± 1.5 (9)	4.5 ± 1.4 (11)
	7.8 ± 1.2 (12)	4.4 ± 1.9 (12)	5.8 ± 1.5 (9)	5.8 ± 1.4 (11)
3	6.5 ± 1.2 (12)	5.6 ± 1.9 (11)	4.9 ± 1.5 (9)	6.4 ± 1.4 (10)
	8.1 ± 1.2 (12)	5.1 ± 1.9 (12)	10.6 ± 1.5 (9)	5.5 ± 1.4 (11)
4	5.5 ± 12. (11)	4.9 ± 1.9 (12)	4.3 ± 1.6 (8)	5.5 ± 1.4 (11)
	6.0 ± 1.2 (12)	7.6 ± 1.9 (12)	6.1 ± 1.5 (8)	5.5 ± 1.4 (11)
5	7.2 ± 1.2 (12)	4.0 ± 1.9 (12)	7.6 ± 1.5 (8)	5.6 ± 1.4 (11)
	6.7 ± 1.2 (12)	3.7 ± 1.9 (12)	6.0 ± 1.5 (8)	5.9 ± 1.4 (11)
6	7.5 ± 1.2 (12)	3.3 ± 1.9 (12)	8.2 ± 1.5 (8)	6.5 ± 1.4 (11)
	6.9 ± 1.2 (12)	2.5 ± 1.9 (12)	8.6 ± 1.5 (8)	4.5 ± 1.4 (11)
7	5.0 ± 1.2 (12)	5.1 ± 1.9 (11)	6.0 ± 1.5 (8)	7.2 ± 1.4 (10)
	3.9 ± 1.2 (12)	5.2 ± 1.9 (12)	4.4 ± 1.5 (8)	5.1 ± 1.4 (11)
8	5.9 ± 1.2 (12)	2.4 ± 1.9 (12)	6.7 ± 1.5 (8)	4.7 ± 1.4 (11)
	6.5 ± 1.2 (12)	4.1 ± 1.9 (11)	6.5 ± 1.5 (8)	6.8 ± 1.4 (10)
9	7.1 ± 1.5 (12)	4.4 ± 2.3 (9)	5.7 ± 2.2 (8)	4.0 ± 2.5 (8)
10	6.5 ± 1.5 (12)	7.7 ± 2.3 (9)	6.3 ± 2.2 (8)	5.0 ± 2.4 (8)
11	6.4 ± 1.5 (12)	5.4 ± 2.3 (9)	6.1 ± 2.2 (8)	6.7 ± 2.2 (9)
12	6.5 ± 1.5 (12)	10.1 ± 2.3 (9)	7.4 ± 2.2 (8)	7.0 ± 2.4 (8)
13	6.0 ± 1.5 (12)	8.2 ± 2.3 (9)	6.6 ± 2.2 (8)	6.5 ± 2.4 (8)
14			8.7 ± 2.2 (8)	7.4 ± 2.2 (9)
15			7.4 ± 2.4 (8)	6.3 ± 2.5 (7)
16			10.1 ± 2.5 (7)	8.5 ± 2.7 (6)
17			6.6 ± 2.4 (8)	13.1 ± 2.5 (7)
18			8.8 ± 2.5 (7)	13.7 ± 2.5 (7)
19			9.1 ± 2.5 (7)	12.9 ± 2.5 (7)
20			6.9 ± 2.5 (7)	13.7 ± 2.5 (7)
21			13.9 ± 3.4 (4)	13.2 ± 2.7 (6)
22			19.0 ± 3.0 (5)	14.9 ± 3.0 (5)

[a]Mean ± S.E., number in parentheses = number of gilts.

[b]During chamber confinement (days 1 to 8) samples were twice daily at 8 AM and 8 PM.

TABLE XXIII

PLASMA LH (NG/ML) IN GILTS DURING AND AFTER EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Day After Estrus	All Gilts		Open Gilts	
	Control	Heat Stressed	Control	Heat Stressed
1[b]	4.7 ± 1.2 (12)[a]	2.4 ± 1.0 (12)	5.5 ± 1.0 (9)	2.6 ± 0.9 (11)
	2.4 ± 1.2 (12)	2.4 ± 1.0 (12)	2.2 ± 1.0 (9)	2.5 ± 0.9 (11)
2	2.4 ± 1.2 (12)	2.2 ± 1.0 (12)	2.1 ± 1.0 (9)	2.1 ± 0.9 (11)
	2.8 ± 1.2 (12)	2.0 ± 1.0 (12)	2.5 ± 1.0 (9)	1.9 ± 0.9 (11)
3	1.8 ± 1.2 (12)	1.3 ± 1.0 (11)	1.5 ± 1.0 (9)	1.4 ± 1.0 (10)
	2.0 ± 1.2 (12)	1.9 ± 1.0 (12)	2.1 ± 1.0 (9)	1.8 ± 0.9 (11)
4	5.3 ± 1.2 (11)	2.0 ± 1.0 (12)	7.3 ± 1.0 (8)	2.1 ± 0.9 (11)
	3.5 ± 1.2 (12)	1.7 ± 1.0 (12)	2.3 ± 1.0 (9)	1.9 ± 0.9 (11)
5	3.2 ± 1.2 (12)	1.9 ± 1.0 (12)	2.4 ± 1.0 (9)	1.7 ± 0.9 (11)
	2.5 ± 1.2 (12)	2.3 ± 1.0 (12)	1.6 ± 1.0 (9)	2.3 ± 0.9 (11)
6	3.5 ± 1.2 (12)	1.4 ± 1.0 (12)	3.1 ± 1.0 (9)	1.4 ± 0.9 (11)
	1.8 ± 1.2 (12)	2.3 ± 1.0 (12)	1.8 ± 1.0 (9)	2.8 ± 0.9 (11)
7	1.7 ± 1.2 (12)	1.9 ± 1.0 (11)	1.3 ± 1.0 (9)	1.7 ± 1.0 (10)
	2.4 ± 1.2 (12)	2.0 ± 1.0 (12)	2.6 ± 1.0 (9)	2.2 ± 0.9 (11)
8	2.6 ± 1.2 (12)	1.6 ± 1.0 (12)	3.0 ± 1.0 (9)	1.5 ± 0.9 (11)
	1.8 ± 1.2 (12)	3.0 ± 1.0 (11)	2.7 ± 1.0 (9)	2.2 ± 1.0 (10)
9	1.4 ± 0.4 (12)	3.3 ± 0.7 (9)	1.5 ± 0.6 (9)	1.4 ± 0.7 (8)
10	2.3 ± 0.4 (12)	1.4 ± 0.7 (9)	1.6 ± 0.6 (9)	1.9 ± 0.6 (8)
11	1.3 ± 0.4 (12)	1.3 ± 0.7 (9)	1.4 ± 0.6 (9)	1.0 ± 0.6 (9)
12	1.1 ± 0.4 (12)	1.6 ± 0.7 (9)	1.2 ± 0.6 (9)	1.4 ± 0.6 (8)
13	1.1 ± 0.4 (12)	1.2 ± 0.7 (9)	0.9 ± 0.6 (9)	1.6 ± 0.6 (8)
14			0.8 ± 0.6 (9)	1.4 ± 0.6 (9)
15			1.5 ± 0.6 (8)	0.7 ± 0.7 (7)
16			1.3 ± 0.7 (7)	1.0 ± 0.8 (6)
17			1.3 ± 0.6 (8)	2.2 ± 0.7 (7)
18			4.9 ± 0.7 (7)	1.7 ± 0.7 (7)
19			1.9 ± 0.7 (7)	2.9 ± 0.7 (7)
20			1.2 ± 0.7 (7)	1.3 ± 0.7 (7)
21			1.7 ± 0.9 (4)	1.6 ± 0.7 (6)
22			1.0 ± 0.8 (5)	1.2 ± 0.8 (5)

[a]Mean ± S.E., number in parentheses = number of gilts.

[b]During chamber confinement (days 1 to 8) samples were twice daily at 8 AM and 8 PM.

TABLE XXIV

PLASMA CORTICOIDS (NG/ML) IN GILTS DURING EXPOSURE TO CONTROL OR ELEVATED AMBIENT TEMPERATURE

Day After Estrus	All Gilts		Open Gilts	
	Control	Heat Stressed	Control	Heat Stressed
1[b]	27.8 ± 7.4 (12)[a]	34.6 ± 10.3 (12)	25.7 ± 6.9 (9)	34.7 ± 6.3 (11)
	22.0 ± 7.4 (12)	35.0 ± 10.3 (12)	16.7 ± 6.9 (9)	25.7 ± 6.3 (11)
2	23.7 ± 7.4 (12)	13.5 ± 10.3 (12)	24.7 ± 6.9 (9)	23.8 ± 6.3 (11)
	18.0 ± 7.4 (12)	21.0 ± 10.3 (12)	17.3 ± 6.9 (9)	41.0 ± 6.3 (11)
3	21.3 ± 7.4 (12)	13.2 ± 10.3 (11)	25.2 ± 6.9 (9)	24.8 ± 6.6 (10)
	20.1 ± 7.4 (12)	10.1 ± 10.3 (12)	24.5 ± 6.9 (9)	20.2 ± 6.3 (11)
4	22.3 ± 7.4 (11)	40.1 ± 10.3 (12)	28.0 ± 7.4 (8)	32.9 ± 6.3 (11)
	25.5 ± 7.4 (12)	16.5 ± 10.3 (12)	22.4 ± 6.9 (9)	21.3 ± 6.3 (11)
5	23.1 ± 7.4 (12)	13.2 ± 10.3 (12)	31.1 ± 6.9 (9)	24.6 ± 6.3 (11)
	26.3 ± 7.4 (12)	10.4 ± 10.3 (12)	27.9 ± 6.9 (9)	18.5 ± 6.3 (11)
6	24.5 ± 7.4 (12)	28.9 ± 10.3 (12)	19.9 ± 6.9 (9)	22.0 ± 6.3 (11)
	22.1 ± 7.4 (12)	18.2 ± 10.3 (12)	17.5 ± 6.9 (9)	20.7 ± 6.3 (11)
7	34.0 ± 7.4 (12)	17.0 ± 10.3 (11)	33.4 ± 6.9 (9)	21.4 ± 6.6 (10)
	41.3 ± 7.4 (12)	12.7 ± 10.3 (12)	37.1 ± 6.9 (9)	22.7 ± 6.3 (11)
8	16.3 ± 7.4 (12)	14.6 ± 10.3 (12)	18.1 ± 6.9 (9)	21.0 ± 6.3 (11)
	24.1 ± 7.4 (12)	6.6 ± 10.3 (11)	23.5 ± 6.9 (9)	13.1 ± 6.6 (10)

[a] Mean ± S.E., number in parentheses = number of gilts.

[b] Samples were collected twice daily at 8 AM and 8 PM.

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