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Effects of adrenocorticotrophin administration during early gestation on conceptus development in swine

Jeffrey D. Arnold

To the Graduate Council:

I am submitting herewith a thesis written by Jeffrey D. Arnold entitled "Effects of adrenocorticotrophin administration during early gestation on conceptus development in swine." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Henry G. Kattesh, Major Professor

We have read this thesis and recommend its acceptance:

Tom T. Chen, R. L. Murphree

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Jeffrey D. Arnold entitled "Effects of Andrenocorticotrophin Administration During Early Gestation on Conceptus Development in Swine." I have examined the final copy of this thesis for form and content and recommend its acceptance for the degree of Master of Science, with a major in Animal Science.

Henry G. Kattesh

Henry G. Kattesh, Major Professor

We have read this thesis
and recommend its acceptance:

R S Murphree

Accepted for the Council:

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Vice Chancellor
Graduate Studies and Research

EFFECTS OF ADRENOCORTICOTROPHIN ADMINISTRATION DURING EARLY GESTATION ON CONCEPTUS DEVELOPMENT IN SWINE

A Thesis

Presented for the

Master of Science

Degree

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ABSTRACT

The objectives of this study were (1) to determine the effect of exogenous ACTH upon maternal and conceptus parameters in pregnant gilts and (2) to validate an assay for cytoplasmic progesterone receptors in order to quantitate levels in uterine endometrium of pregnant gilts receiving ACTH injections.

Seventy-two gilts of similar age and breeding were mixed and randomly placed in groups of 12 in one of six pasture lots and observed through one normal estrous cycle. Upon detection of the second estrus, gilts were double mated at 12 and 24 hours after estrus detection using a different boar at each breeding. Mating was accomplished by artificial insemination using fresh semen. Breeding continued until 48 gilts were obtained. Once bred, gilts were randomly assigned to one of 3 treatments and one of 4 injection periods. Treatments consisted of a single, daily, intramuscular injection of either 0, 40 or 80 USP units of corticotrophin in an aqueous suspension containing zinc hydroxide for repository action, for a period of 5 days. Control gilts received an injection of vehicle containing zinc hydroxide with no corticotrophin activity. The injection periods were 1-5, 6-10, 11-15, or 16-20 days of gestation with day one corresponding to 48 hours postestrus detection. All gilts were slaughtered at approximately 37 days of gestation.

Forty-two of the 48 gilts bred conceived; there was no difference ($P>.10$) in conception rate among the 12 treatment-period combinations. Injections of ACTH, regardless of dosage or period given had no apparent effects on fetal survival or any of the maternal or conceptus parameters measured.

Conceptus data were pooled and analyzed for differences according to position within the uterus. The three positions analyzed were the utero-tubal portion, the middle portion and the cervical portion. Average allantoic fluid volume was significantly ($P<.05$) greater, fetal wet weights were heavier ($P<.10$) and degenerating fetal numbers were greater ($P<.05$) in the middle portion while placental lengths were significantly ($P<.05$) longer in the ovarian portion than the cervical portion of the uterus.

Validation of an assay for quantitating uterine cytoplasmic progesterone receptors indicated that a specific protein receptor for progesterone existed having a K_d of 26.3 pM and a binding capacity of 3.4 pmol/mg tissue. It was also determined that 12.5 mg of uterine tissue appears to be saturated at a concentration of 15-20 nM ^{3}H-progesterone for an incubation period of 10 to 14 hours.

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CHAPTER I

INTRODUCTION

An important factor in livestock production today is the ability of the dam to produce maximum numbers of viable offspring in a minimum amount of time. One of the goals of the swine industry is to improve reproductive efficiency or the number of pigs born alive. Reproductive efficiency is decreased and profits are lost when for any reason the time from weaning of one litter to weaning of the next is increased, or low numbers of piglets are weaned.

Many factors can affect the ability of the gilt or sow to become pregnant and carry a litter to term. One factor that can affect viability and survival of embryos and fetuses is severe stress acting on the female. It has been estimated that one-third to one-half of all gestation failures are due to embryonic and fetal mortality. The fertilization rate for swine was found to be 95% to 99% while average embryonic loss was 25% to 40% of the total number of ovulations at 30 days of gestation.

Many physiological, nervous, endocrine, neurohumoral and motor responses come into play when a body is under stress. The hypothalamic-pituitary-adrenal axis has been identified as playing a major role in embryonic and fetal survival as well as maintenance of pregnancy. The actual mechanisms by which the involvement of this axis elicits its detrimental effects is still unclear.

The objective of this experiment was to investigate further the actions of exogenous ACTH upon pregnant swine during early gestation.

A second objective was to validate an assay for cytoplasmic progesterone receptor in order to quantitate receptor levels in uterine endometrial tissue of swine.

CHAPTER II

LITERATURE REVIEW

I. STRESS AND FEMALE REPRODUCTION

Stress, as defined by Moberg (1976), is "an event (physical, environmental, etc.) which significantly challenges the homeostasis of the animal." Stress brings into play several physiologic and motor functions combined to restore a constant energy metabolism and behavior to survive in a new environment.

Events that can upset the normal functions of an animal can be a collection of many stresses. Stress as it affects livestock production, specifically reproduction, can be classified into two general categories (Moberg, 1976): Environmental stress (temperature, humidity, wind, photoperiod) and management related stress. Examples of management related stress are intraspecies interaction (crowding, social status), animal manipulation and handling, interspecies interaction, physical trauma and psychological distress (due to lack of water, feed, etc.).

The effects of environmental stress on reproduction have been well documented in many species. High environmental temperature has been shown to be detrimental during pregnancy in swine (Warnick et al., 1965; Tompkins et al., 1967; Edwards et al., 1968; Teague et al., 1968; Omtvedt et al., 1971; Wildt et al., 1975), sheep (Yeates, 1953; Dutt, 1963; Woody and Ulberg, 1964; Thwaites, 1969), cattle (Stott and Williams, 1962; Labhstwar et al., 1963; Dunlap and Vincent, 1971; Ingrahm et al., 1974; Moberg, 1976) rats (MacFarlane et al., 1957;

Fernandez-Cano, 1958; Edwards, 1968; Fried et al., 1970), rabbits (Shah, 1956), mice (Ogle, 1934) and guinea pigs (Edwards, 1969). Likewise high humidity exerts adverse effects during early gestation in swine (Teague et al., 1968) and cattle (Ingrahm et al., 1974). Euker and Riegle (1973) demonstrated that restraint stress would cause embryonic degeneration and resorption in the pregnant rat. Crowding in mice may cause pregnancy to be compromised (Christian and Lemunyan, 1958). Hypoxia also was shown to cause fetal mortality in rats (Fernandez-Cano, 1958).

Ovulation

Ovulation rates expressed as the total number of corpora lutea (CL) formed were not significantly affected in heat stressed swine compared to controls although there tended to be more CL in those groups of females maintained at cooler temperatures (Warnick et al., 1965; Edwards et al., 1968). Teague et al. (1968) reported significantly fewer CL in gilts maintained at 33C for one estrous cycle prior to breeding. MacFarlane et al. (1957) found that rats acclimated for 2-3 weeks at 35C formed significantly fewer CL. Others found that neither heat stressed sheep (Dutt et al., 1959; Alliston and Ulberg, 1961; Dutt, 1963) nor rats experiencing restraint stress (Euker and Riegle, 1973) exhibited differences in the number of CL formed when compared to controls.

Unfertilized Ova

Woody and Ulberg (1964) performed transfers of unfertilized sheep ova from donors maintained at either 21C or 32C to recipients at 21C.

When the surrogate mothers were checked 25 to 30 days later, equal numbers of normal embryos from both treatment groups were found thus confirming that heat stress did not affect the viability of the ova prior to fertilization. Swine that were subjected to high environmental temperatures during proestrus to 3 days postbreeding were found to have as good a conception rate as control gilts (Warnick et al., 1965). The authors thus assumed that effects of high temperature on females did not affect unfertilized ova and any detrimental effects must occur after fertilization.

Fertilization and Cleavage

Austin and Braden (1954) working with rats found that by applying heat to the oviduct post-ovulation, fertilization occurred but the second polar meiosis was inhibited and cleavage did not take place. Ulberg and Burfening (1967) theorized that an increase in the body core temperature, specifically the uterus and oviduct, of an animal under heat stress would cause sperm to lose its potency. The results shown by Austin and Braden (1954) were produced by extreme measures and may only account for a small amount of pregnancy failure.

Female rats experiencing severe heat days 1 to 4 postcoitum (Fernandez-Cano, 1958) and heifers subjected to high temperatures 1 to 3 days postbreeding (Dunlap and Vincent, 1971) contain fertilized ova that proceed through cleavage but exhibit high incidences of embryo degeneration. Alliston and Ulberg (1961) demonstrated by ova transfer that embryos collected from ewes under high environmental temperature and transplanted into uteri of recipient ewes not stressed showed a significant increase in embryonic death. This is in accordance with

what others found in sheep (Dutt et al., 1959; Alliston et al., 1961; Dutt, 1963). As pointed out earlier, heat stress does not appear to affect fertilization and early cleavage in swine with any significant effects. In contrast, fertilized ova residing in the oviduct of sheep, cattle and rats are greatly affected.

Early Gestation

Shah (1956), working with rabbits, examined whether the effects of stress was working directly upon the ova or if it was working through maternal origins. He collected embryos from unstressed does at six days postcoitum and placed them in does that were stressed the first six days. After the transplants, he moved the recipients to a normal environment. At 12 to 14 days postcoitum, rabbits were sacrificed and uteri were checked for embryos. Implantation had occurred but 90% of the embryos were resorbed or degenerating. The conclusions were that something occurred in the female tract during heat stress that later affected the transplanted embryos.

Sheep which are introduced to high temperatures during later stages of cleavage and embryo development prior to implantation (when the embryos have reached the uterus) still show significant reductions in embryonic survival although the reductions are not as critical as seen during earlier stages of cleavage (Dutt et al., 1959; Alliston et al., 1961; Woody and Ulberg, 1964). In comparison, according to Warnick et al. (1965), heat stress does not affect fertilization and early cleavage in swine (while the blastocysts are residing in the oviduct). However, high temperatures do significantly reduce the number of viable embryos by eight days postbreeding (Tompkins et al., 1967;

Omtvedt et al., 1971). Omtvedt et al. (1971) also found that swine were most susceptible to high temperature during implantation stage. More embryonic mortality occurred when gilts were heat stressed 8 to 16 days postbreeding, a time when recognization of pregnancy occurs.

Pregnant rats (Edwards, 1968) and guinea pigs (Edwards, 1969) exposed to stress during early gestation show embryonic and fetal mortality. Exposure during implantation and postimplantation will not affect survival but will lead to congenital malformations. These malformations are not due to maternal effects.

Mid- and Late Gestation

High environmental temperature during midgestation in swine severe enough to kill the mother did not affect fetal survival (Heitman et al., 1951; Omtvedt et al., 1971; Kattesh et al., 1980). However, gilts that experience high temperatures during late gestation (days 102 to 110 of gestation) farrow fewer live pigs and more stillborn pigs were compared to controls (Omtvedt et al., 1971).

Although not much work has been done with gestating cattle under stress, Boyd et al. (1969) found that the expected amount of embryonic death occurred mostly by 9 to 14 days postbreeding. It was assumed that most embryonic loss would occur in cows and heifers by day 26 postbreeding (through the preimplantation stage).

This research points out critical periods during gestation when stress may adversely affect pregnancy in a variety of species. Although under laboratory conditions the results appear valid, they may be greatly exaggerated for practical conditions. Thwaites (1969) pointed out that ewes exposed continuously to heat stress exhibited 83% embryonic

death while a 19% embryo loss occurred in ewes exposed to diurnal stress (8 hours heat, 16 hours of cooler temperatures) that might be expected under normal environmental conditions.

II. ENDOCRINE ASPECTS INVOLVED WITH STRESS AND FEMALE REPRODUCTION

The physiological and endocrinological mechanisms by which stressful situations adversely affect reproduction are unclear. One possible source is the adreno-cortical hyperactivity described by Selye (1946) in his "General Adaptation Syndrome."

Early researchers found that various damaging agents (i.e. ether and other anesthetics, caustic agents, etc.) and crowded living environments accorded rats and mice led to increased adrenal gland weights, gonadal atrophy and cessation of the estrous cycle in females (Selye, 1939; Christian, 1955; Christian and Lemunyan, 1958; Christian et al., 1965). These authors theorized that a lack of gonadotrophins from the pituitary led to decreased function of the gonads and reproductive tract. But increased adrenal weights indicated that the pituitary was secreting increasing levels of adrenocorticotrophic hormone and not lying dormant. To Selye (1939) it appeared that the pituitary was compensating the abnormal metabolism by producing more adrenocorticotrophic hormone (ACTH) in order to sustain normal body function; the necessity to preserve normal sex functions was only of secondary importance.

ACTH and Corticosteroid Release During Stress

The effects of high temperature and humidity on swine significantly raised and maintained high plasma levels of ACTH for several days (Marple et al., 1972a; 1972b). These researchers also found that

the stressful conditions imposed affected the adrenals such that after several days the plasma cortiocosteroid levels were lower. Sebranek et al. (1973) found the maximum adrenal response in pigs occurred 60 minutes after ACTH administration. Corticosteroid levels declined after that. A similar response was also found in swine after exposure to high temperatures (Aberle et al., 1974).

These experiments agree with those of Collins and Weiner (1968) who stated that heat adaptation leads to increased levels of ACTH while the adrenal glands become less responsive and a reduction of glucocorticoid secretion ensues. Chronic stimulation of the adrenals by ACTH during severe stress leads to an initial surge of glucocorticoids after which stores are depleted. That hypertrophy follows suggests the adrenals are not altogether unresponsive to further stimulation by adrenocorticotrophin.

Research by Kattesh et al. (1980) showed that heat stressed pregnant gilts exhibited lower plasma glucocorticoid levels as well as lower glucocorticoid binding globulin (CBG) content. This suggests that stressed and acclimated swine maintain lower blood levels of glucocorticoids and metabolize the adrenal steroids at a faster rate than swine maintained under normal conditions.

ACTH and Corticosteroids Affecting Pregnancy

Past research has shown that ACTH will interrupt pregnancy and increase embryonic mortality in rabbits (Robson and Sharaf, 1952), rats (Velardo, 1957; Yang et al., 1969) and mice (Robson and Sharaf, 1952; Kittinger et al., 1980) but does not affect fertilization, cleavage or tubal transport. From this research it appears that ACTH effects embryos only after they have descended into the uterus.

Dupouy et al. (1980) produced evidence that no transplacental passage of adrenocorticotrophin occurs between mother and fetus in the rat. The adverse effects of ACTH must be produced through maternal tissues and do not work directly on the conceptus.

Lorenzen et al. (1977) suggested there is competition in the pituitary to produce and secrete ACTH and luteinizing hormone (LH). During stress LH secretion would be depressed while adrenocorticotrophic hormone synthesis and release is increased many fold. If this were the case, the lack of luteinizing hormone would compromise the integrity of pregnancy. Increased levels of ACTH and corticosteroids would only be a consequence of metabolic inbalance and administration of either would not significantly affect the intrauterine population of gestating females.

However, it has been demonstrated that cortisone administration affects pregnancy similar to ACTH when given to gestating rats (MacFarlane et al., 1957) and sheep (Howarth and Hawk, 1968). Yang et al. (1969) found no embryonic death in adrenalectomized rats treated with ACTH, indicating that ACTH mediates its effects via adrenal secretions.

Adrenocorticotrophin has been shown to increase adrenal secretion of progesterone in swine (Liptrap, 1970; Close and Liptrap, 1975; Benjaminsen and Lunaas, 1980) and androgens in swine (Liptrap, 1970) and humans (Facchinetti et al., 1980) as well as corticosteroids. It is quite possible that these steroids, by negative feedback, could block or reduce LH secretion from the pituitary.

It is well known that a spike of LH is important for ovulation to occur. Research now shows that if pregnant rabbits (Spies and Quadri,

1967) or rats (Loewit et al., 1969; Madhwa and Moudagal, 1970; Nishi et al., 1976) are immunized against LH or its releasing factor (from the hypothalmus) during early gestation (when LH levels are low) pregnancy is terminated, but not before day 3 of gestation. Pregnancy was maintained in immunized females when progesterone was administered. This indicates that LH, even in low levels is important for corpus luteum maintenance and progesterone secretion.

Recent evidence indicates that glucocorticoids may initiate pregnancy termination at parturition. Administration of dexamethasone, a synthetic glucocorticoid, induced premature parturition in swine (First and Staigmiller, 1973; North et al., 1973; Coggins and First, 1977; First and Bosc, 1979). Nara and First (1981) found that sows treated with dexamethasone demonstrated increased plasma levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a known luteolytic and parturient inducing agent.

Brunner et al. (1969) showed that heifers injected with ACTH exhibited significant decreases in corpus luteum weight, but was ineffective when injected into hysterectomized females. It appears that a uterine luteolytic mechanism is necessary for luteal regression.

New research has demonstrated an endometrial progesterone receptor protein in rats (Walters and Clark, 1977; 1978), sheep (Kontula, 1975), swine (Diekman and Anderson, 1979), cattle (Atkins et al., 1980) and many other lab animals. Evidence suggests that the progesterone receptor has many characteristics similar to that of CBG which is found both in tissue and plasma and which has demonstrated a low affinity for some adrenal steroids (Milgrom and Baulieu, 1968; 1970; Walters and Clark, 1977; Atkins et al., 1980).

Based on these recent findings it may be suggested that a large release of adrenal corticosteroids during stress may become localized in the uterus, could bind to the progesterone receptors and prevent progesterone binding within the uterus. This would reduce progesterone induced uterine protein secretion important for growth of the developing conceptus (Knight et al., 1973b; Knight et al., 1974; Bazer, 1975; Chen et al., 1975). Corticosteroids may also adversely affect progesterone action which has been shown to enhance placental development and fetal fluid accumulation (Knight et al., 1973a; Knight et al., 1974).

Prolactin Affecting Pregnancy

Prolactin, another pituitary hormone, has been suggested as having detrimental effects on pregnancy. Ensor (1978) cites evidence that stress will stimulate increased levels of prolactin that may produce alterations in gonadal function. Kittinger et al. (1980) found chronic administration of ACTH produced hyperprolactinemia and reduced nidation sites in pregnant mice. Ensor (1978) related how increases in prolactin paralleled increases of ACTH in animals under stress. Clark et al. (1978) found that sheep immunized against luteinizing hormone releasing hormone (LHRH) exhibited decreased plasma levels of LH and increased levels of prolactin. Prolactin does possess some luteolytic properties, although its mechanism of action is presently unclear (Ensor, 1978).

Hyperprolactinemic women exhibiting dysmenorrhea were found to have increased levels of adrenal androgens in circulation (Jones et al., 1980). It was thought that prolactin, acting synergistically with ACTH, stimulated adrenal secretion of androgens and that these androgens were blocking the release of LH from the pituitary causing these women to be acyclic.

Hormones Involved in Ovulation Inhibition

Besides its effects on pregnancy, ACTH was also found to slow sexual maturation (Velardo and Sturgis, 1956; Christian, 1964), inhibit ovulation, cause cystic follicles and promote an aberrant estrus (Christian 1964; Jarret, 1965; Hagino et al., 1969; Liptrap, 1970; Liptrap, 1973), although ovulation occurred in adrenalectomized swine (Liptrap, 1973) and rats (Hagino et al., 1969) injected with ACTH. Corticosteroids injected into intact swine (Liptrap, 1970) and rats (Hagino et al., 1969) did not inhibit ovulation. Wagner et al. (1977) found that carotid infusion or ovarian perfusion of ACTH in bovine produced no significant decrease in CL activity, specifically secretion of progesterone.

Swine (Liptrap, 1973) and rats (Hagino et al., 1969) in which ovulation was inhibited responded to injections of human chorionic gonadotropin (HCG) which possesses LH activity. Madan and Johnson (1973) similarly found heat stress reduced peak levels of LH and reduced or suppressed estrus in cattle.

ACTH appears to work through the adrenal gland to inhibit or reduce the preovulatory spike of LH, but corticosteroids do not appear to be involved. ACTH and prolactin may stimulate androgen secretion from the adrenal cortex as discussed earlier, and by negative feedback mechanism inhibit LH release from the pituitary.

Ulberg et al. (1951) found that progesterone injected into gilts on days 15 or 19 through day 28 of gestation blocked ovulation and resulted in cystic follicles, similar to the effects recently found by administering ACTH. Adrenocorticotrophin stimulated adrenal glands in swine to increase output of progesterone (Liptrap, 1970; Close and

Liptrap, 1975; Benjaminsen and Lunaas, 1980) and may be the factor involved in ovulation inhibition.

CHAPTER III

MATERIALS AND METHODS

I. ANIMALS AND DATA COLLECTION

Seventy-two six month old gilts of mixed breeding (a combination of Landrace, Yorkshire and Duroc breeds) were weighed, mixed and relocated in groups of 12 to one of 6 one acre pasture lots. Each lot of 12 gilts was separated by a half-lot of 3 mature, crossbred boars. The twelve boars were used to detect standing heat in gilts and to collect fresh semen for artificial insemination. Gilts were allowed access to an open front building for shade, and water was provided ad libitum. Approximately four pounds of a 16% gestation ration was fed per head per day to all animals.

All gilts were observed through one normal estrous cycle (18 to 24 days in length) following relocation to the pasture lots. Gilts were checked for estrus activity twice daily (morning and evening) with the aid of a boar. Upon detection of the second estrus, gilts were double mated at 12 and 24 hours after the onset of estrus, using a different boar each time. Breeding was accomplished by artificial insemination using fresh semen. The boar used to detect heat was allowed to mount the gilt in estrus and semen was collected in an insulated Nalgene bottle by the gloved hand method (Hancock and Hovell, 1959).

Collected semen was immediately strained through three layers of cheesecloth to remove the gel fraction. Each collection was then evaluated microscopically for percent live-dead cells, motility, morphology,

volume and sperm cell concentration. Only those ejaculates which exhibited 70% live, morphologically normal cells of good motility or better were used. Semen was extended in BL-1 extender (Pursel et al., 1973) to a concentration of 8 to 10 billion cells per 100 ml of extended semen. Each insemination was accomplished with approximately 100 ml of extended semen.

After breeding, gilts were randomly assigned to one of twelve treatment period groups in a 3 x 4 factorial experiment (Table 1). Treatment consisted of a single, daily intramuscular injection of either 0 (control), 40 (low or L) or 80 (high or H) USP units of an aqueous suspension of corticotrophin with zinc hydroxide for repository action[1] for a period of five days. Control gilts received a daily injection for their respective injection period of vehicle containing zinc hydroxide with no corticotrophin activity present. The injection periods were either 1-5, 6-10, 11-15 or 16-20 days of gestation. Day one was considered to begin 48 hours after the onset of standing heat.

After breeding all gilts were maintained in their respective pasture lots and checked daily with the aid of a boar for return to estrus. At approximately 37 days of gestation gilts were weighed and slaughtered by exsanguination. The uteri and adrenal glands from all 48 gilts, pregnant or nonpregnant, were obtained, tagged for identification and maintained 15C until examined. All specimens were evaluated within 8 hours.

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[1]The author acknowledges with appreciation the donation of Corticotrophin^{R} - Zinc from Organon Pharmaceutical Inc., West Orange, New Jersey.

TABLE 1. DESIGN OF THE EXPERIMENT

Treatment[b]	Injection Period Post-breeding (days)[a]			
	1-5	6-10	11-15	16-20
Control ACTH (0)	4[c]	4	4	4
Low ACTH (40 USP)	4	4	4	4
High ACTH (80 USP)	4	4	4	4

[a]Represents days of gestation (inclusive), at which time the treatments were administered, with day 1 corresponding to 48 hours after detection of estrus.

[b]Treatment consisted of a single daily intramuscular injection of an aqueous suspension of corticotrophin with zinc hydroxide for repository action (Corticotrophin R-Zinc, Organon Pharmaceuticals Inc, West Orange, N.J.) in two levels of concentration. Control groups received an injection of vehicle containing zinc hydroxide with no corticotrophin activity.

[c]Represents number of gilts.

Uteri of open gilts were examined for anatomical defects or other abnormalities. Each pregnant uterus was cut free of all attached ligaments and stretched longitudinally. The ovaries were excised, weighed and corpora lutea were counted. The uterine horns were then sliced open longitudinally along the mesometrial border from the utero-tubal junction to the cervix. Each conceptus was exposed beginning from the utero-tubal end and conceptus measurements were taken as indicated in Table 2. Once conceptus data were obtained, the uterus was measured for uterine horn length, average horn width and weight.

Tissue samples were taken at the utero-tubal, middle and cervical portions of each uterine horn (see Figure 1) where fetal attachment had taken place. Two tissue samples were also taken from each horn at a site of nonattachment. Samples were obtained, the endometrium was separated from the myometrium and the endometrium was frozen for later analysis.

II. STATISTICAL ANALYSIS

The experiment was arranged in a 3 x 4 factorial (Table 1) using a completely randomized design. The data was statistically analyzed by least squares analysis of variance using a mixed effects model (Goodnight, 1979). The model used was as follows:

$$Y_{ijk} = t_i + p_j + (tp)_{ij} + e_{ijk}$$

where:

y_{ijk} = dependent variable

t_i = treatment

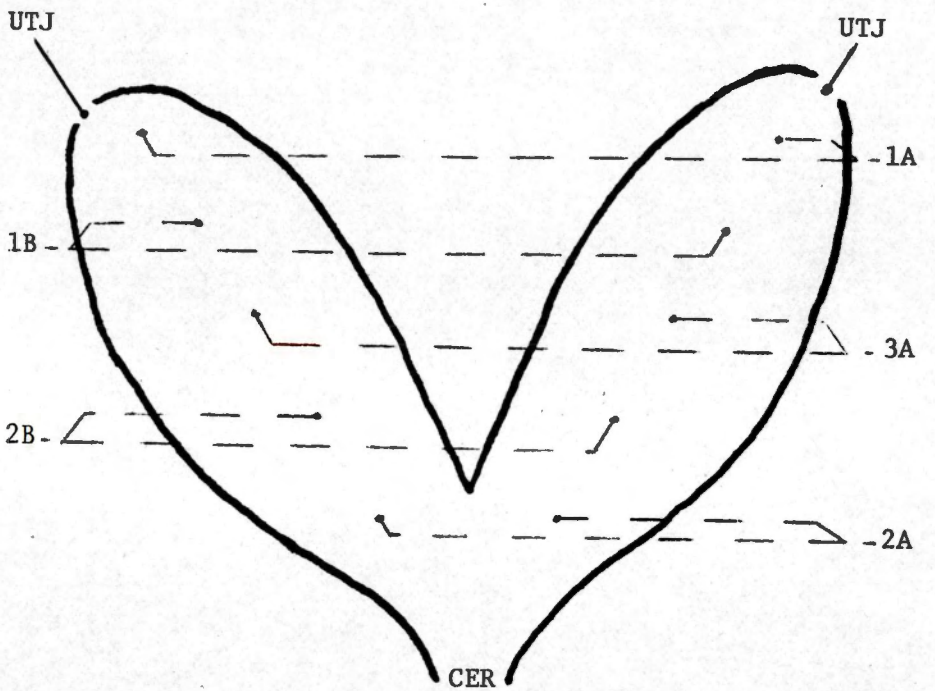
p_j = (injection) period

$(tp)_{ij}$ = treatment by period interaction

e_{ijk} = residual error

TABLE 2. CONCEPTUS MEASUREMENTS.

Number of live fetuses
Number of dead and degenerating fetuses
Placental length, cm
Placental wet weight, gm
Fetal crown-rump length, cm
Fetal wet weight, gm
Allantoic fluid volume, ml
Amnionic fluic volume, ml



Uterine samples taken at sites of attachment: 1A, 2A, 3A

Uterine samples taken at sites of nonattachment: 1B, 2B

Utero-tubal Juncion (UTJ)

Cervix (CER)

FIGURE 1. UTERINE ENDOMETRIUM SAMPLES TAKEN AT SITES OF FETAL ATTACHMENT AND NONATTACHMENT

The dependent variables analyzed are shown in Table 3. The effects of treatment, period and treatment by period interaction were tested using the residual mean square.

All conceptus data was pooled and analyzed according to uterine position using a randomized complete block design. The three uterine positions examined were the utero-tubal position, the middle position and the cervical portion (see Figure 2). The model used was as follows:

y_{ijk} = dependent variable

a_i = uterine position

g_j = individual gilt (block)

e_{ijk} = residual error

The dependent variables analyzed were placental length and weight, fetal crown-rump length, fetal wet weight, allantoic fluid volume, amnionic fluid volume, number of live fetuses and number of degenerating fetuses. Duncan's multiple range test (1955) was used to determine the significance between three positions. Correlations were run among the dependent variables to detect relationships.

III. CYTOPLASMIC PROGESTERONE RECEPTOR ASSAY

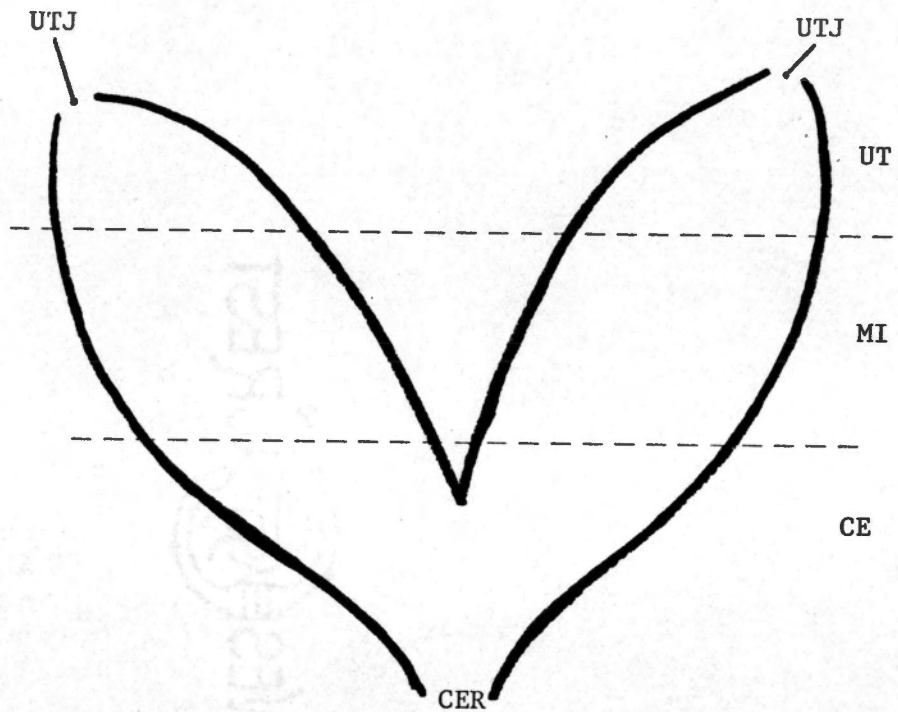
Materials

A 30% Tris-glycerol (TG) (Walters and Clark, 1977) buffer solution (0.01 M Tris 30% v/v glycerol at pH 7.4) was prepared using the following ingredients:

158 mg Tris(hydroxymethyl)aminomethane hydrochloride
100 mg Sodium azide
100 ml Distilled water
43 ml Glycerol

TABLE 3. DEPENDENT VARIABLES.

Total number of corpora lutea
Total number of live fetuses
Total number of dead and degenerating fetuses
Corrected fetal number (total live + total dead)
Percent fetal survival (total live/total CL)
Average fetal crown-rump length, cm
Average fetal wet weight, gm
Average placental wet weight, gm
Average placental length, cm
Average allantoic fluid volume, ml
Average amnionic fluid volume, ml
Average total ovarian weight, gm
Average uterine weight, gm
Average uterine surface area, cm^2
Average combined adrenal weight, gm
Adrenal weight/body weight X 100 (percent of body weight)



Uterine Position: Utero-tubal Portion (UT), Middle Portion (MI), Cervical Portion (CE)

Utero-tubal Junction (UTJ)

Cervix (CER)

FIGURE 2. UTERINE POSITION BY WHICH CONCEPTUS DATA WAS ANALYZED.

Dextran coated charcoal solution was prepared using the following ingredients:

250 mg Norit A charcoal
50 mg Dextran T-70
100 ml 30% TG buffer

All other solvents were reagent grade and used without any further purification.

Progesterone (Δ^4-pregnene-3,20-dione) and hydrocortisol (Δ^4-pregnene-11β,17,21-triol-3,20-dione) were purchased from Sigma Chemical Company and used without further purification. [1α,2α(n)-^{3}H] progesterone was purchased from Amersham and used without any further purification. All hormone preparations were made up in absolute alcohol and stored at 0-4C.

A toluene based counting fluor was prepared as follows:

4 liters Scintanalyzed toluene
16 g 2.5-diphenyloxazole (PPO)
0.32 g p-bis-o-methylstyryl benzene (bis-MSB)

A mixture of 2.5 ml of counting fluor and 0.5 ml Scintanalyzed 1,4-dioxane was added to the prepared tissue solution just prior to counting.

Tissue Preparation

The uterine tissue preparation for the subsequent cytoplasmic progesterone receptor assay was adapted from Walters and Clark (1977) and performed in the following manner:

1. Collect uterine tissue samples as soon after death as possible. Slice tissue into small pieces, separate the endometrium from myometrium and place the endometrium in ice cold 30% TG buffer. Tissue must be maintained at 0-4C when working with it.

2. Blot dry each endometrial slice, weigh and chop with a razor blade in a chilled petri dish. Homogenize in a precooled Downs glass homogenizer. Homogenize one slice at a time in 1-2 ml of 30% TG buffer. Use 6 to 10 strokes each for both A and B pestles.
3. Strain the homogenates through three layers of cheesecloth and pool all homogenates from all slices for each gilt. The final tissue dilution should be 50 mg tissue/ml buffer.
4. Add homogenates to 12 x 75 mm glass culture tubes and centrifuge in a precooled (4C) Beckman model TJ-6R for 15 minutes at 1800 x g (3000 rpm).
5. Decant supernatant (cytosol fraction) into 12 x 75 mm polypropylene culture tubes. If a nuclear progesterone receptor analysis is to be assayed the pellet should be saved. Centrifuge the cytosol in a precooled Beckman model J-21C at 25000 x g (16,500 rpm) for 30 minutes. Decant the supernatant and freeze for later analysis; discard the pellet.

The tissue solution may be used fresh instead of frozen if so desired. In this case, place the tissue on ice in preparation for the receptor analysis.

Receptor Analysis

Saturation analyses were performed using solutions of [^{3}H] - progesterone ranging from 1-30nM in the presence or absence of 2 µM unlabeled progesterone. Two µM of unlabeled hydrocortisol was added to correct for the glucocorticoid cross-reactive binding in those tubes in which unlabeled progesterone was absent.

A 0.25 ml aliquot of tissue solution was added to chilled (0-4 C) 12 x 75 mm culture tubes in which hormone preparations in alcohol were previously added and the alcohol was dried off. The determinations of total and nonspecific binding were always done in triplicate to yield a single average value for each binding parameter. All tubes were vortexed and allowed to incubate in the refrigerator for 12 hours.

Incubation was terminated by the addition of 0.5 ml of cold dextron-coated charcoal for 5 minutes. Each tube, maintained in an ice-water bath during exposure of the charcoal suspension, was then vortexed and centrifuged in a Beckman TJ-6R at 1800 x g (3000 rpm) for 10-15 minutes. The supernatant was then decanted into scintillation vials and 0.5 ml of 1,4-dioxane and 2.5 ml of the toluene base counting fluor was added to each vial. Scintillation vials were capped, vortexed and radioactivity determinations were made in a Searle model 6868 liquid scintillation counter (Isocap 300).

Specifically bound [^{3}H] -progesterone was determined by the subtraction of nonspecific binding ([^{3}H] -progesterone bound in the presence of excess unlabeled progeterone) from total [^{3}H] -progesterone binding (bound in the absence of unlabeled progesterone).

Protein determinations were made according to the method described by Lowry et al. (1951).

CHAPTER IV

RESULTS AND DISCUSSION

I. EVALUATION OF MATERIAL AND CONCEPTUS DATA

Treatment Effects

Forty-two of the 48 gilts bred, or 88 percent conceived. There were no significant ($P>.10$) differences among the 12 treatment-period combinations in conception rate. Of the six open gilts, four appeared normal in all respects concerning the physical appearance of the uterus and ovaries. Two of the animals were L treatment (40 units ACTH) at 6-10 days, one was L treatment 11-15 days and one was H treatment (80 units ACTH) 16-20 days. One gilt receiving H treatment 1-5 days had extensive infection within the body cavity at slaughter although the reproductive tract appeared normal. One gilt receiving H treatment 6-10 days was noted to have hydrosalpinx of the right oviduct near the ovary. The right ovary appeared nonfunctional with no corpora lutea (CL) and very small follicles; however, the left ovary appeared to be normal and remnants of degenerating placenta were found in the uterus.

Of the forty-two gilts which did conceive there was no significant ($P>.10$) difference among the twelve treatment period groups in ovulation rate recorded as corpora lutea per gilt. The average ($\bar{X} \pm SD$) number of corpora lutea per gilt for all 42 gilts was 13.0 ± 2.0.

No significant ($P>.10$) differences were noted among treatment groups or injection periods for the average number of live fetuses per gilt or the corrected fetal number per gilt (average number of live fetuses per gilt plus the average number of degenerating fetuses per

gilt or total number of fetuses, both live and dead; Table 4). There was a significant ($P<.05$) treatment by period interaction for both the average number of live fetuses and the corrected fetal number per gilt (Figure 3) but no apparent patterns could be established by treatment for any of the injection periods. The average ($\bar{X} \pm SD$) number of live fetuses and the corrected fetal number per gilt for all 42 gilts was 9.7 ± 2.8 and 11.2 ± 2.9, respectively. Results by Knight et al. (1977) showed that the number of live fetuses per gilt at day 35 of gestation was approximately 11.

Fetal survival (number of live fetuses per gilt divided by the number of CL per gilt) was not significantly ($P>.10$) different among the four injection periods (Table 4) but was significant for the treatment by period interaction ($P<.05$) as well as for treatment ($P<.10$). Once again no pattern was found between the control, L or H treatments (Table 5) or between the treatments based on the period of injection (Figure 4). The average ($\bar{X} \pm SD$) for all gilts was $74.5 \pm 18.7\%$ fetal survival and 1.5 ± 1.2 degenerating fetuses per gilt.

Perry and Rowlands (1962) found that embryonic loss in gilts at 18 days of pregnancy was 28% of the total ovulations; by date 40 only 65% of the total number of ovulations survived as viable fetuses, lower than what was found by this study. Knight et al. (1977) reported a fetal survival of 81% in 35 day old fetuses and 0.5 dead fetuses per gilt. Anderson and Parker (1976) reported a survival rate of 89% in 14-34 day old pig embryos.

No significant ($P>.10$) differences among the twelve treatment period combinations were seen for any of the conceptus measurements taken (Table 6). The fetal crown-rump length and fetal wet weight,

TABLE 4. LEAST SQUARES ANALYSIS OF VARIANCE VALUES FOR TOTAL NUMBER OF LIVE FETUSES, CORRECTED FETAL NUMBER, NUMBER OF DEGENERATING FETUSES AND PERCENT FETAL SURVIVAL PER GILT.

PARAMETER	Treatment (TRT)		(Injection) Period		TRT by Period		Error	
	DF	MS	DF	MS	DF	MS	DF	MS
Total Number of Live Fetuses per Gilt	2	10.25184	3	6.55442	6	15.96105[a]	30	6.52500
Corrected Fetal Number per Gilt	2	13.41122	3	8.64218	6	17.39668[a]	30	6.40000
Number of Degenerating Fetuses per Gilt	2	0.21453	3	2.00726	6	0.69124	30	1.86944
Fetal Survival	2	0.06365[b]	3	0.04157	6	0.07927[a]	30	0.02411

[a]Indicates significant ($P<.05$) difference was detected.

[b]Indicates significant ($P<.10$) difference was detected.

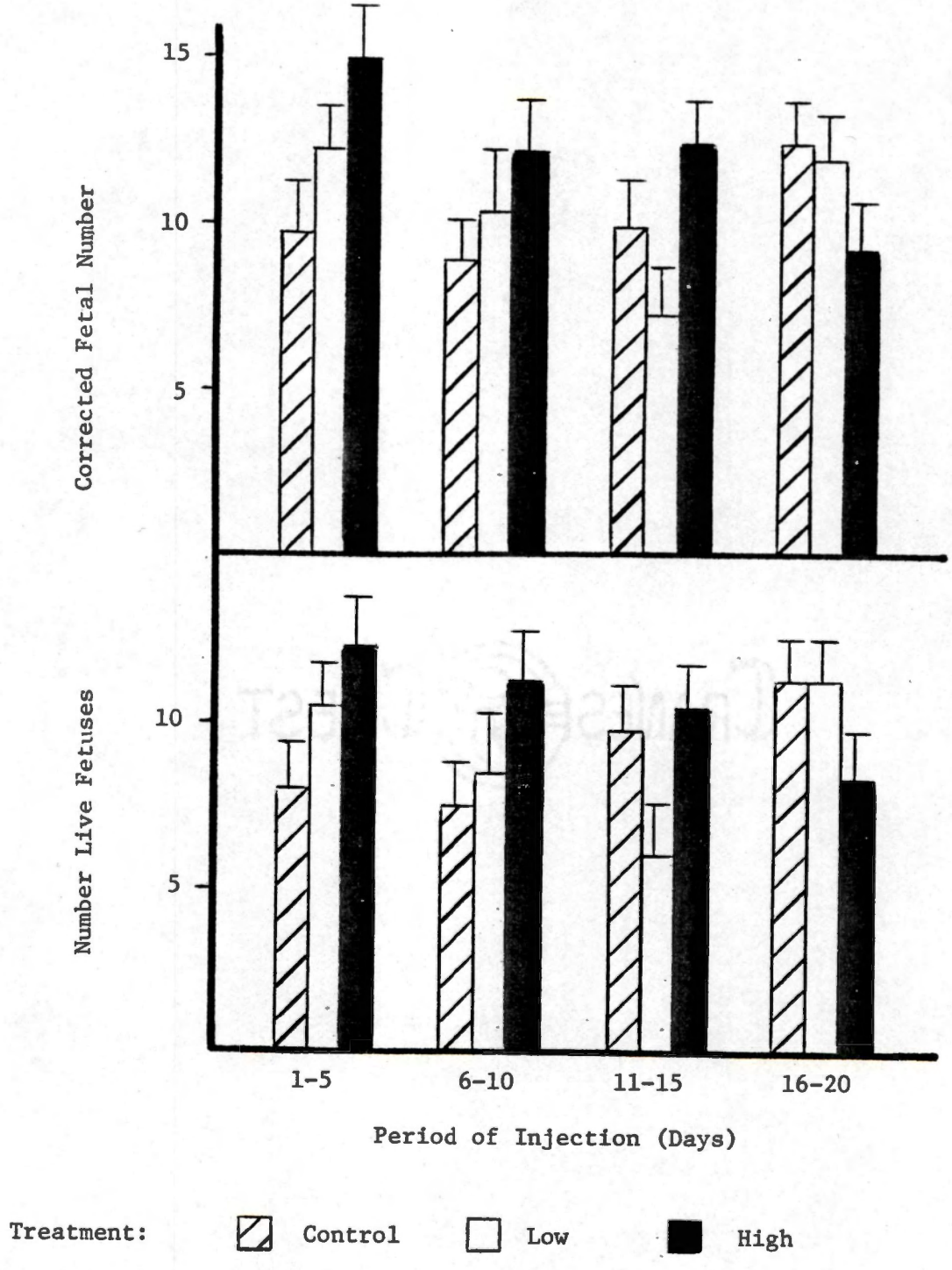


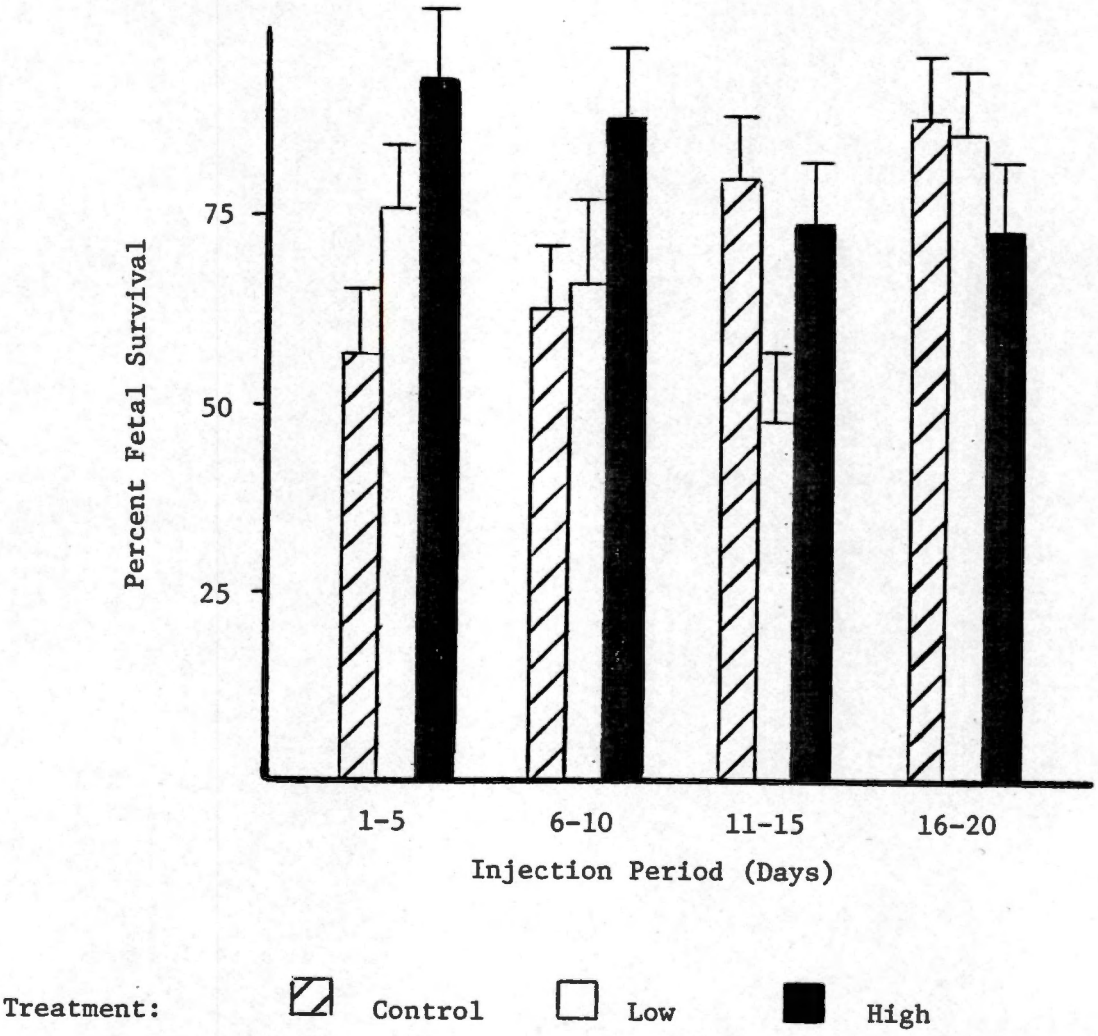
FIGURE 3. NUMBER OF LIVE FETUSES PER GILT AND CORRECTED FETAL NUMBER FOR TREATMENT-PERIOD INTERACTION ($\bar{X} \pm$ SEM).

TABLE 5. PERCENT FETAL SURVIVAL FOR CONTROL, L AND H TREATMENTS IN PREGNANT GILTS DURING EARLY GESTATION

Treatment	Percent Survival[a]
Control (0) ACTH	71.7 ± 3.9%[bc]
Low (40) ACTH	68.8 ± 4.5%[b]
High (80) ACTH	82.2 ± 4.3%[c]

[a]Total number of live fetuses per gilt divided by total number of corpora lutea per gilt x 100; $\bar{X}$ ± SEM.

[b,c]Means with different letters are significantly ($P<.05$) different.



[a]Number of Live Fetuses/Number of Corpora Lutea x 100.

FIGURE 4. PERCENT FETAL SURVIVAL[a] ($\bar{X}$ + SEM) FOR TREATMENT-PERIOD INTERACTION.

TABLE 6. LEAST SQUARES ANALYSIS OF VARIANCE VALUES FOR AVERAGE FETAL CROWN-RUMP LENGTH AND FETAL WET WEIGHT, PLACENTAL LENGTH AND WEIGHT, AMNIONIC AND ALLANTOIC FLUID VOLUMES PER GILT.

PARAMETER	Treatment (TRT)		(Injection) Period		TRT by Period		Error	
	DF	MS	DF	MS	DF	MS	DF	MS
Average Fetal Crown-Rump Length, cm	2	0.00491	3	0.01675	6	0.16481	30	0.36413
Average Fetal Wet Weight, gm	2	0.29376	3	0.62759	6	3.86519	30	6.47855
Average Placental Length, cm	2	33.90182	3	47.43595	6	31.54099	30	59.70317
Average Placental Weight, gm	2	38.56440	3	385.15146	6	33.11124	30	179.76089
Average Amnionic Fluid Volume, ml	2	1.55011	3	0.58379	6	1.64949	30	3.76319
Average Allantoic Fluid Volume, ml	2	966.69735	3	103.85383	6	405.24758	29	829.56250

averaged ($\bar{X} \pm$ SD) for 407 fetuses was 4.7 $\pm$ 0.5 cm and 8.8 $\pm$ 2.3 gm, respectively. Knight et al. (1977) found that 40 day old fetuses averaged 5.1 cm for crown-rump length and 9.4 gm for fetal wet weight. Our study also showed that average ($\bar{X} \pm$ SD) placental wet weights (66.2 $\pm$ 13.0 gm), placental length (45.0 $\pm$ 7.2 cm), allantoic fluid volume (61.2 $\pm$ 26.8 ml) and amnionic fluid volume (10.1 $\pm$ 1.7 ml) of 37 day old fetuses were similar to that reported by Knight et al (1977) for 40 day old fetuses.

Research by Edwards et al. (1968), Omtvedt et al. (1971) and Wildt et al. (1975) indicated that heat stress experienced by gestating swine the first two weeks following mating adversely affects embryo survival by reducing it 40 to 60%. Yet no significant differences were found between treated and control gilts for average embryo (fetal) wet weight and crown-rump length of viable embryos (fetuses). Their data is similar to that reported by Knight et al. (1977) for the respective embryo (fetal) ages (Table 7).

Measurements taken on the maternal reproductive tracts exhibited no significant ($P>.10$) differences with respect to treatment, injection period or treatment by injection period as indicated in Table 8. The combined adrenal gland weights showed a significant ($P<.05$) difference when analyzed by injection period (Table 8). The least square means ($\bar{X} \pm$ SEM) for adrenal weights analyzed by injection period are as follows: Days 1-5, 4.9 $\pm$ 0.3 gm; days 6-10, 5.4 $\pm$ 0.3 gm; days 11-15, 4.7 $\pm$ 0.3 gm; days 16-20, 6.0 $\pm$ 0.3 gm. Since ACTH is known to increase the weight of, and produce hypertrophy of the adrenal cortex, specifically the zona fasciculata and zona reticularis (Turner and Bagnara, 1976; Nelson, 1980) injections at 16-20 days would allow approximately 17 days after the last

TABLE 7. APPROXIMATE EMBRYO CROWN-RUMP LENGTH AND WET WEIGHT AMONG DIFFERENT EXPERIMENTS OF STRESS ON EARLY GESTATING SWINE.

Article	Embryo Age (days)	Crown-Rump Length (cm)	Embryo Weight (gm)
Warnick et al., 1965	25	1.8	0.45
Edwards et al., 1968	30-35	3.1	—
Omtvedt et al., 1971	30-36	3.3	—
Wildt et al., 1975	42	—	11
Knight et al., 1977	25	1.8	0.53
	35	3.5	3.70
	40	5.1	9.40

TABLE 8. LEAST SQUARES ANALYSIS OF VARIANCE VALUES FOR TOTAL OVARIAN WEIGHT, TOTAL UTERINE WEIGHT, TOTAL UTERINE SURFACE AREA AND TOTAL ADRENAL WEIGHT PER GILT.

PARAMETER	Treatment (TRT)		(Injection) Period		TRT by Period		Error	
	DF	MS	DF	MS	DF	MS	DF	MS
Total Ovarian Weight per Gilt, gm	2	5.76	3	5.60	6	4.76	28	3.88
Total Uterine Weight, gm	2	24783.09	3	148302.53	6	109225.14	28	81326.73
Total Uterine Surface Area, cm^2	2	793893.90	3	575456.53	6	873612.00	29	747981.65
Total Adrenal Weight per Gilt, gm	2	0.012	3	0.033[a]	6	0.005	29	0.010

[a]Indicates significant ($P<.05$) difference for period.

injection to slaughter compared to 32, 27 and 22 days for the first three injection periods. The longer time span between the last injection of the first period to the time of slaughter would allow more time for adrenal return after injections were discontinued. When adrenal weights were analyzed as a percent of the body weight no significant ($P>.10$) differences were found.

These analyses indicate that no apparent differences existed between the control, L or H treatment, the four periods in which these treatments were given, or in the treatment by period combination for any of the maternal or conceptus parameters measured. It may be that an insufficient amount of ACTH was administered to elicit an adrenal response. A corticotrophin-zinc hydroxide preparation, similar to the preparation used in this study, was tested in humans by injecting subcutaneously at levels of 40 or 80 USP units (Goldfield and Forsham, 1972). Plasma levels of ACTH showed a significant rise from baseline values for both treatment levels which peaked at 3 hours post injection. Forty units of corticotrophin produced an average increase in plasma levels of 11-hydroxycorticosteroids from a baseline of 11 µg/ml to peak value of 45 µg/ml. Eighty units produced a similar increase in 11-hydroxycorticosteroids to 56 µg/ml but prolonged the return to baseline levels by 14 hours compared to injections of 40 units.

Data from this lab for nonpregnant gilts injected with corticotrophin containing zinc hydroxide for repository action appears to indicate that injections of 40 USP units did not differ from injections of 0 USP units for plasma levels of glucocorticoids (Figure 5). Injections of 80 USP units showed peak values of 118-119 ng glucocorticoids/ml plasma at four hours postinjection on day 1 but a similar response was

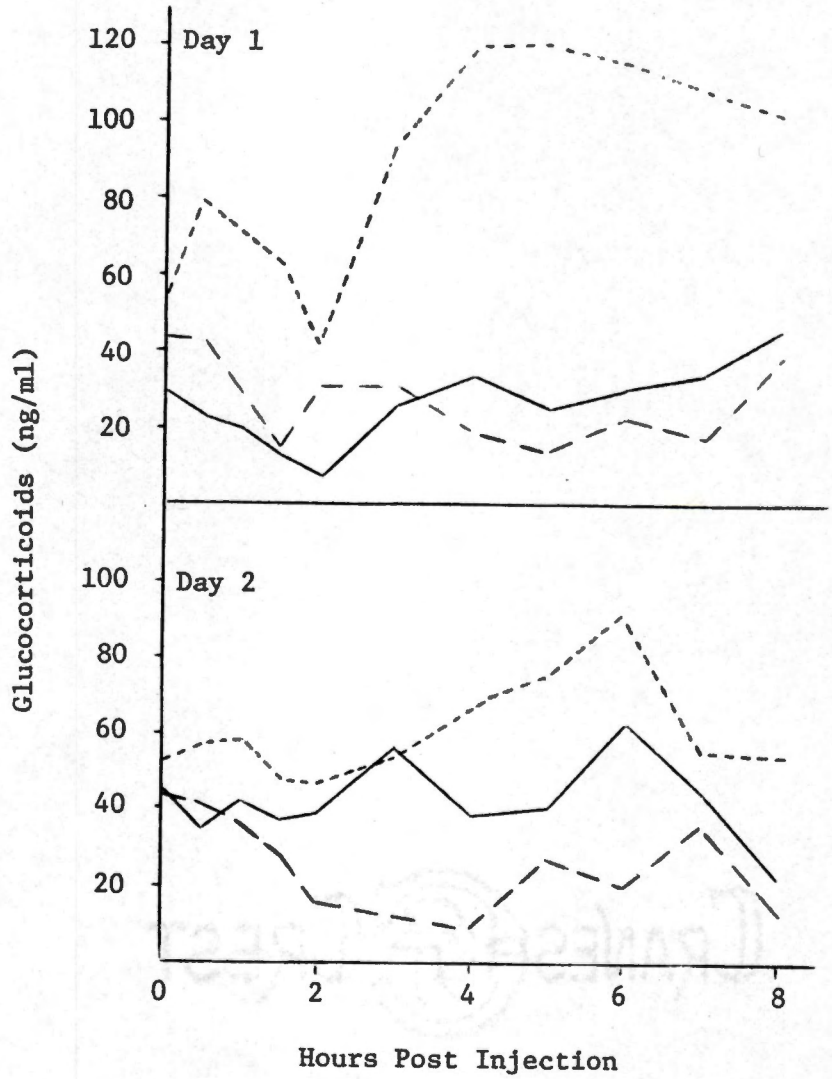


FIGURE 5. PLASMA GLUCOCORTICOID LEVELS AFTER INJECTING NONPREGNANT GILTS WITH CORTICOTROPHIN AT LEVELS OF 0, 40 OR 80 USP UNITS.

not seen on day 2. This pattern is similar to that shown by Aberle et al. (1974) when heat stress was introduced to swine and plasma corticoids were measured.

Also, it is possible that the relatively high temperatures which were prevalent at the time this experiment was conducted (average high temperature, 85.9 ± 1.4F; average low temperature, 61.5 ± 0.8F; average relative humidity, 800.0 ± 0.9%; $\bar{X}$ ± SEM)[2] may have already produced a maximal adrenal response in the gilts which subsequently depleted the glucocorticoid stores. Aberle et al. (1974) demonstrated that plasma adrenal corticoids increased significantly during heat stress, but when the stress was repeated the pattern of corticoid release was not repeated and plasma adrenal corticoids remained low.

Uterine Positional Differences on Conceptus Measurements

The analysis of the pooled conceptus showed a significant ($P<.05$) difference among the three positions for placental length (table 9). Placental length at the utero-tubal position (46.92 ± 1.24 cm; $\bar{X}$ ± SEM) was significantly ($P<.05$) longer than that of the cervical portion (42.53 ± 1.24 cm). No difference was found for placental length between the cervical or utero-tubal portions and the middle (44.22 ± 1.28 cm; $\bar{X}$ ± SEM) portion of the uterus. Allantoic fluid volume and amnionic fluid volume were significantly ($P<.01$) correlated ($r = .32$ and .24, respectively) to placental length. It has been shown that a rapid increase in allantoic fluid volume and placental length occurs between days 20 to 30 and again days 40 to 60 of gestation in swine (Knight,

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[2]Weather data for Aug. 8 to Oct. 8, 1980, was provided by U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Atmospheric Turbulence and Diffusion Laboratory, Oak Ridge, TN.

Table 9. DIFFERENCES BY POSITION IN THE UTERUS FOR PLACENTAL LENGTH AND FETAL WET WEIGHTS.

Position[a]	Placental Length, cm[b]	Fetal Wet Weight, g[b]
Utero-tubal	46.9 ± 1.2[c]	8.71 ± 0.10[e,f]
Middle	44.2 ± 1.3[c,d]	8.97 ± 0.10[e]
Cervical	42.5 ± 1.2[d]	8.69 ± 0.10[f]

[a]Position within the uterus.

[b]$\bar{X}$ ± SEM

[c,d]Means with different letters are significantly (P<.05) different.

[e,f]Means with different letters are significantly (P<.10) different.

et al., 1977). The early increase of allantoic fluid volume was associated with expansion of the chorioallantoic membranes, increasing placental surface area and number of areola which makes intimate contact with uterine endometrium. Placental development preceeds fetal development and under conditions where placental development is limited, fetal survival may be reduced (Knight et al., 1974; Knight et al., 1977).

Placental weight did not differ significantly ($P>.10$) according to uterine position in 37 day old pig fetuses but was highly ($P<.01$) correlated ($r = .71$) with placental length. McLaren (1965) found significant differences in placental weight according to uterine position in mice; placentas nearer the ovarian end were heavier and weights decreased toward the cervical end of the uterine horn. LeGault and Leuillet (1973) found that placenta weights were higher for conceptuses located nearer the utero-tubal and cervical ends of the uterine horn by day 30 of pregnancy. This agrees with work done by Waldorf et al. (1957) with fetuses of 102-108 days gestation.

Perry and Rowell (1969) working with swine found that position had no effect on fetal wet weights when there were five or fewer fetuses in the horn, but as the number increased above five those at the extreme ends tended to have an increasing advantage with those at the utero-tubal end weighing slightly more than those at the cervical end. These differences remained constant throughout the period of 31 to 113 days of gestation. These findings are similar to results reported by Waldorf et al. (1957). In contrast to the findings of Perry and Rowell (1969), the results of the present study suggest that 37 day old fetuses tend ($P<.10$) to be heavier toward the middle of the uterine horn compared to the utero-tubal and cervical ends (Table 9). We can offer no explanation for the contrast with other authors on fetal wet weights.

McLaren (1965) and others have suggested that hemodynamic factors may be responsible for differences seen in fetal and placental growth according to position. It was thought that the anatomical arrangement of the blood vessels, the uterine artery feeding in near the cervix and the ovarian artery supplying the uterus beginning from the utero-tubal region, positively affected conceptus growth and those conceptuses in the middle portion may be somewhat deprived. Results from Perry and Rowell (1969) suggested that differences in porcine fetal weights between positions could not be explained in terms of the uterine vascular architecture. Research by Ford and Christenson (1979) shows that the presence of conceptuses in the uterus of sows triggers a significant increase in uterine blood flow beginning on days 12 to 13 of gestation (pregnancy recognization in swine; Dhindsa and Dziuk, 1968) but no association was found between the number of embryos in a uterine horn and the magnitude of blood flow to that horn during the first 30 days of pregnancy.

Our study indicated no significant ($P>.10$) differences according to position for fetal crown-rump length, but fetal crown-rump length was significantly ($P<.001$) correlated ($r = .95$) with fetal wet weight. Knight et al. (1977) found that fetal wet weight was highly correlated with fetal crown-rump length ($r = .92$) throughout gestation.

In swine (Waldorf et al., 1957) and in mice (McLaren, 1965) a positive correlation was found between fetal and placental wet weights but fetal and placental weights were inversely related to litter size. Conceptuses of 12 and 18 days of age in the utero-tubal area of the uterine horn in swine contained more protein than conceptus in other areas of the uterus (Anderson, 1978) but days 14 to 34 nitrogen content of fetuses and placenta did not differ according to position (Anderson

and Parker, 1976). As the number of embryos in a horn increased the proportion of nitrogen for each embryo remained similar. Weight differences of fetuses and placentas according to position are thus due to water content and not total protein content.

Data from the present study shows that allantoic fluid volume was greatest at the middle portion of the uterine horn compared to the utero-tubal portion (Table 10), and was negatively correlated ($P<.01$) with fetal wet weight ($r = -.25$) and fetal crown-rump length ($r = -.33$). Amnionic fluid volume was not significantly ($P>.10$) different according to position (Table 10), but was positively correlated ($P<.001$) with fetal wet weight ($r = .64$) and fetal crown-rump length ($r = .64$). Knight et al. (1977) found that allantoic fluid volume increased rapidly between days 20 to 30 of gestation, decreased to day 40 and increased again to day 60 where it decreased through the remainder of gestation in the pig. Amnionic fluid volume increases from days 30 to 70, while fetal wet weight and fetal crown-rump length increase as the fetus grows. This explains why our study showed a negative correlation for allantoic fluid volume and fetal growth parameters, while amnionic fluid volume displayed a positive correlation with fetal wet weight and crown-rump length at 37 days gestation.

The number of live fetuses per position was not significantly ($P>.10$) different. All gilts averaged ($\bar{X} \pm SD$) 3.3 ± 1.1 fetuses per position for both uterine horns. The number of degenerating fetuses per position was significant ($P<.05$); a greater number of degenerating fetuses were found at the middle position of the uterine horn (Table 10). Perry and Rowell (1969) found that fetuses in crowded horns had a smaller chance of survival. A negative correlation has been shown between litter

TABLE 10. DIFFERENCE BY POSITION IN THE UTERUS FOR ALLANTOIC FLUID VOLUME, AMNIONIC FLUID VOLUME AND NUMBER OF DEGENERATING FETUSES.

Position[a]	Allantoic Fluid Volume, ml[b]	Degenerating Fetal Number[b]	Amnionic Fluid Volume, ml[b]
Utero-tubal	62.8 ± 2.5^{c}	0.31 ± 0.10^{c}	10.1 ± 0.2^{c}
Middle	72.8 ± 2.6^{d}	0.69 ± 0.10^{d}	10.0 ± 0.2^{c}
Cervical	$65.9 \pm 2.5^{c,d}$	$0.43 \pm 0.10^{c,d}$	10.2 ± 0.2^{c}

[a]Position within the uterus.

[b]$\bar{X} \pm$ SEM

[c,d]Means with different letters are significantly ($P<.05$) different.

size and percent survival in swine (Waldorf et al., 1957) and mice (McLaren, 1965).

This study comprises a time in fetal pig development when placental length and weight, fetal length and weight and amnionic fluid volume are progressively increasing while allantoic fluid volume is decreasing (Knight et al., 1977). The average ($\bar{X} \pm SD$) number of live fetuses per uterine horn for this study was 4.9 ± 1.6, with an average ($\bar{X} \pm SD$) per gilt of 9.7 ± 2.8. Although there was not a significant difference among the three positions for number of live fetuses, there were more degenerating fetuses in the middle portion. This suggests that possibly greater fetal growth is taking place and crowded conditions within the middle of the uterus may be fatal to some fetuses. Placental lengths were significantly longer and the number of degenerating fetuses was lower in the utero-tubal portion of the uterine horns, indicating that crowding was not as much of a problem at this portion.

Anderson (1978) found that in early development of the pig, spacing of the embryos was proportional regardless of the number found in the horn, and no overlap occurred. Webel and Dziuk (1974) indicated that embryonic mortality before day 30 in the pig was not associated with limited intrauterine space, but after day 30 it may be a factor in fetal death, especially when large numbers of fetuses are present or when intrauterine space is restricted. This also agrees with research by Monk and Erb (1974). Data by Knight et al. (1977) suggests that placental insufficiency was the cause of increased fetal death after day 35 of gestation in the pig.

II. EVALUATION OF PROGESTERONE RECEPTOR ANALYSIS

The incubation time used in this study for the cytoplasmic progesterone receptor analysis was 12 hours. Figure 6 shows that peak values for specific binding appears to occur at 10 hours of incubation time and is stable for the subsequent 4 hours after which time specific counts bound decreases. Walters and Clark (1977) found that specific binding for rat uterine tissue was complete by 4 hours of incubation and remained stable for the following 44 hours.

A saturation curve for specific counts bound was drawn for data from which 12.5 mg of tissue in 250 µl of 30% TG buffer was incubated with ^{3}H-progesterone at concentrations ranging from 1-28 nM and 2 µM of unlabeled progesterone (Figure 7). The values for specific binding were much lower than those shown by Walters and Clark (1977) but the indications are similar in that saturation appears to be complete with 15 nM of ^{3}H-progesterone and single concentration analyses could be carried out using concentrations of ^{3}H-progesterone ranging from 16 to 20 nM. This analysis reveals that an average of 4.6 pM of specifically bound ^{3}H-progesterone occurred per 1 mg of porcine uterine endometrium (Table 11 and Figure 8) or 0.11 pM of specifically bound ^{3}H-progesterone occurred per µg of protein (Table 11 and Figure 9).

A Scatchard analysis of our data yielded a K_d of 26.3 pM and a binding capacity of 3.4 pmol/mg of tissue. Walters and Clark (1977) found that uterine tissue of estrogen primed rats yielded a K_d of 3.3 nM while Diekman and Anderson (1979) working with porcine endometrium reported a K_d of 1.9 nM and a binding capacity of 1.0 fmol/mg tissue. This is an indication that more work needs to be done before using this

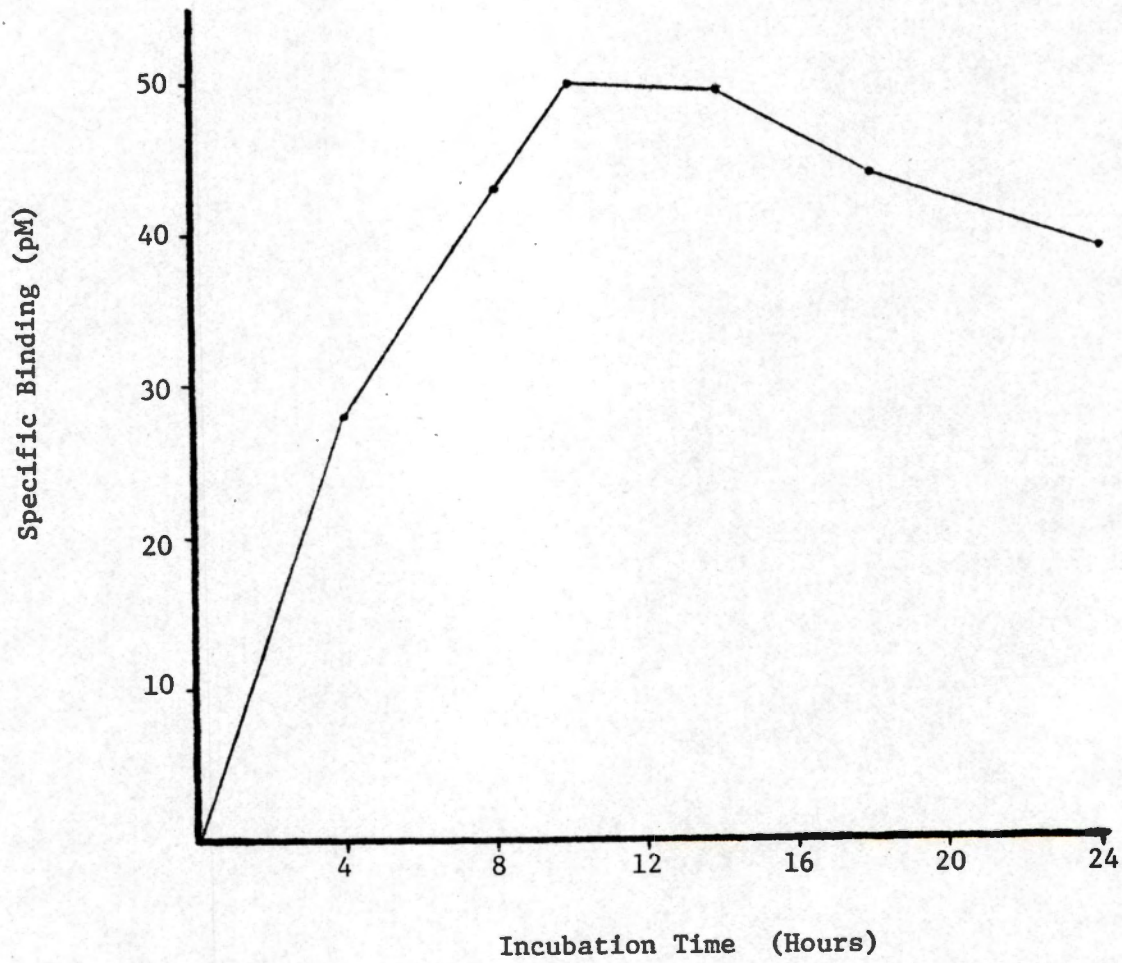

FIGURE 6. THE EFFECT OF INCUBATION TIME ON THE AMOUNT OF SPECIFICALLY BOUND ^{3}H-PROGESTERONE (pM).

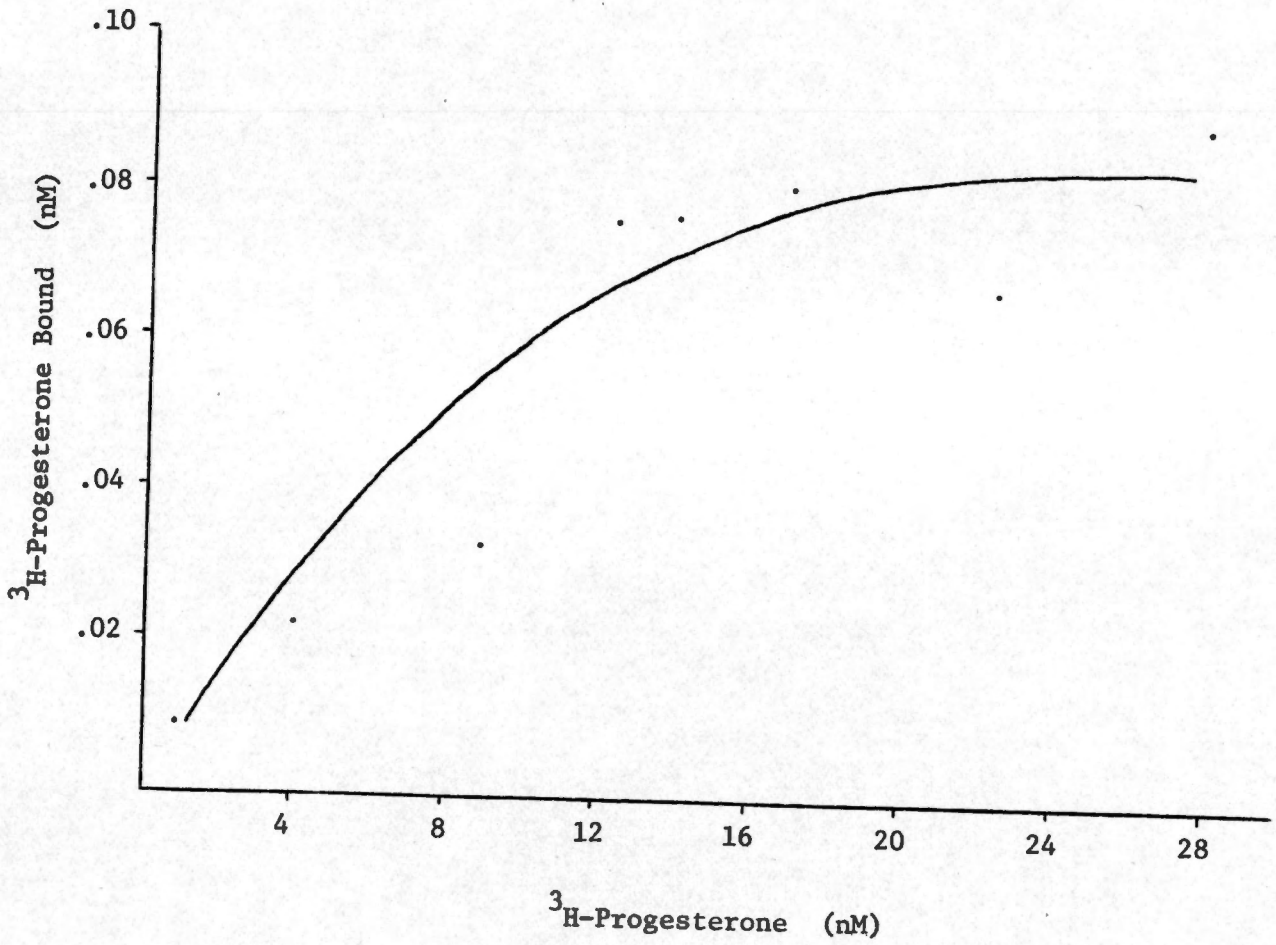

FIGURE 7. SATURATION CURVE DEPICTING AMOUNT OF SPECIFICALLY BOUND ^{3}H-PROGESTERONE PER TOTAL AMOUNT ^{3}H-PROGESTERONE ADDED.

TABLE 11. THE AMOUNT OF TISSUE (mg) AND PROTEIN (µg) ANALYZED, THE TOTAL VALUES FOR ^{3}H-PROGESTERONE SPECIFICALLY BOUND AND ^{3}H-PROGESTERONE SPECIFICALLY BOUND PER mg OF TISSUE OR PER µg OF PROTEIN IN SOLUTION.

Tissue, mg	Protein, µg	^{3}H-Progesterone Specifically Bound, pM	^{3}H-Progesterone Specifically Bound Per mg Tissue[a] (pM/mg)	^{3}H-Progesterone Specifically Bound Per µg Protein[b] (pM/µg)
50.0	2041	240.2	4.8	.12
25.0	1014	107.8	4.3	.11
12.5	522	53.6	4.3	.10
6.25	257	32.8	5.2	.13
3.125	128	14.6	4.7	.11

[a] $\bar{X}$ = 4.6 pM ^{3}H-progesterone specifically bound/mg tissue.

[b] $\bar{X}$ = .11 pM ^{3}H-progesterone specifically bound/µg protein.

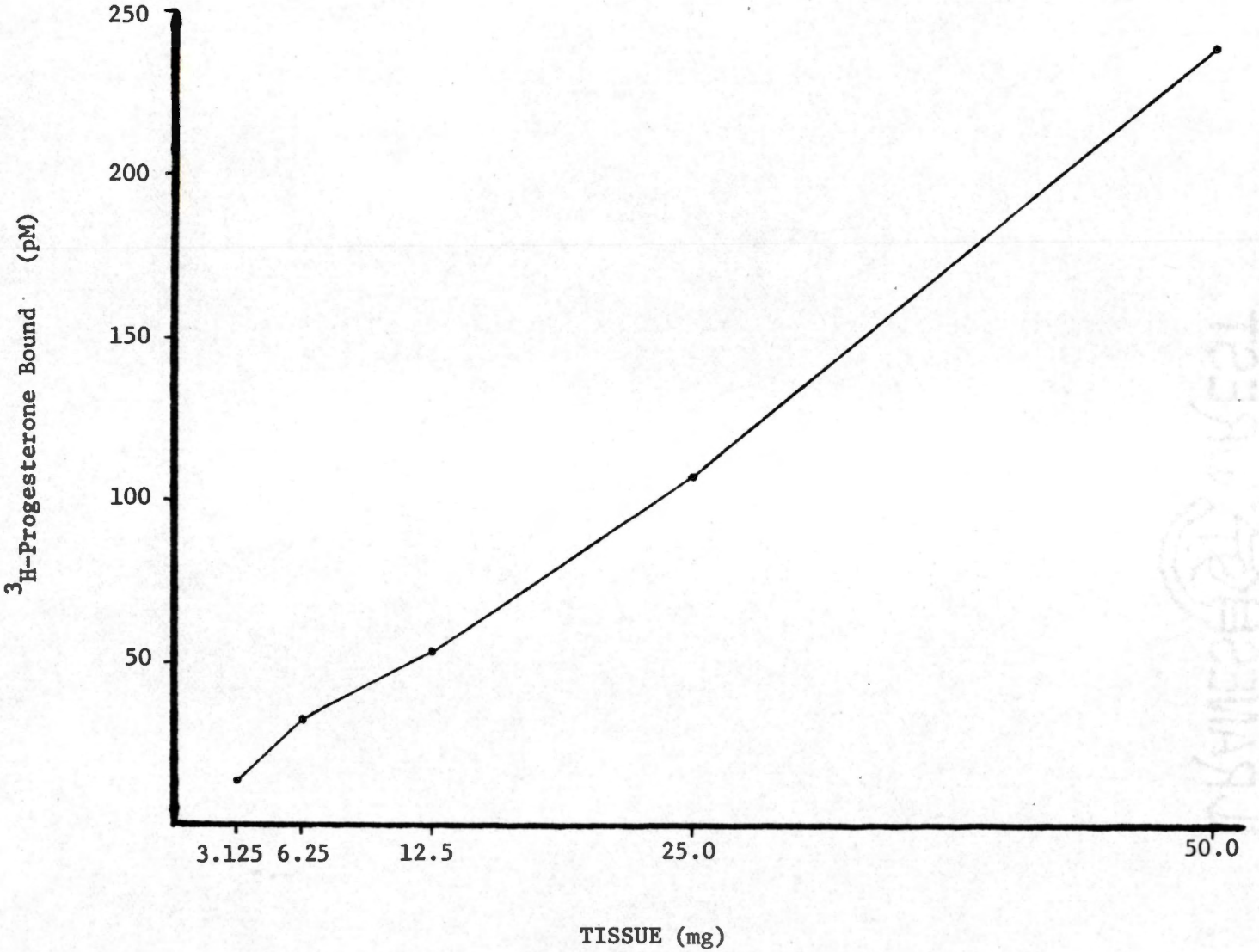

FIGURE 8. ^{3}H-PROGESTERONE SPECIFICALLY BOUND TO VARIOUS AMOUNTS OF TISSUE (mg).

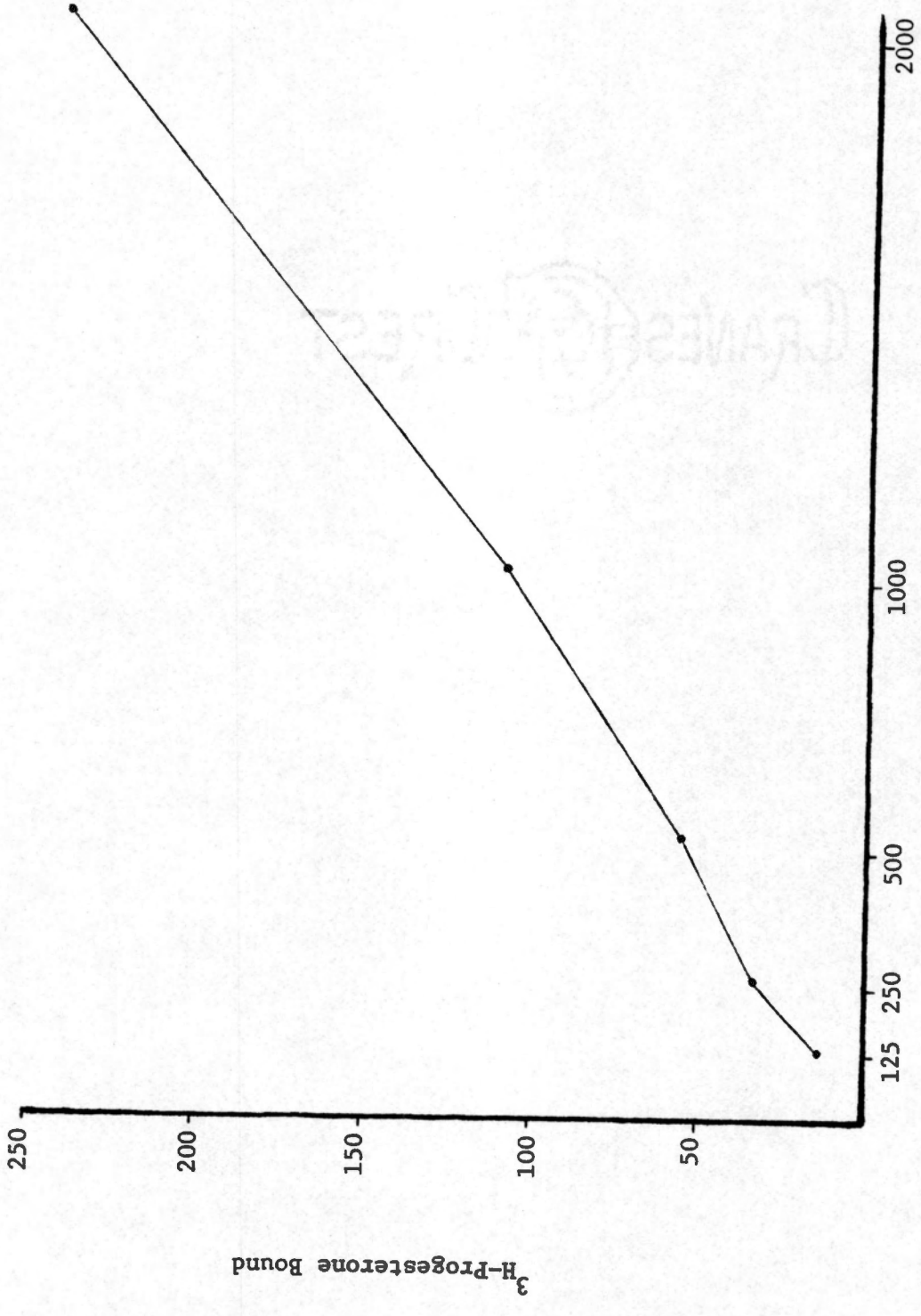

FIGURE 9. ^{3}H-PROGESTERONE SPECIFICALLY BOUND TO VARIOUS AMOUNTS OF PROTEIN (µg).

analysis. It is possible that using radiolabeled R5020 instead of ^{3}H-progesterone may improve the specific binding. Also more binding may be realized by homogenizing both myometrium and endometrium first in a Waring blender and then in a Downs glass homogenizer after which it would be filtered through cheesecloth.

Cytoplasmic progesterone receptors were not quantitated in the harvested uterine endometrial tissue from gilts injected with control or ACTH treatments because of the lack of a valid assay procedure, poor techniques in collection and preparation of the tissue (unknown to us at the time of collection) and finally, the lack of differences found between the various treatments in terms of fetal survival and other parameters measured.

Milgrom and Baulieu (1968; 1970) were the earliest to report on finding a specific protein receptor for progesterone in the uterus of rats. This receptor contains chemical and biological characteristics similar to those of transcortin but could be separated from plasma proteins by density gradient centrifugation. The receptor is characterized by thermolability, sensitivity to a sulfhydryl-blocking agent and a high affinity for progesterone (Milgrom et al., 1970; Leavitt et al., 1974; Walters and Clark, 1977).

The subcellular distribution of the progesterone receptor has been described in the uterus of the guinea pig (Milgrom et al., 1973; Feil and Bardin, 1975; Saffran et al., 1976). Progesterone binds to cytoplasmic receptors and is translocated to the nucleus. Stormshak (1978) gives a good general discussion on this. This phenomenon is observed as a concomitant cytosol depletion and nuclear accumulation of receptors. Cytoplasmic receptor replenishment was not observed 24 hours

after progesterone injection as is the case with estrogen receptors in estrogen primed rats.

Walters and Clark (1978) provided evidence to indicate that estrogen stimulates the synthesis of its own receptors as well as increasing concentrations of progesterone receptors in the cytoplasm of rat uterine endometrium. In contrast progesterone interferes with mechanisms involved in replenishment of cytoplasmic estrogen and progesterone receptors. This agrees with work done in ewes quantitating numbers of estrogen and progesterone receptors in the cytoplasm of uterine endometrium throughout gestation (Miller et al., 1977). Maximal concentrations of both were found during estrus and the following 2 to 5 days corresponding to high levels of plasma estrogen and low plasma levels of progesterone. Subsequently numbers of both estrogen and progesterone receptors declined to low levels on day 14 of the estrous cycle in conjunection with high plasma progesterone concentration and low estrogen levels in the blood. In contrast, Diekman and Anderson (1979) found no differences in cytoplasmic and nuclear concentrations of progesterone and estrogen receptors throughout the estrous cycle and on day 30 of gestation in the pig. Brodie and Green (1978) found that the concentration of cytoplasmic progesterone receptors was high on day one of pregnancy and dropped to low levels by day 3 of gestation and remained so through day 10 of pregnancy in the rat.

Puri and Roy (1980) found that in rabbit oviducts, nuclear progesterone receptor numbers were steady at 14 hours postcoitum in the ampulla then decreased, while values for the ampulla-isthmic junction were low until 24 hours postcoitum and then increased. Values for nuclear receptor numbers remained unaltered in the isthmus until 70 hours

postcoitum after which the concentration increased. It was concluded that the increased effects of progesterone is required for transport of the egg through the oviduct.

Logeat et al. (1980) found that the numbers of cytoplasmic and nuclear progesterone receptors at nidation sites in endometrium of the pregnant rat was twice the number found at sites of fetal nonattachment. Atkins et al. (1980) found that the number of nuclear progesterone receptor sites per endometrial cell from the uterine horn ipsilateral to the corpus luteum in gestating cows was twice the number found in endometrium of the uterine horn contralateral to the corpus luteum.

Ogle (1980) has provided evidence that progesterone receptors are present in cytosol of rat placental tissue on day 12 of pregnancy. This strengthens the hypothesis that the action of progesterone on the maintenance of placental function may be mediated (via mechanisms consistent with the general concept of steroid hormone action) within the cell of placental tissue as well as regulating uterine secretions that affect conceptus growth and development.

CHAPTER V

SUMMARY AND CONCLUSIONS

The first objective of this study was to determine the effects of exogenous ACTH upon maternal and conceptus parameters in pregnant swine during early gestation. A second objective was to validate an assay for cytoplasmic progesterone receptor in order to quantitate levels in uterine endometrial tissue from pregnant gilts receiving injections of ACTH.

Forty-eight pregnant gilts were randomly allotted in a 3 x 4 factorial design to one of three treatments and one of four injection periods. Treatments consisted of a single, daily, intramuscular injection of either 0, 40 or 80 USP units of corticotrophin in an aqueous suspension containing zinc hydroxide for repository action for a period of five days. The injection periods were 1-5, 6-10, 11-15 or 16-20 days of gestation with day one corresponding to 48 hours after estrus detection.

Of the 48 gilts bred, 42 conceived for an overall conception rate of 87.5%. There were no significant ($P>.10$) differences in conception rate among any of the 12 treatment-period groups. Injections of ACTH, regardless of dosage or period given, had no apparent effects on fetal survival or any of the maternal or conceptus parameters measured.

Conceptus data were then pooled and analyzed for differences according to position within the uterus. The uterine positions analyzed were the utero-tubal portion, the middle portion and the cervical portion of the uterus. Placental length was found to be significantly ($P<.05$) longer in the utero-tubal portion (46.9 $\pm$ 1.2 cm; $\bar{X} \pm$ SEM) than in the

cervical portion (42.5 ± 1.2 cm). No significant difference was found between placental lengths in the middle portion (44.2 ± 1.3 cm; $\bar{X}$ ± SEM) and the utero-tubal or cervical portions. Allantoic fluid volume and number of degenerating fetuses were significantly ($P<.05$) greater in the middle portion (72.8 ± 2.6 ml and 0.69 ± 0.10, respectively; $\bar{X}$ ± SEM) compared to the utero-tubal portion (62.8 ± 2.5 ml and 0.31 ± 0.10, respectively). Allantoic fluid volume and degenerating fetal numbers from the cervical portion (65.9 ± 2.5 ml and 0.43 ± 0.10, respectively; $\bar{X}$ ± SEM) was not significantly different from those measurements in the other two positions. Fetal wet weights located in the middle portion (8.97 ± 0.10 gm; $\bar{X}$ ± SEM) tended ($P<.10$) to be heavier than fetal wet weights from the cervical portion (8.69 ± 0.10 gm). Fetal wet weights from the utero-tubal portion (8.71 ± 0.10 gm) was not significantly different from the other uterine positions.

A cytoplasmic progesterone receptor analysis was developed to analyze receptors in uterine endometrial tissue from swine. In the validation of this assay, a saturation curve revealed that 12.5 mg of tissue in 250 µl of 30% TG buffer was saturated in a concentration of 15 to 20 nM ^{3}H-progesterone for an incubation period of 10 to 14 hours. A Scatchard analysis indicated a K_d of 26.3 pM and a binding capacity of 3.4 pmol/mg tissue.

LITERATURE CITED

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Aberle, E. D., R. A. Merkel, J. C. Forrest and C. W. Alliston. 1974. Physiological response of stress susceptible and stress resistant pigs to heat stress. J. Anim. Sci. 38:954.

Alliston, C. W., Gene E. Egli and L. C. Ulberg. 1961. Loss of potential young in the ewe due to high ambient temperature. J. Applied Physiol. 16:253.

Alliston, C. W. and L. C. Ulberg. 1961. Early pregnancy loss in sheep at ambient temperatures of 70° and 90°F as determined by embryo transfer. J. Anim. Sci. 20:608.

Anderson, L. L. 1978. Growth, protein content and distribution of early pig embryos. Anat. Rec. 190:143.

Anderson, L. L. and R. O. Parker. 1976. Distribution and development of embryos in the pig. J. Reprod. Fert. 46:363.

Atkins, D. T., D. C. Kraemer, P. G. Harms and J. L. Fleeger. 1980. Progesterone-receptor complex in the nuclear fraction of the bovine endometrium. Biol. Reprod. 23:317.

Austin, C. R. and A. W. H. Braden. 1954. Induction and inhibition of the second polar division in the rat egg and subsequent fertilization. Aust. J. Biol. Sci. 7:195.

Bazer, Fuller W. 1975. Uterine protein secretions: relationship to development of the conceptus. J. Anim. Sci. 41:1376.

Benjaminsen, E. and T. Lunaas. 1980. Plasma progesterone increments in sows treated with ACTH. Acta Vet. Scand. 21:143.

Boyd, Hugh, Paul Bacsich, Archibald Young and J. A. McCracken. 1969. Fertilization and embryonic survival in dairy cattle. Br. Vet. J. 125:87.

Brodie, James Y. and Brian Green. 1978. Progesterone binding in rat uterine cytosol during early pregnancy. Biochem. Soc. Trans. (London) 6:773.

Brunner, M. A., L. E. Donaldson and William Hansel. 1969. Exogenous hormones and luteal function in hysterectomized and intact heifers. J. Dairy Sci. 52:1849.

Chen, T. T., Fuller W. Bazer, B. M. Gebhardt and R. M. Roberts. 1975. Uterine secretions in mammals: synthesis and placental transport of a purple acid phosphatase in pigs. Biol. Reprod. 13:304.

Christian, John J. 1955. Effect on population size on the adrenal glands and reproductive organs of male mice in populations of fixed size. Am. J. Physiol. 182:292.

Christian, John J. 1964. Effect of chronic ACTH treatment on maturation of intact female mice. Endocr. 74:669.

Christian, John J. and Cobert D. Lemunyan. 1958. Adverse effects of crowding on lactation and reproduction of mice and two generations of their progeny. Endocr. 63:517.

Christian, John J., James A. Lloyd and David E. Davis. 1965. The role of endocrines in the self-regulation of mammalian populations. Recent Prog. Horm. Res. 21:501.

Clarke, I. J., H. M. Fraser and A. S. McNeilly. 1978. Active immunization of ewes against luteinizing hormone releasing hormone, and its effects on ovulation and gonadotrophin, prolactin and ovarian steroid secretion. J. Endocr. 78:39.

Close, R. W. and R. M. Liptrap. 1975. Plasma progesterone levels in sows with induced cystic ovarian follicles. Res. Vet. Sci. 19:28.

Coggins, E. G. and N. L. First. 1977. Effect of dexamethisone, methallibure and fetal decapitation on porcine gestation. J. Anim. Sci. 44:1041.

Collins, K. J. and J. S. Weiner. 1968. Endocrinological aspects of exposure to high environmental temperatures. Physiol. Rev. 48:785.

Dhindsa, D. S. and P. J. Dziuk. 1968. Effect on pregnancy in the pig after killing embryos or fetuses in one uterine horn in early gestation. J. Anim. Sci. 27:122.

Diekman, M. A. and L. L. Anderson. 1979. Quantitation of nuclear and cytoplasmic receptors for estradiol 17β, and progesterone in the uterus, hypothalmus and pituitary of the pig throughout the estrous cycle and day 30 of pregnancy. Biol. Reprod. 20 (Suppl 1):14A.

Dunlap, S. E. and C. K. Vincent. 1971. Influence of postbreeding thermal stress on conception rate in beef cattle. J. Anim. Sci. 32:1216.

Duncan, D. B. 1955. Multiple range and F tests. Biometrics 11:1.

Dupouy, Jean-Paul, Alain Chatelain and Patrick Allume. 1980. Absence of transplacental passage of ACTH in the rat: direction experimental proof. Biol. Neonate 37:96.

Dutt, R. H. 1963. Critical period for early embryo mortality in ewes exposed to high ambient temperature. J. Anim. Sci. 22:713.

Dutt, R. H., Earl F. Ellington and William C. Carlton. 1959. Fertilization rate and early embryo survival in sheared and unsheared ewes following exposure to elevated air temperature. J. Anim. Sci. 18:1308.

Edwards, M. J. 1968. Congenital malformations in the rat following induced hyperthermia during gestation. Teratology 1:173.

Edwards, M. J. 1969. Congenital defects in guinea pigs: fetal resorptions, abortions and malformations following induced hyperthermia during early gestation. Teratology 2:313.

Edwards, R. L., I. T. Omtvedt, E. J. Turman, D. F. Stephens and G. W. A. Mahoney. 1968. Reproductive performance of gilts following heat stress prior to breeding and in early gestation. J. Anim. Sci. 27:1634.

Ensor, D. M. 1978. Comparative Endocrinology of Prolactin. Chapman and Hall Ltd, London.

Euker, Jeans. and Gail D. Riegle. 1973. Effects of stress on pregnancy in the rat. J. Reprod. Fert. 34:343.

Facchinetti, F., P. Inaudi and A. R. Genazzani. 1980. Adrenal response to ACTH in hyperprolactinemic amenorrhea: effect of bromocryptine treatment. pp 95. In A. R. Genazzani, J. H. H. Thijssen and P. K. Siiteri (Ed.) Adrenal Androgens, Raven Press, New York.

Feil, P. D. and C. W. Bardin. 1975. Cytoplasmic and nuclear progesterone receptors in the guinea pig uterus. Endocr. 97:1398.

Fernandez-Cano, Luis. 1958. Effects of increase or decrease of body temperature and hypoxia on pregnancy in the rat. Fert. and Steril. 9:455.

First, N. L. and Michel J. Bosc. 1979. Proposed mechanisms controlling parturition and the induction of parturition in swine. J. Anim. Sci. 48:1407.

First, N. L. and R. B. Staigmiller. 1973. Effects of ovariectomy, dexamethasone and progesterone on the maintenance of pregnancy in swine. J. Anim. Sci. 37:1191.

Ford, S. P. and R. K. Christenson. 1979. Blood flow to uteri of sows during the estrous cycle and early pregnancy: local effect of the conceptus on the uterine blood supply. Bio. Reprod. 21:617.

Fried, D. A., R. L. Brent and R. P. Jensh. 1970. The developmental effects of elevated uterine temperature in the rat. Teratology 3:200.

Goldfield, Edythe B. and Peter H. Forsham. 1972. Pharmacodynamics of repository adrenocorticotropic hormone preparations in man. Arch. Derm. 106:828.

Goodnight, James W. 1979. GLM procedure reference. pp 245. In Janet T. Helwig and Kathryn A. Council (Ed.) SAS User's Guide, SAS Institute Inc., Cary, North Carolina.

Hagino, Nobuyoshi, Mitsutoshi Watanabe and Joseph W. Goldzieher. 1969. Inhibition by adrenocorticotrophin of gonadotrophin-induced ovulation in immature female rats. Endocr. 84:308.

Hancock, J. L. and G. J. R. Hovell. 1959. The collection of boar semen. Vet. Rec. 71:664.

Heitman, Hubert, J., E. H. Hughes and C. T. Kelly. 1951. Effects of elevated ambient temperatures on pregnant sows. J. Anim. Sci. 10:907.

Howarth, B., Jr. and H. W. Hawk. 1968. Effect of hydrocortisone on embryonic survival in sheep. J. Anim. Sci. 27:117.

Ingrahm, R. H., D. D. Gillette and W. D. Wagner. 1974. Relationship of temperature and humidity to conception rate of holstein cows in subtropical climate. J. Dairy Sci. 57:476.

Jarrett, R. J. 1965. Effects and mode of action of adrenocorticotrophic hormone upon the reproductive tract of the female mouse. Endocr. 76:434.

Jones, D. L., H. S. Jacobs and V. H. T. James. 1980. The relationship between plasma prolactin and dehydroepiandrosterone and dehydroepiandrosterone sulphate levels in patients with hyperprolactinemia. pp 83. In A. R. Genazzani, J. H. H. Thijssen and P. K. Siiteri (Ed.), Adrenal Androgens, Raven Press, New York.

Kattesh, H. G., E. T. Kornegay, J. W. Knight, F. G. Gwazdauskas, H. R. Thomas and D. R. Notter. 1980. Glucocorticoid concentrations, corticosteroid binding protein characteristics and reproduction performance of sows and gilts subjected to applied stress during mid-gestation. J. Anim. Sci. 50:897.

Kittinger, J. W., R. M. Gutierrez-Cernosek, S. F. Cernosek, Jr. and J. N. Pasley. 1980. Effects of adrenocorticotropin on pregnancy and prolactin in mice. Endocr. 107:616.

Knight, J. W., Fuller W. Bazer, W. W. Thatcher, D. E. Franke and H. D. Wallace. 1977. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J. Anim. Sci. 44:620.

Knight, J. W., Fuller W. Bazer and H. D. Wallace. 1973a. Porcine conceptus response to progesterone therapy. J. Anim. Sci. 36:211.

Knight, J. W., Fuller W. Bazer and H. D. Wallace. 1973b. Porcine uterine response to progesterone therapy. J. Anim. Sci. 36:211.

Knight, James W., Fuller W. Bazer and H. D. Wallace. 1974. Effect of progesterone induced increase in uterine secretory activity on development of the porcine conceptus. J. Anim. Sci. 39:743.

Knight, James W., Fuller W. Bazer, H. D. Wallace and C. J. Wilcox. 1974. Dose-response relationships between exogenous progesterone and estradiol and porcine uterine protein secretions. J. Anim. Sci. 39:747.

Kontula, Kimmo. 1975. Progesterone binding proteins form endometrium and myometrium of sheep uterus: a comparative study. Acta Endocrin. 78:593.

Labhsetwar, Anant P., W. J. Tyler and L. E. Casida. 1963. Genetic and environmental factors affecting quiet ovulations in holstein cattle. J. Dairy Sci. 46:843.

Leavitt, W. W., D. W. Toft, C. A. Strott and B. W. O'Malley. 1974. A specific progesterone receptor in the hamster uterus: physiologic properties and regulation during the estrous cycle. Endocr. 94:1041.

LeGault, C. and M. Leuillet. 1973. Ètude de quelques facteurs de variation du poids de l'embryon et du placenta chez la truie primipare an trentième jour de la gestation. Ann. Biol. anim. Bioch. Biophys. 13:25.

Liptrap, R. M. 1970. Effect of corticotrophin and corticosteroids on oestrus, ovulation and oestrogen excretion in the sow. J. Endocr. 47:197.

Liptrap, R. M. 1973. Oestrogen excretion by sows with induced cystic ovarian follicles. Res. Vet. Sci. 15:215.

Loewit, K., S. Badawy and K. Laurence. 1969. Alteration of corpus luteum function in the pregnant rat by antiluteinizing serum. Endocr. 84:244.

Logeat, Frédérique, Pierre Sartor, Mai Thu Vu Hai and Edwin Milgrom. 1980. Local effect of the blastocyst on estrogen and progesterone receptors in the rat endometrium. Science (NY) 207:1083.

Lorenzen, Janice R. 1977. The effect of displacement of time of adrenalectomy on gonadotropin release following castration in male rats. 10th Annual Society for the Study of Reproduction, Austin, TX, pp 34.

Lowry, Oliver H., Nira J. Tosebrough, A. Lewis Farr and Rose J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265.

MacFarlane, W. V., Pamela R. Pennycuik and E. Thrift. 1957. Resorption and loss of foetuses in rats living at 35°C. J. Physiol. 135:451.

Madan, M. L. and H. D. Johnson. 1973. Environmental heat effects on bovine luteinizing hormone. J. Dairy Sci. 56:1420.

Madhwa Raj, H. G. and N. R. Moudgal. 1970. Hormonal control of gestation in the intact rat. Endocr. 86:874.

Marple, D. N., E. D. Aberle, J. C. Forrest, W. H. Blake and M. D. Judge. 1972a. Effects of humidity and temperature on porcine plasma adrenal corticoids, ACTH and growth hormone levels. J. Anim. Sci. 34:809.

Marple, D. N., E. D. Aberle, J. C. Forrest, W. H. Blake and M. D. Judge. 1972b. Endocrine responses of stress susceptible and stress resistant swine to environmental stressors. J. Anim. Sci. 35:576.

McLaren, Ann. 1965. Genetic and environmental effects on fetal and placental growth in mice. J. Reprod. Fert. 9:79.

Milgrom, Edwin and Etienne-Emile Baulieu. 1968. Endocrinology-specific binding of progesterone to protein in the uterus. C. R. Acad. Sci., Paris 267:2005.

Milgrom, Edwin and Etienne-Emile Baulieu. 1970. Progesterone in uterus and plasma. I. Binding in rat uterus 105,000 g supernatant. Endocr. 87:276.

Milgrom, E., M. Perrot, M. Atger and E. E. Baulieu. 1972. Progesterone in uterus and Plasma: V. An assay of the progesterone cytosol receptor in the guinea pig uterus. Endocr. 90:1064.

Milgrom, E., Luu Thi, M. Atger and E. E. Baulieu. 1973. Mechanisms regulating the concentration and the conformation of progesterone receptor(s) in the uterus. J. Biol. Chem. 248:6366.

Miller, B. G., L. Murphy and G. M. Stone. 1977. Hormone receptor levels and hormone, RNA and protein metabolism in the genital tract during the oestrous cycle of the ewe. J. Endocr. 73:91.

Moberg, Gary P. 1976. Effects of environment and management stress on reproduction in the dairy cow. J. Dairy Sci. 59:1618.

Monk, Edison L. and R. E. Erb. 1974. Effect of unilateral ovariectomy and hysterectomy on reproductive parameters in the gilt during early pregnancy. J. Anim. Sci. 39:366.

Nara, B. S. and N. L. First. 1981. Effect of indomethacin on dexamethasone induced parturition in swine. J. Anim. Sci. 52:788.

Nelson, Don H. 1980. ACTH, MSH and β-lipotrophin. pp 32. In Lloyd H. Smith, Jr. (Ed.) The Adrenal Cortex: Physiological Function and Disease, W. B. Saunders Co., Philadelphia, PA.

Nishi, N., A. Arimura, K. G. de la Cruz and A. V. Schally. 1976. Termination of pregnancy by sheep anti-LHRH gammaglobulin in rats. Endocr. 98:1024.

North, S. A., E. R. Hauser and N. L. First. 1973. The induction of parturition in swine and rabbits with corticosteroid dexamethasone. J. Anim. Sci. 36:1170.

Ogle, Cordelia. 1934. Adaptation of sexual activity to environmental stimulation. Am. J. Physiol. 107:628.

Ogle, Thomas F. 1980. Characteristics of high affinity progesterone binding to rat placental cytosol. Endocr. 106:1861.

Omtvedt, I. T., R. E. Nelson, Ron L. Edwards, D. F. Stephens and E. J. Turman. 1971. Influence of heat stress during early, mid and late pregnancy of gilts. J. Anim. Sci. 32:312.

Perry, J. S. and J. G. Rowell. 1969. Variations in fetal weight and vascular supply along the uterine horn of the pig. J. Reprod. Fert. 19:527.

Perry, J. S. and I. W. Rowlands. 1962. Early pregnancy in the pig. J. Reprod. Fert. 4:175.

Puri, Raj K. and S. K. Roy. 1980. Progesterone nuclear receptors in different parts of rabbit fallopian tube and uterus during ovum transport. Indian J. Exp. Biol. 18:533.

Pursel, V. G., L. A. Johnson and L. L. Schulman. 1973. Fertilizing capacity of boar semen stored at 15C. J. Anim. Sci. 37:532.

Robson, J. M. and A. A. Sharaf. 1952. Effect of adrenocorticotrophic hormone (ACTH) and cortisone on pregnancy. J. Physiol. 116:236.

Saffran, J., B. K. Loeser, S. A. Bohnett and L. C. Faber. 1976. Binding of progesterone receptor by nuclear preparations of rabbit and guinea pig uterus. J. Biol. Chem. 251:5607.

Sebranek, J. G., D. N. Marple, R. G. Cassens, E. J. Briskey and L. L. Kastenschmidt. 1973. Adrenal responses to ACTH in the pig. J. Anim. Sci. 36:41.

Selye, Hans. 1939. The effect of adaptation to various damaging agents on the female sex organs in the rat. Endocr. 25:615.

Selye, Hans. 1946. The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocr. 6:117.

Shah, M. K. 1956. Reciprocal egg transplantations to study the embryo uterine relationship in heat-induced failure of pregnancy. Nature 177:1134.

Spies, H. G. and S. K. Quadri. 1967. Regression of corpora lutea and interruption of pregnancy in rabbits following treatment with rabbit serum to ovine LH. Endocr. 80:1127.

Stormshak, Fredrick. 1978. Uterine estrogen and progesterone receptors. Beltsville Symposia in Agriculture Research, Number 3, Animal Reproduction.

Stott, G. H. and R. J. Williams. 1962. Causes of low breeding efficiency in dairy cattle associated with seasonal high temperatures. J. Dairy Sci. 45:1369.

Teague, H. S., W. L. Roller and A. P. Grifo, Jr. 1968. Influence of high temperature and humidity on the reproductive performance of swine. J. Anim. Sci. 27:408.

Thwaites, C. J. 1969. Embryo mortality in heat stressed ewe. J. Reprod. Fert. 19:255.

Tompkins, E. C., C. J. Heindenreich and Martin Stob. 1967. Effect of post breeding thermal stress on embryonic mortality in swine. J. Anim. Sci. 26:377.

Turner, C. D. and J. T. Bagnara. 1976. General Endocrinology. W. B. Saunders Co., Philadelphia, PA.

Ulberg, L. C. and P. J. Burfening. 1967. Embryo death resulting from adverse environment on spermatozoa or ova. J. Anim. Sci. 26:571.

Ulberg, L. C., R. H. Grummer and L. E. Casida. 1951. The effects of progesterone upon ovarian function in gilts. J. Anim. Sci. 10:665.

Velardo, Joseph T. 1957. Action of adrenocorticotropin on pregnancy and litter size in rats. Am. Physiol. 191:319.

Velardo, J. T. and S. H. Sturgis. 1956. Suppression of uterine growth by purified hydrocortisone acetate, 9α-fluorohydrocortisone acetate and corticotropin. J. Clin. Endocr. 16:496.

Wagner, W. C., R. E. Strohbehn and P. A. Larson. 1977. Effect of local or parenteral application of ACTH or hydrocortisone on bovine corpus luteum function. Acta Endocr. 85:158.

Waldorf, D. P., W. C. Foote, H. L. Self, A. B. Chapman and L. E. Casida. 1957. Factors affecting fetal pig weight late in gestation. J. Anim. Sci. 16:976.

Walters, M. R. and J. H. Clark. 1977. Cytosol progesterone receptors of the rat uterus: assay and receptor characteristics. J. Steroid Biochem. 8:1137.

Walters, Marian R. and James H. Clark. 1978. Cytosol and nuclear compartmentalization of progesterone receptors of the rat uterus. Endocr. 103:601.

Warnick, A. C., H. D. Wallace, A. Z. Palmer, E. Sosa, D. J. Duerre and V. E. Caldwell. 1965. Effect of temperature on early embryo survival in gilts. J. Anim. Sci. 24:89.

Webel, S. K. and P. J. Dziuk. 1974. Effect of stage of gestation and uterine space on prenatal survival in the pig. J. Anim. Sci. 38:960.

Wildt, D. E., G. D. Riegle and W. R. Dukelow. 1975. Physiological temperature response and embryonic mortality in stressed swine. Am. J. Physiol. 229:1471.

Woody, C. O. and L. C. Ulberg. 1964. Viability of one-cell sheep ova as affected by high environmental temperature. J. Reprod. Fert. 7:275.

Yang, W. H., W. P. Yang and L. L. Lin. 1969. Interruption of pregnancy in the rat by administration of ACTH. Endocr. 84:1282.

Yeates, N. T. M. 1953. The effect of high air temperature on reproduction in the ewe. J. Agric. Sci. 43:199.

VITA

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In August 1973 Jeffrey began college at Northeast Technical Community College and Nebraska Christian College simultaneously. He received an Associate of Arts degree in Mathematics from NTCC in July 1976 and entered the University of Nebraska-Lincoln in the fall of the same year. The degree of Bachelor of Science in Animal Husbandry was awarded to Jeffrey in December 1978. Jeffrey then entered the University of Tennessee, Knoxville in September 1979 and worked toward the degree of Master of Science in Animal Science which was awarded in December 1981.