To the Graduate Council:

I am submitting herewith a thesis written by Michael J. Behrens entitled " Hormonal and Uterine Changes in Pregnant and Pseudopregnant Gilts Treated With Hydrocortisone Acetate." I have examined the final 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

H. G. Kattesh, Major Professor

We have read this thesis
and recommend its acceptance:

George Allan Brumbach

Frank B Masincupp

Accepted for the council :

C W Minkel

The Graduate School

# HORMONAL AND UTERINE CHANGES IN PREGNANT AND PSEUDOPREGNANT GILTS TREATED WITH HYDROCORTISONE ACETATE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Michael J. Behrens

August 1991

AG-VET-MED.
THESIS
91
.B344

## ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks and appreciation to the following persons, for assisting in the preparation of this thesis and in making his graduate study possible.

Dr. H. G. Kattesh, major professor and adviser, for his patience, guidance, professional expertise, friendship, and assistance throughout the course of this study and in the preparation of this thesis.

Dr. G. A. Baumbach, for his friendship, professional expertise, assistance in collecting data and serving on the committee.

Dr. F. B. Masincupp, for his friendship, knowledge of physiology, and serving on the committee.

John Schneider, statistician, for his assistance involving analyses of data included in this thesis.

Teresa Herring, for her attitude, humor and especially her patience in typing the tables for this thesis.

Barbara Gillespie and Mary Roberts, for there expert teaching, guidance, and assistance in performing laboratory techniques utilized in obtaining data for this study.

David Thomas, for his friendship, encouragement, and assistance during the course of this study.

Paul and Nancy Behrens, parents of the author, for their love, support, encouragement, but most of all for providing the author the opportunity to pursue higher education.

Finally, the author wishes to thank his fiancee, Anita Genova, for her love, understanding and support that gave him the strength to overcome the obstacles during the course of this study.

## ABSTRACT

In the first experiment 11 gilts were injected with 5 mg of estradiol benzoate on d 11-15 of the estrous cycle to induce pseudopregnancy. Twice daily on d 21-30 gilts were administered either 5 mg per kg bodyweight (avg. wgt 120 kg) hydrocortisone acetate (HA) in sesame oil (5 ml) or sesame oil (control) subcutaneously (SQ). Blood samples (20 ml) were collected via jugular puncture on d 11, 21, and 31. Uterine flushings were obtained surgically the day following the last day of treatment (d 31).

Twice daily injection of HA on d 21-30 significantly ($p<.001$) elevated cortisol levels above that of control animals in plasma and uterine flushing on d 31. The percent distribution of cortisol in plasma [% unbound (UB-C), % corticosteroid binding globulin (CBG) bound (CBG-C), and % albumin bound(Alb-C)] was not different between treatments. Plasma CBG binding capacity (CBG-BC) was lower ($p<.001$) following 10 d of treatment with HA compared to control gilts (7.4 versus 38.7 pmol/ml). Plasma progesterone ($P_4$) levels were significantly ($p<.01$) lower in HA treated gilts (8.9 ng/ml) compared to control gilts (17.8 ng/ml). Uterine flush $P_4$ levels were also decreased ($p<.001$) compared to control gilts. Total plasma protein and albumin concentrations were similar ($p>.05$) to control gilts. Total proteins in the uterine

flush were lower ($p<.001$) in HA treated gilts. Corpora lutea (CL) number and $P_4$ concentrations were not affected by treatment. Total CL weight was significantly ($p<.01$) lower in HA treated gilts compared to control animals.

In the second experiment 18 crossbred gilts exhibiting 2 normal estrous cycles (18-23 d) were naturally bred to a mature boar and randomly assigned to receive 5 mg/kg bodyweight (BW) HA (Trt 1), 2.5 mg/kg BW HA (Trt 2), or 5 ml sesame oil (control; Trt 3) twice daily on d 9-13 of pregnancy. Blood samples were collected on d 9, 11, 13, and 20 of pregnancy. On d 46$\pm$2 gilts were slaughtered and reproductive tracts collected. CL number and CL weights were obtained. Number, weight, crown rump length, placental weight, allantoic and amniotic fluid volume of fetuses were measured and recorded.

Plasma cortisol levels were increased ($p<.05$) due to treatment on day 13. The distribution of cortisol (% UB-C, % CBG-C, and % Alb-C) was not different ($p>.05$) between treatments. CBG-BC, plasma $P_4$ levels, total proteins, CL number, CL weight, fetal number, fetal length, and placental weight were not affected by treatment. Fetal weights in Trt 2 (2.5 mg/kg HA) were significantly ($p<.05$) lower compared to Trt 1 (5 mg/kg HA) and control gilts. Allantoic and amniotic fluid volumes were lower in Trt 2 ($p<.001$ and $p<.05$, respectively) compared to Trt 1 and control gilts.

These results suggest that : a) HA affected $P_4$ production in the ovary by retarding CL development, B) the HA treatment lowered total $P_4$ output, as reflected by lowered $P_4$ concentration in the plasma, and affected protein secretion in the uterus, C) uterine protein secretions were preferentially reduced in the component contributed by $P_4$-induced, locally synthesized proteins but not by serum transudate, D) HA did not affect liver function as measured by total plasma protein concentration, E) HA affected CBG as evident by the decrease in CBG-BC concentrations and F) reduction in uterine secretory protein output may result in lowered fetal weight due to poor nutrition <u>in utero</u>.

# TABLE OF CONTENTS


| CHAPTER | | PAGE |
|---|---|---|
| I. | INTRODUCTION | 1 |
| II. | LITERATURE REVIEW | 4 |
| | Stress and Swine | 4 |
| | Stress and Female Reproductive Performance | 6 |
| | Endocrine Mechanism of Stress-Impaired Female Reproduction | 9 |
| III. | MATERIALS AND METHODS | 13 |
| | Animals and Data Collected | 13 |
| | Experiment One | 13 |
| | Experiment Two | 15 |
| | Hormone Radioimmunoassays | 16 |
| | Statistical Analysis | 18 |
| IV. | RESULTS | 21 |
| | Experiment One | 21 |
| | Total Plasma Cortisol | 21 |
| | Percent Unbound Cortisol | 21 |
| | Percent CBG and Albumin Bound Cortisol | 25 |
| | CBG Binding Capacity | 25 |
| | Plasma Progesterone | 25 |
| | Total Plasma Protein | 28 |
| | Albumin | 28 |
| | Uterine Flush Cortisol | 28 |
| | Uterine Flush Progesterone | 28 |
| | Total Uterine Proteins and Albumin | 29 |
| | Corpora Lutea Number and Weight | 29 |
| | Corpora Lutea Progesterone | 29 |
| | Experiment Two | 29 |
| | Total Plasma Cortisol | 30 |
| | Percent Unbound Cortisol | 30 |
| | Percent CBG and Albumin Bound Cortisol | 35 |
| | CBG Binding Capacity | 35 |
| | Plasma Progesterone | 35 |
| | Total Plasma Protein | 39 |
| | Corpora Lutea Number and Weight | 39 |
| | Fetal Number, Weight, and Length Placental Weight, Allantoic and Amniotic Fluid Volume | 39 |

V. DISCUSSION..... 42
Experiment One..... 42
Experiment Two..... 47

VI. SUMMARY..... 51
Experiment One..... 51
Experiment Two..... 52

LITERATURE CITED..... 54

APPENDICES..... 64

A. PROCEDURE FOR DETERMINATION OF PERCENT DISTRIBUTION OF BOUND AND UNBOUND CORTISOL IN PLASMA..... 64
B. PROCEDURE FOR DETERMINATION OF CBG BINDING CAPACITY IN PLASMA..... 69
C. PROCEDURE FOR QUANTIFICATION OF TOTAL PLASMA PROTEIN CONCENTRATION..... 73
D. PROCEDURE FOR QUANTIFICATION OF ALBUMIN IN PLASMA..... 77

VITA..... 78

# LIST OF TABLES


TABLE PAGE

1. Total plasma cortisol, % unbound cortisol (UB-C), % CBG-bound cortisol (CBG-C), % albumin bound cortisol (Alb-C), and CBG binding capacity (CBG-BC) in gilts treated twice daily with 5 mg/kg body weight hydrocortisone acetate (HA; n=5) or sesame oil (control;n=6) on d 21-30 of pseudopregnancy..... 22

2. Plasma progesterone, total protein, and albumin concentrations in gilts treated twice daily with 5 mg/kg body weight hydrocortisone acetate (HA; n=5) or sesame oil (control;n=6) on d 21-30 of pseudopregnancy..... 27

3. Total cortisol, progesterone, total proteins, and albumin concentrations recovered from uterine flush of gilts treated twice daily with 5 mg/kg body weight hydrocortisone acetate (HA; n=5) or sesame oil (control;n=6) on d 21-30 of pseudopregnancy..... 29

4. Total CL number, CL weight, and CL progesterone concentration in gilts treated with 5 mg/kg bodyweight hydrocortisone acetate (HA; n=5) or sesame oil (control;n=6) on d 21-30 of pseudopregnancy..... 30

5. Total plasma cortisol, % unbound cortisol (UB-C), % CBG-bound cortisol (CBG-C), % Albumin bound cortisol (Alb-C), and CBG binding capacity (CBG-BC) in Trt 1 (5 mg/kg HA;n=4); Trt 2 (2.5 mg/kg HA;n=6); Trt 3 (control; n=5) on d 9-13 of pregnancy..... 33

6. Plasma progesterone, total plasma protein, and albumin concentrations in gilts treated with 5 mg/kg bodyweight (BW) hydrocortisone acetate (HA; Trt 1;n=4), 2.5 mg/kg BW HA (Trt 2;n=5), or sesame oil (control;Trt 3;n=5) on d 9-13 of pregnancy.. 38

7. Total CL number, CL weight, fetal number, fetal weight, fetal length, placental weight, amniotic and allantoic fluid volume in gilts treated with 5 mg/kg bodyweight (BW) hydrocortisone acetate (HA; Trt 1;n=4), 2.5 mg/kg BW HA (Trt 2;n=5), or sesame oil (control;Trt 3;n=5) on d 9-13 of pregnancy.. 41

# LIST OF FIGURES

FIGURE PAGE

1. Total plasma cortisol (A) and percent unbound cortisol (B) in pseudopregnant gilts following twice daily administration of hydrocortisone acetate (5 mg/kg bodyweight; Trt 1) or sesame oil (control; Trt 2) on d 21-30 of pseudopregnancy... 23

2. Percent cortisol bound to CBG (A) and albumin (B) in pseudopregnant gilts following twice daily administration of hydrocortisone acetate (5 mg/kg bodyweight; Trt 1) or sesame oil (control; Trt 2) on d 21-30 of pseudopregnancy ..... 24

3. CBG binding capacity (A) and total plasma progesterone levels (B) in pseudopregnant gilts following twice daily administration of hydrocortisone acetate (5 mg/kg bodyweight; Trt 1) or sesame oil (control; Trt 2) on d 21-30 of pseudopregnancy ..... 26

4. Total plasma cortisol (A) and percent unbound cortisol (B) in pregnant gilts following twice daily administration of 5 mg/kg hydrocortisone acetate (HA; Trt 1), 2.5 mg/kg HA (Trt 2), or sesame oil (control; Trt 3) on d 9-13 of pregnancy..... 34

5. Percent cortisol bound to CBG (A) and percent cortisol bound to albumin (B) in pregnant gilts following twice daily administration of 5 mg/kg hydrocortisone acetate (HA; Trt 1), 2.5 mg/kg HA (Trt 2), or sesame oil (control; Trt 3) on d 9-13 of pregnancy..... 36

6. CBG binding capacity (A) and progesterone levels (B) in pregnant gilts following twice daily administration of 5 mg/kg hydrocortisone acetate (HA; Trt 1), 2.5 mg/kg HA (Trt 2), or sesame oil (control; Trt 3) on d 9-13 of pregnancy..... 37

# CHAPTER I

## INTRODUCTION

Reproductive efficiency is a major concern of swine producers. The producer's major objective is to get as many piglets per sow as possible. If the gilt's or sow's ability to reproduce is impaired, for any reason, the producer's profits are reduced. During the first twenty-five days of pregnancy 30% of conceptuses will normally not survive (Perry and Rowlands, 1962). Environmental stress (i.e., temperature extremes and humidity) and conditions related to management (i.e., handling, overcrowding, isolation, and nutrition) can have profound effects on the fertility of gilts and sows. Early embryonic mortality can reach 50% or greater in swine placed in a stressful environment (Tompkins et al., 1967).

The term stress implies any nonspecific response of the body to a specific demand (stressor) made upon it. Selye (1956) was the first to describe an animal's physiological response to a stress. His theory had three stages: 1)emergency reaction, 2) stage of adaptation, and 3) stage of exhaustion. In 1985, Moberg expanded Selye's theory and reorganized it into three categories: 1) recognition of a threat to homeostasis, 2) the response to the stress, and 3)

the biological effects of stress.

The exact physiological mechanism by which stress increases early embryonic mortality in swine is unclear. The perception and response to a stressor are reflected by increased concentrations of adrenocorticotropic hormone (ACTH), which stimulates the release of glucocorticoids from the adrenal gland. An increase in plasma adrenal glucocorticoid levels either by direct (exogenously administered) or indirect (ACTH administration or following acute stress) means has been associated with hindered reproductive ability in several species (Andrews, 1977; Fuquay, 1981). Limited information is available on how adrenal glucocorticoids actually effect reproduction.

The objectives of this study were to document changes in: (1) selected hormones (cortisol and progesterone) in the plasma and uterine flush of pigs; (2) plasma and uterine protein changes in pseudopregnant and pregnant gilts; and (3) changes in fetal pig parameters (i.e. fetal length, fetal number, and fetal weight) following hydrocortisone acetate administration (to simulate conditions of elevated glucocorticoids in response to stress).

# CHAPTER II

# LITERATURE REVIEW

## STRESS AND SWINE

Any external stimulus that challenges homeostasis can be viewed as a stressor, and the changes in biological function that occur as the animal attempts to maintain homeostasis is referred to as the stress response (Moberg, 1985). For most stressors the prudent response for the animal is to alter its behavior, by simply removing itself from the threat. For example, an animal will escape the heat of the summer sun by finding shade, and when challenged by a dominant peer the animal will move or look away (Moberg, 1985). Elevated temperature, crowding, inadequate nutrition, and illness have been linked to decreased growth, increased gluconeogenesis, suppressed immune function, and altered metabolism (Barnett et al.,1982; Munck et al.,1984; Dantzer and Mormede, 1987; Hemsworth et al., 1987; Rajeeve et al., 1990; Dreiling et al., 1990).

The neuroendocrine response to a stress offers the greatest potential for understanding the impact of stress on an animals well-being (Moberg, 1985). In that the hypothalamus regulates the pituitary gland, a direct connection exists between the endocrine system and the

central nervous system (CNS). The neuroendocrine system affects the functions of virtually every physiological system. Neuroendocrine hormones regulate reproduction, influence growth, alter metabolism and directly or indirectly effect immunity and behavior (Moberg, 1985).

In animals, corticotropin-releasing hormone (CRH) controls the release of ACTH from the anterior pituitary gland. CRH is produced by neurosecretory neurons in the hypothalamus and secreted into the hypothalamo-hypophysial portal vessels to initiate release of ACTH from pituitary corticotropes (Sanson, 1988). ACTH stimulates synthesis of glucocorticoids, progesterone, and androgens from the adrenal cortex (Kaplan, 1988). The adrenal-cortical response to a stimulus is measured by an increase in circulating levels of glucocorticoids to indicate that stress has occurred (Moberg, 1985).

In swine the primary glucocorticoid is cortisol (Yousef, 1985). Cortisol receptors are found in every mammalian tissue, and nearly all respond to cortisol in some way. In animals cortisol regulates normal biological functions such as metabolism, lactation, immunity and possibly reproduction (Moberg, 1987).

The majority of cortisol is bound to a specific glycoprotein known as corticosteroid binding globulin (CBG). The remaining cortisol is bound to albumin or is unbound (Tait and Burnstein, 1964). Of the two proteins CBG (MW 52

k in man; Westphal, 1983) has a higher affinity but lower capacity for cortisol and serves to prevent the rapid hepatic catabolism of cortisol (Sitteri et al., 1982). Both CBG and albumin bound corticosteroids provide an available pool of free hormone when needed (Sitteri et al., 1982). Unbound cortisol (free form) is the biologically active form of the hormone (Tait and Burnstein, 1964). Unbound cortisol ranges from 2 to 5 ng/ml in swine (Barnett et al., 1981a,b; Kattesh et al.,1990)

Barnett and coworkers (1981a) found that gilts penned individually had higher total cortisol concentrations and lower maximum corticosteroid binding capacity (MCBC) compared to gilts penned in groups, which resulted in an increase in free cortisol. Thus individual penning resulted in chronic stress. In a similar study, gilts housed in pairs exhibited higher levels of free cortisol and lower MCBC compared to gilts penned in groups (Barnett et al., 1983). Hemsworth and coworkers (1987) showed there was no significant difference in MCBC in 7 wk old gilts following short term exposure to different types of handling (pleasant, unpleasant, inconsistent, and minimal). However, free cortisol levels were elevated and growth rate decreased in the unpleasantly and inconsistently handled groups. The authors concluded that poor human animal relationship caused a chronic stress response which resulted in decreased growth (Hemsworth et al., 1987)

Results from earlier studies (Kattesh et al., 1980; Barnett et al., 1981a,b) indicated that chronic stress was capable of decreasing corticosteroid binding capacity (CBC) or MCBC. Kattesh et al. (1980) reported that the coefficient of variation was smaller for CBC values than for cortisol (16.5 % and 45%, respectfully), and therefore, CBC may be a better measure of chronic stress in swine when infrequent blood sampling is used. They showed that CBC levels were higher in sows compared to gilts.

## STRESS AND FEMALE REPRODUCTIVE PERFORMANCE

In the pig, maternal recognition of pregnancy occurs around day 10 to 11 post-breeding (Bazer et al., 1977). Maternal recognition of pregnancy can be defined as the manner by which the conceptus prolongs the functional lifespan of corpora lutea (CL) (Geisert et al., 1990). Bazer and Thatcher (1977) provided evidence that indicated the involvement of conceptus estrogen synthesis and release for maintenance of CL function in the pig. They suggested that developing conceptuses may maintain plasma progesterone through an antiluteolytic action on the endometrium to prevent transfer of luteolytic agents to the ovaries. This theory is strengthened by the fact that administration of estrogen (5 mg) on days 11-15 of the estrous cycle will prolong luteal function (pseudopregnancy) in pigs (Bazer et

al., 1977). The exogenous estrogen appears to mimic the estrogen released from the blastocyst at this time (Bazer et al., 1977).

Corpora lutea are the principal source of progesterone throughout gestation in pigs (Bazer et al., 1977). In cycling gilts progesterone concentrations increase slowly from around day 4 to a maximum level around day 12 (Bazer et al, 1977). By day 14 progesterone concentrations decrease sharply and reach basal levels by day 18. In pregnant gilts progesterone increases from day 4 to day 14, then decreases slightly to day 20 were it remains elevated above basal levels until day 98 of pregnancy (Robertson and King, 1974). Comparison of plasma progesterone levels in pregnant and pseudopregnant gilts on days 8-27 indicated that progesterone levels were slightly lower in pseudopregnant gilts (Zeicik et al., 1986; King and Rajamahendran, 1988). Zeicik et al. (1986) suggested that the large doses of estradiol used to induce pseudopregnancy may have reduced the production or secretion of $P_4$ from luteal cells.

A high percentage (80%) of prepuberal gilts exposed to elevated environmental temperatures beginning at 140 days of age fail to reach puberty by 230 days (Flowers et al., 1988). Wettermann et al. (1985) showed that elevated environmental temperatures can cause periods of infertility in mature swine.

Chronic heat stress (24 hour duration) on day 1, 5 or 20 of pregnancy increased early embryonic mortality 27% compared to control animals (Tompkins et al, 1967). Wildt and coworkers (1975) showed that acute heat stress (2 hour duration) applied on days 2-13 increased early embryonic loss 25% compared to control animals. Arnold and coworkers (1982) showed that pregnant gilts administered a long lasting preparation of ACTH (40 U.S.P. units) on days 11-15 of pregnancy had a lower percentage of fetal survival (48.0$\pm$ 9 %) compared to control gilts (71.7$\pm$ 3.8 %). Pregnancy failures and small litters have been observed in heat-stressed sows (Omtvedt et al., 1971; Christenson, 1980). Howarth and Hawk (1968) showed that four days of exogenous ACTH or corticosteroids starting 10 to 16 hours after estrus disrupted implantation and fetal development, and increased early embryonic mortality 30% above normal in sheep.

Gilts exposed to high environmental temperatures during the first eight days post breeding had altered levels of progesterone and estradiol hormones which were related to establishment and maintenance of pregnancy (Hoagland and Wetterman, 1984; Flint et al., 1984; Stone and Seamark, 1985). Hoagland and Wetterman (1984) suggested that luteal function or progesterone metabolism and clearance was altered in heat stressed gilts resulting in the disruption of pregnancy. A significant increase in estradiol concentrations preceded the decrease in progesterone, and

may have had detrimental effects on conceptus development. Pregnant gilts exposed to elevated temperatures (37± 1·C) on day 8-16 of pregnancy had conceptuses that appeared to be fragmenting on day 16 of pregnancy compared to control animals (Wetterman et al., 1988). It is evident that chronic thermal stress can increase early embryonic loss and prevent prepuberal gilts from cycling normally.

Detrimental effects of stress on reproduction have also been documented in sheep (Braden et al., 1964; Prezkop et al., 1985; Behrens and Parvizi, 1988), cattle (Moberg, 1976; Gwazdauskas et al., 1985; Coubrough, 1985), rats (Rivier et al., 1986), hamsters (Wise and Eldred, 1986), and llama (Johnson, 1989),

## ENDOCRINE MECHANISM OF STRESS-IMPAIRED FEMALE REPRODUCTION

The exact pathophysiological mechanisms by which stress affects reproduction is not fully understood. Selye (1959), hypothesized that the increase in adrenal activity associated with stress acts to return the animal to homeostasis, frequently at the cost of normal reproduction.

Stress induced release of adrenal glucocorticoids can affect the synthesis and secretion of gonadotropins (Moberg, 1990). Glucocorticoids have been shown to alter the secretion of gonadotropin releasing hormone (GnRH), release of gonadotropins, and synthesis of gonadal steroids (Moberg,

1987). Moberg (1987) also suggested that CRH and ACTH may have an independent effect on the regulation of gonadotropins. In rats (Rivier and Vale, 1984) and monkeys (Olster and Ferin, 1987) CRH inhibits the secretion of GnRH by the hypothalamus. CRH may indirectly inhibit the secretion of GnRH by way of opioid neurons projecting upon the neurosecretory cells in the hypothalamus (MacLusky et al., 1988; Petraglia et al.,1986). Naylor and coworkers (1990) found that exogenous CRH in ewes failed to inhibit GnRH, and actually stimulated the secretion of LH. This suggests that the effect of CRH on GnRH secretion may be species specific.

There is considerable evidence to suggest that glucocorticoids can act directly on the pituitary to hinder the secretion of gonadotropins, mainly luteinizing hormone (Moberg, 1987). Glucocorticoids may affect the ability of GnRH to stimulate the secretion of LH. Restraint stress (40 minutes) in sheep (Moberg, 1987) and transportation stress in cattle (Dobson, 1987) increased plasma glucocorticoid concentrations and decreased the pituitary's response to exogenous GnRH.

Another potential mechanism for glucocorticoids to alter gonadotropin secretion is to modify the effects of gonadal steroids (estrogen and testosterone) on gonadotrophes. Estrogen and testosterone have two distinct effects on gonadotroph regulation; the sensitization of the

gonadotrophes by estrogen in females and the inhibition of gonadotrophin secretion by negative feedback on the pituitary and/or hypothalamus (Moberg, 1987). In rats corticosteroids appear to inhibit estrogen from sensitizing the pituitary and, maybe through this mechanism, corticosteroids prevent the preovulatory surge of LH (Baldwin, 1979). This hypothesis could explain why corticosteroids effect the preovulatory surge of LH, but have limited effects on synthesis and basal secretion of LH.

Information on corticosteroid effects on the negative feedback of gonadal steroids on the pituitary is limited. In one study using adrenalectomized rats, neither glucocorticoids nor testosterone alone affected gonadotropins, but co-administration decreased plasma LH (373 to 9 ng/ml) and FSH (1549 to 354 ng/ml) (Vreeburg et al., 1984). The authors concluded that corticosteroids may sensitize gonadotrophes to the negative feedback effects of testosterone. More research needs to be done in domestic animals to determine the exact gonadal mechanisms affected by glucocorticoids.

In castrated male pigs a single injection of ACTH (20 IU) had no effect on circulating concentrations of LH (Juniewicz and Johnson, 1981). However, Barb et al. (1982) showed that prolonged treatment (12 days) with ACTH or cortisol blocked the preovulatory surge of LH. In a study by Li and Wagner (1987), cortisol inhibited the ability of

estrogen to stimulate LH secretion in porcine pituitary cell cultures. In sheep the influence of estrogens on the gonadotrophes was not affected by elevated glucocorticoid levels (Moberg et al., 1981). Once again, species differences are apparent and should be taken into consideration when attempting to make species comparisons.

Limited data are available on the effects corticosteroids have on FSH secretion. Corticosteroids appear to have a positive effect on FSH secretion (Suter and Schwartz, 1985). It was reported that corticosteroid treatment for 48 hours enhanced the basal secretion of FSH in cultured female rat pituitary cells, directly effecting FSH synthesis by a mechanism independent of LH synthesis (Suter and Schwartz, 1985).

# CHAPTER III

# MATERIALS AND METHODS

## ANIMALS AND DATA COLLECTED

### Experiment One

Twenty crossbred gilts (a combination of Yorkshire, Landrace, and Duroc breeding) approximately 120 kg in weight and eight months of age were ear tagged and moved to a one-half acre pasture lot in close proximity to 1-2 mature boars. Each gilt was fed approximately 2.2 kg of a 16% crude protein corn-soybean meal diet once a day, and water was provided ad libitum.

Eleven of the 20 gilts, demonstrating two estrous cycles of 18 to 23 days, were moved to pens located in an open-front building (2-3 gilts/pen) on d 8 of the estrous cycle (d 0 = first day of standing estrus). On d 11-15 of the cycle, gilts were injected (0800 hrs) subcutaneously (SQ) with 5 mg of estradiol benzoate (Sigma Chemical Co., St Louis, Mo.) in sesame oil to induce pseudopregnancy (Frank et al., 1977). Feed and water were provided as described earlier.

On d 21-30 of pseudopregnancy gilts were randomly selected to receive one of the following treatments

administered SQ twice daily (0800 and 2000 h): (1) 600 mg (5 mg/kg bodyweight) hydrocortisone acetate (HA) (Sigma Chemical CO., St. Louis, MO.) dissolved in sesame oil (n=5) or (2) 5 ml sesame oil alone (n=6).

Two to three days prior to the last day of treatment (d 30), gilts were moved to holding pens directly adjacent to surgery facilities. On d 31 gilts were anesthetized by administering an ear vein injection of sodium thiamylal and anesthesia was maintained throughout surgery by closed circuit halothane administered by a gas inhalation machine. Each uterine horn was exposed by midventral laparotomy and flushed with 20 ml 0.33% hypotonic saline solution as described previously (Murray et al., 1972). The recovered fluid was stored at -20.C until analyzed for cortisol (total, % unbound, % CBG bound, and % albumin bound), progesterone, albumin, and total protein concentrations. Ovaries were removed, weighed, and individual corpora lutea (CL) were excised and counted. CL obtained from the left ovary were frozen at -70.C until analyzed for progesterone.

Blood samples (20 ml) were collected by jugular venipuncture on d 11, 21, and 31 immediately prior to administrating injections. Each sample was collected into a 20 ml heparinized syringe, centrifuged at 3000 X g for 20 minutes and plasma collected and stored at -20.C until analyzed for cortisol (total, % unbound, % CBG bound, and % albumin bound), CBG binding capacity, progesterone, albumin,

and total protein concentrations.

Experiment Two

Forty crossbred gilts of similar age, weight, and breeding to those used in the previous experiment were ear tagged and moved to a one-half acre pasture lot in close proximity to 1-2 mature boars. Management procedures and pasture conditions were as described previously.

Eighteen gilts demonstrating two estrous cycles of 18 to 23 days were bred naturally to a mature boar on two consecutive days (0800 hr) of estrus. Gilts were moved to pens located in an open front building (2-3 gilts/pen) on d 7. Gilts were randomly assigned to one of the following treatment groups: (1) control, injected with sesame oil (5 ml; SQ), (2) 300 mg HA in sesame oil (5 ml; SQ), (3) 600 mg HA in sesame oil (5 ml; SQ). Treatments were administered twice daily (0800 and 2000 hr) on d 9-13. Blood samples (20 ml) were collected via jugular puncture immediately prior to administration of treatment on d 9, 11, 13, and 20. Plasma was recovered and stored as described previously for later analysis of cortisol (total, % unbound, % CBG bound, and % albumin bound), CBG binding capacity, progesterone, and total protein concentrations.

On day 46 $\pm$ 2 of pregnancy gilts were slaughtered and reproductive tracts were collected and placed on ice for dissection. Number, weight, and crown rump length, and

placental weight of each fetus was recorded. Allantoic and amniotic fluid volumes were also measured. Ovaries were collected, CL excised, counted, weighed and stored at -70.C for later analysis of progesterone (left ovary only).

## HORMONE RADIOIMMUNOASSAYS

### Total Cortisol, Progesterone

Total cortisol and progesterone, in the plasma and uterine flushings, were determined using a commercial radioimmunoassay kit for the particular hormone measured (COAT A COUNT [125 I] Diagnostic Products Corporation, Los Angeles, CA.). Bound radioactivity was measured in a gamma counter and calculated as total nanograms per milliliter of plasma or flush by extrapolating from a standard curve. The intra- and inter assay coefficients of variation were 4.0 and 11.9 % for cortisol and 7 and 13.1 % for progesterone.

CL (left ovary) were minced on ice and suspended in 10 ml of cold phosphate buffered saline (PBS). Homogenate was centrifuged at 1000 x g for 30 min, the supernatant recovered and assayed for progesterone using the procedure stated above.

### Percentage Distribution of Cortisol

Estimation of the percentage of free cortisol (UB-C) in

undiluted plasma was determined by using the procedure of Hammond et al. (1980), described previously for use in swine (Kattesh et al, 1990) and outlined in Appendix A. The intra- and inter- assay coefficients of variation of the percentage free cortisol in a reference pig sample (n=12) were 1.7 and 12.8 %, respectively. The percentage of cortisol bound to CBG (CBG-C) was determined for each plasma sample using the procedure outlined in Appendix A following prior heating of samples at 60.C for 1 hour (Sitteri et al., 1982). The percentage of cortisol bound to albumin (Alb-C) was determined as follows:

$$\% \text{ Alb-C} = 100 - (\% \text{ UB-C} - \% \text{ CBG-C})$$

CBG Binding Capacity

The binding capacity of CBG for cortisol was determined using the procedure of Hammond et al. (1985), as modified in our laboratory for use in swine. The procedure is outlined in Appendix B. The intra- and inter- assay coefficients of variation were 4.8 and 9.3 % ,respectively.

Plasma and Uterine Proteins

Total protein concentration was determined in plasma samples (100 ul) using a dye-binding assay (Pierce Chemical Company, Rockford, IL). The assay is based on an absorbance shift from 465 to 595 nm when Coomassie blue G-250 binds to protein in an acidic solution. The assay procedure is outlined in Appendix C. Plasma was diluted with deionized

water to adjust for increasing levels of total protein occurring in pig plasma over the sampling period. A standard curve of 12.5, 25, 50, 75, 100, and 200 mg/ml of albumin (bovine albumin Fraction V, Pierce Chemical Company, Rockford, IL) was prepared by using deionized water as the diluent. Protein concentrations were calculated from a standard curve using absorbance values read on a spectrophotometer at 595 nm. Albumin concentrations were determined using 10 ul of plasma in an assay (Sigma Diagnostics, St. Louis, MO) based on the binding of endogenous albumin to bromcresol green to produce a blue green color with a maximum absorbance of 628 nm. The assay procedure is outlined in Appendix D. Samples were read on a spectrophotometer at an absorbance of 628 nm.

## STATISTICAL ANALYSIS

The effects of treatment and day on plasma hormone and protein changes were tested by least-squares analysis of variance procedures according to Goodnight (1983) for a randomized complete block design with unequal subclasses. The model fitted was as follows:

$$Y_{ijk} = T_i + D_j + TD_{ij} + E_{ijk}$$

where:

$Y_{ijk}$ = dependent variable

$T_i$ = treatment

$D_j$ = day of sampling

$TD_{ij}$ = treatment*day interaction

$E_{ijk}$ = residual error

Mean separation procedures were performed on the main effects using Duncans multiple range test (1955). Mean separation of the interaction was carried out by running a second model which contained only treatments, and run separately for each day. Another mean separation on the interaction was carried out by running a third model with only days, and run separately for each treatment.

All ovarian, uterine and fetal data were analyzed by least-squares analysis of variance using the following model:

$$Y_{ijk} = T_i + E_{ijk}$$

where:

$Y_{ijk}$ = dependent variable

$T_i$ = treatment

$E_{ijk}$ = residual error

In experiment one the dependent variables analyzed were total plasma cortisol concentration (total, % unbound cortisol, % CBG bound, % albumin bound), progesterone concentration, CBG binding capacity, total protein and albumin concentration. Uterine flush dependent variables were total cortisol, % unbound cortisol, progesterone, total proteins and albumin concentrations. Ovarian parameters included CL number, CL weight, and CL $P_4$.

In experiment two, dependent variables analyzed were the same as in experiment one (excluding uterine flushings),

as well as the following fetal parameters: fetal number, fetal weight, fetal length, placental weight, and amniotic and allantoic fluid volumes.

# CHAPTER IV

# RESULTS

## EXPERIMENT ONE

The eleven animals used in this study (HA=5; Control=6) were confirmed as pseudopregnant based upon the absence of signs of estrus on d 18-22.

### Total Cortisol

Total plasma cortisol levels in control and HA treated gilts measured on d 11, 21 and 31 of pseudopregnancy are given in Table 1. Cortisol levels were not different between gilts on d 11 and 21. Following treatment on d 21-30 HA-treated gilts had significantly ($p < .001$) higher total cortisol levels (295 $\pm$ 30 ng/ml) compared to control gilts (35.6 $\pm$ 9.1 ng/ml) as measured on day 31 (Figure 1).

### Percentage Unbound Cortisol

The percentage of unbound cortisol (UB-C) was not different ($p > .05$) between treatments (Table 1). UB-C levels were similar on d 11 and 21 and increased ($P < .01$) from d 21-31 in all gilts (Figure 1). UB-C values averaged 12.6 % for all gilts on d 31 compared to 8.3 % for d 11 and 21.

Table 1. Total Plasma Cortisol, % unbound cortisol (UB-C), % CBG-bound cortisol (CBG-C) , % albumin bound cortisol (ALB-C), and CBG binding capacity (CBG-BC) in gilts treated twice daily with 5 mg/kg body weight hydrocortisone acetate (HA; n=5) or sesame oil (control;n=6) on d 21-30 of pseudopregnancy†.

| Item | Day of Pseudopregnancy 11 | 21 | 31 |
|---|---|---|---|
| **Cortisol (ng/ml)** | | | |
| HA | $27^{d} \pm 12$ | $36^{d} \pm 12$ | $296^{e} \pm 13$ |
| Control | $32 \pm 11$ | $33 \pm 11$ | $36 \pm 11$ |
| HA vs Control | NS | NS | *** |
| **UB-C (%)** | | | |
| HA | $8.7^{a} \pm 2.3$ | $6.2^{a} \pm 2.4$ | $13.5^{b} \pm 2.3$ |
| Control | $9.8^{a} \pm 4.9$ | $8.3^{a} \pm 2.1$ | $11.7^{b} \pm 2.1$ |
| HA vs Control | NS | NS | NS |
| **CBG-C (%)** | | | |
| HA | $59.3 \pm 5.6$ | $74.7 \pm 5.6$ | $59.7 \pm 7.3$ |
| Control | $59.9 \pm 5.6$ | $62.7 \pm 5.1$ | $62.6 \pm 5.6$ |
| HA vs Control | NS | NS | NS |
| **Alb-C (%)** | | | |
| HA | $32.0 \pm 4.2$ | $19.0 \pm 4.2$ | $31.7 \pm 5.4$ |
| Control | $32.0 \pm 4.2$ | $28.9 \pm 3.8$ | $27.5 \pm 4.2$ |
| HA vs Control | NS | NS | NS |
| **CBG-BC (pmol/ml)** | | | |
| HA | $32^{a} \pm 6$ | $18^{ab} \pm 6$ | $7^{b} \pm 6$ |
| Control | $23 \pm 6$ | $30 \pm 6$ | $38 \pm 6$ |
| HA vs control | NS | NS | *** |

† Data presented as least squares means ± SEM

a,b,c Numbers with different superscripts in the same row are different ($p < .01$)

d,e Numbers with different superscripts in the same row are different ($p < .001$)

*** Significantly different at $p < .001$

NS Not significant

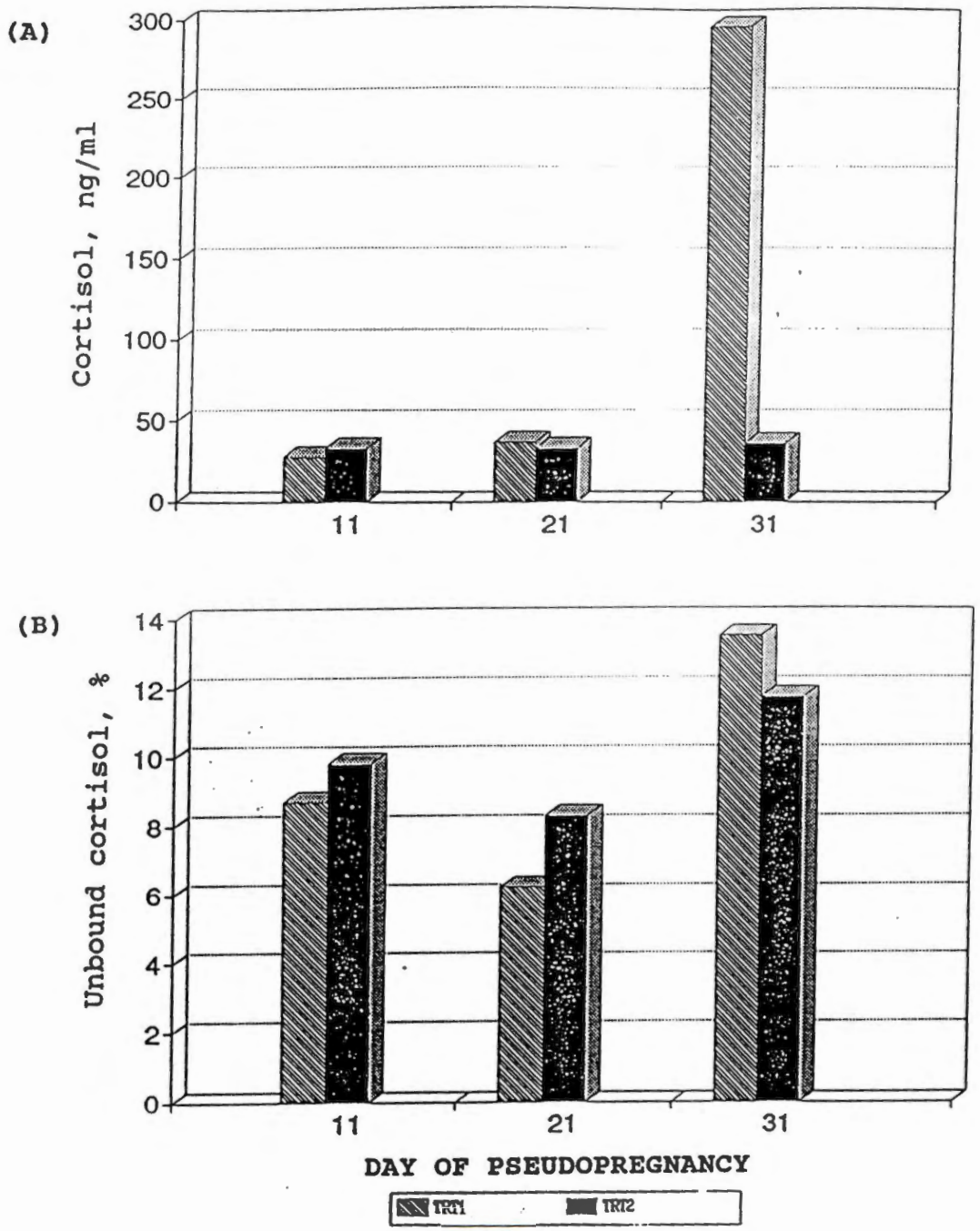


Figure 1. Total plasma cortisol (A) and percent unbound cortisol(B) in pseudopregnant gilts following twice daily administration of hydrocortisone acetate (5 mg/kg; Trt1) or sesame oil (control; Trt 2) on d 21-30 of pseudopregnancy.

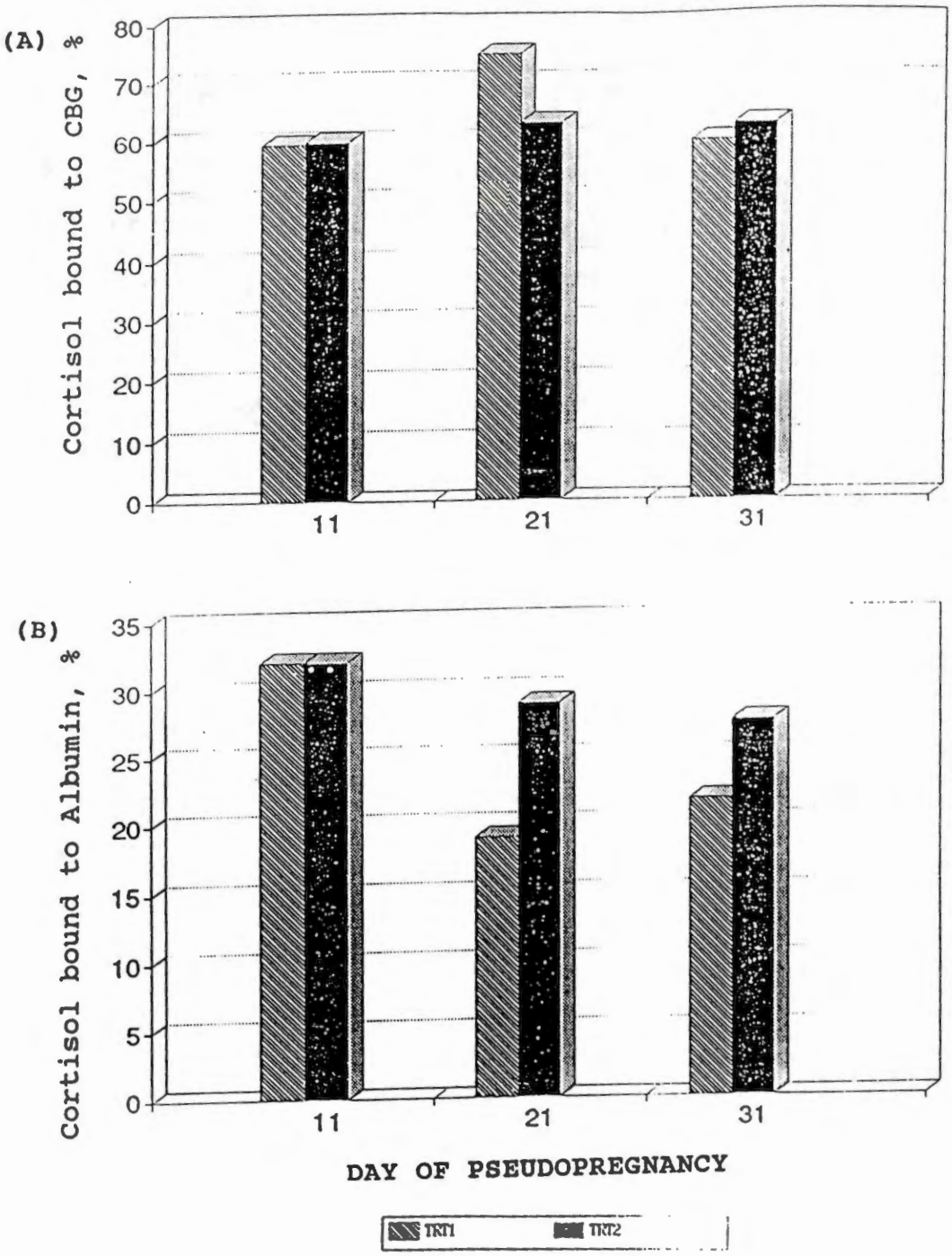


Figure 2. Percent cortisol bound to CBG (A) and albumin (B) in pseudopregnant gilts following twice daily administration of hydrocortisone acetate (HA) or sesame oil (control) on d 21-30 of pseudopregnancy.

Percentage CBG and Albumin Bound Cortisol

Both CBG bound cortisol (CBG-C) and albumin bound cortisol (Alb-C) were unchanged ($p>.05$) as a result of treatment, day of pseudopregnancy, or treatment by day interaction (Table 1). CBG-C values were consistent between treatments averaging 63 % during the entire study (Figure 2). Albumin bound cortisol (Alb-C) was derived from the difference of 100 % minus the sum of CBG-C and UB-C. Hence, Alb-C values were highly correlated (overall $r= -.98$) with CBG-C values (Table 1).

CBG Binding Capacity

The CBG binding capacity (CBG-BC) for cortisol was similar in all animals on days 11 and 21, but on d 31 HA treatment reduced ($p<.001$) CBG-BC compared to control animals (Table 1). HA-treated gilts had lower ($p<.01$) CBG-BC values on d 31 compared to d 11 and 21 for all gilts. CBG-BC values decreased from 18 pmol/ml on day 21 to 7 pmol/ml on d 31 in HA-treated gilts but was not significant (Figure 3).

Plasma Progesterone

Plasma progesterone ($P_4$) values were similar between treatments on d 11 and 21 for all gilts (Table 2). On d 31 plasma $P_4$ concentrations were lower ($p<.01$) in HA treated

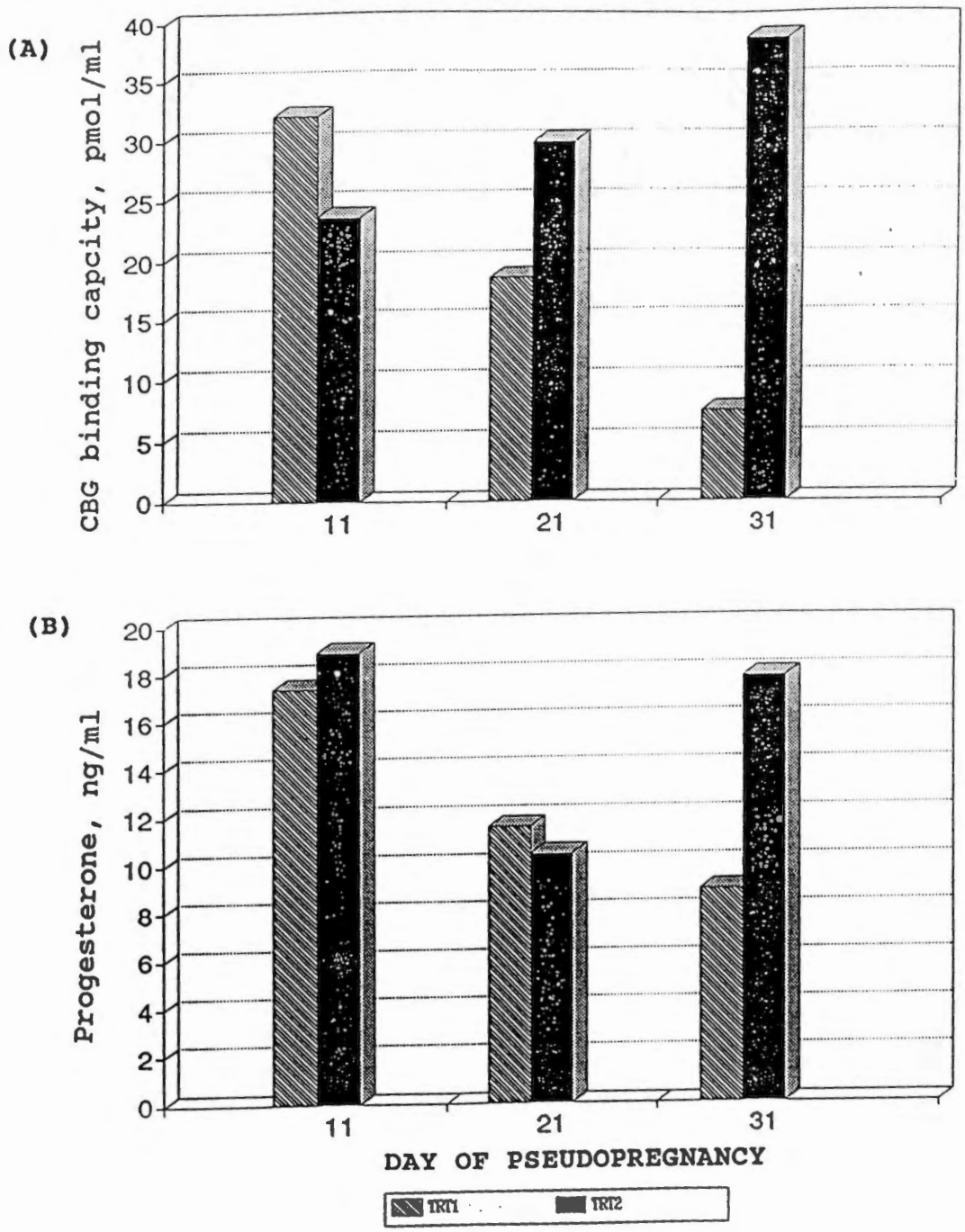


Figure 3. CBG binding capacity (A) and total plasma progesterone (B) in pseudopregnant gilts following twice daily administration of hydrocortisone acetate (5 mg/kg ;Trt 1) or sesame oil (control; Trt 2) on d 21-30 of pseudopregnancy.

Table 2. Plasma progesterone ($P_4$), total protein (Prot) and albumin (Alb) concentrations in gilts treated twice daily with 5 mg/kg body weight hydrocortisone acetate (HA;n=5) or sesame oil (control;n=6) on d 21-30 of pseudopregnancy†.

| Item | Day of Psuedopregnancy 11 | 21 | 31 |
|---|---|---|---|
| **$P_4$ (ng/ml)** | | | |
| HA | $17.4^{c} \pm 1.5$ | $11.6^{d} \pm 1.5$ | $8.9^{d} \pm 1.5$ |
| Control | $18.9^{c} \pm 1.4$ | $10.4^{d} \pm 1.4$ | $17.8^{c} \pm 1.4$ |
| HA vs control | NS | NS | ** |
| **Prot. (mg/ml)** | | | |
| HA | $7.1^{a} \pm .9$ | $9.3^{b} \pm 1.0$ | $5.5^{a} \pm 1.0$ |
| Control | $7.6^{a} \pm 1.0$ | $9.2^{b} \pm 1.0$ | $6.4^{a} \pm 1.0$ |
| HA vs control | NS | NS | NS |
| **Alb (g/dl)** | | | |
| HA | $6.6 \pm .4$ | $6.8 \pm .4$ | $6.7 \pm .4$ |
| Control | $6.5 \pm .3$ | $7.3 \pm .3$ | $7.4 \pm .3$ |
| HA vs control | NS | NS | NS |

† Data presented as least squares means ± SEM

a,b Numbers with different superscripts are significantly different ($p<.01$)

c,d,e Numbers with different superscripts in same row are significantly different ($p< .001$)

** Significantly different at $p< .01$

NS Not significant

gilts (8.9 ng/ml) compared to control gilts (17.8 ng/ml) as illustrated in Figure 3. Regardless of treatment $P_4$ levels were lower ($p<.001$) on d 21 compared to d 11.

## Total Plasma Proteins and Albumin

Total plasma proteins were not affected by treatment (Table 2). However, there was a significant ($p< .01$) day affect. Total proteins increased from d 11 to 21, and then decreased from d 21 to 31 in all animals. Plasma albumin concentrations were not affected ($p>.05$) by HA treatment or day of pseudopregnancy (Table 2).

## Uterine Flush Cortisol

The concentration of cortisol in uterine flushings was significantly ($p<.001$) different due to treatment (Table 3). Uterine flushings from HA treated gilts had nearly twice the cortisol concentration as control animals (9.9 versus 5.7 ng/ml). Cortisol concentrations were not different between uterine horns in individual animals.

## Uterine Flush Progesterone

Uterine progesterone levels were significantly lower ($p<.001$) in HA treated versus control gilts (Table 3). HA treated gilts had one-third as much progesterone (2.1 ng/ml) as control gilts (6.9 ng/ml). Values obtained from both uterine horns were similar.

Table 3. Total Cortisol, progesterone ($P_4$), total protein (Prot) and albumin (Alb) recovered from uterine flush of gilts treated twice daily with 5 mg/kg bodyweight hydrocortisone acetate (HA;n=5) or sesame oil (control;n=6) on d 21-30 of pseudopregnancy†.

| Item | Day 31 of Pseudopregnancy | | |
|---|---|---|---|
| | Left Horn | Right Horn | Average |
| **Cortisol (ng/ml)** | | | |
| HA | 10.6 ± .7 | 9.2 ± .9 | 9.9 ± .8 |
| Control | 5.6 ± .8 | 5.7 ± .8 | 5.6 ± .8 |
| HA vs Control | *** | *** | *** |
| **$P_4$ (ng/ml)** | | | |
| HA | 2.2 ± .9 | 2.0 ± 1.0 | 2.1 ± 1.0 |
| Control | 6.4 ± .9 | 7.3 ± 1.0 | 6.8 ± 1.0 |
| HA vs Control | *** | *** | *** |
| **Prot (mg/ml)** | | | |
| HA | 8.7 ± 3.2 | 7.8 ± 3.2 | 8.2 ± 3.2 |
| Control | 21.2 ± 2.9 | 21.6 ± 2.9 | 21.4 ± 2.9 |
| HA vs Control | *** | *** | *** |
| **Alb (g/dl)** | | | |
| HA | 14.8 ± 2.5 | 12.2 ± 2.5 | 13.5 ± 2.5 |
| Control | 12.7 ± 2.8 | 10.4 ± 2.8 | 11.5 ± 2.8 |
| HA vs Control | NS | NS | NS |

† Data presented as least squares means ± SEM.
*** Significantly different at $p < .001$
NS Not significant

Table 4. Total CL number, CL weight, and CL progesterone ($P_4$) concentration in gilts treated with 5 mg/kg bodyweight hydrocortisone acetate (HA;n=5) or sesame oil (control;n=6) on d 21-30 of pseudopregnancy†.

| | Day 31 of Psuedopregnancy | | |
|---|---|---|---|
| Item | Left Ovary | Right Ovary | Avg. |
| **CL Number** | | | |
| HA | 6.6 ± 1.1 | 4.4 ± 1.1 | 5.5 ± 1.1 |
| Control | 7.6 ± 1.0 | 6.3 ± 1.0 | 6.8 ± 1.0 |
| HA vs Control | NS | NS | NS |
| **CL Weight** (g) | | | |
| HA | 2.4 ± .4 | 1.5 ± .4 | 2.0 ± .4 |
| Control | 2.7 ± .4 | 2.6 ± .4 | 2.7 ± .4 |
| HA vs Control | NS | ** | ** |
| **$P_4$ ($gP_4$/gCL)** | | | |
| HA | .40 | - | - |
| Control | .35 | - | - |
| HA vs Control | NS | | |

† Data presented as least squares means ± SEM.
** Significantly different at P <. 01
NS Not Significant

Total Uterine Protein and Albumin

Total protein concentration in the uterus was significantly ($p < .001$) lower in HA treated gilts (16.5 mg/ml) compared to control gilts (42.8 mg/ml; Table 3). Both uterine horns responded similarly. Uterine albumin concentrations were not affected by treatment (Table 3).

Corpora Lutea (CL) Number and Weight

Total CL number as well as CL number for each ovary was not different between treatments (Table 4). Total CL weight was significantly ($p < .01$) different between treatments (Table 4). Total CL weight from the left ovary was less ($p < .01$) for HA-treated gilts (1.5 mg) compared to control gilts (2.7 mg).

CL Progesterone

CL progesterone (g $P_4$/g CL) for CL collected from the left ovary was not different between treatments (Table 4). HA treated gilts had .40 g $P_4$/g CL compared to .35 g $P_4$/g CL for control gilts.

EXPERIMENT TWO

Of the eighteen gilts assigned in equal numbers to receive 5 mg/kg HA (Trt 1), 2.5 mg/kg HA (Trt 2), or sesame oil alone as a control (Trt 3), 15 completed the experiment. Two of the 3 gilts (Trt 1 and 3) were not pregnant as

evident by the presence of multiple follicles and/ or corpora hemorrhagica. The third gilt (Trt 1) had large cystic-like structures on both ovaries and never exhibited signs of returning to estrus during the study. Blood samples were not obtained on d 11 for 2 gilts in Trt 2 (2.5 mg kg HA) and 2 gilts on d 20; one in Trt 1 (5 mg/kg HA) and the other in Trt 2 (2.5 mg/kg HA).

Total Plasma Cortisol

Total plasma cortisol was significantly different ($p< .01$) between animals treated with 5 mg/kg HA (Trt 1), and 2.5 mg/kg HA (Trt 2) versus control gilts (Trt 3) on d 13 (Table 5). On day 13 total plasma cortisol values were higher ($p<.01$) in Trt 1 and Trt 2 (73.1 and 66.5 ng/ml respectively) compared to control gilts (36.3 ng/ml). There was a significant ($p<.01$) increase in cortisol over days in Trt 1 (5 mg/kg HA) and Trt 2 (2.5 mg/kg HA), which peaked on day 13 then declined by day 20 to levels comparable to control gilts (Figure 4).

Unbound Cortisol

Overall percentage of unbound cortisol (UB-C) was not affected ($p>.05$) by either HA treatment ($p>.05$). There was a day effect ($p<.01$) in Trt 1 and Trt 2 (Table 5). UB-C values increased from d 9-13 in Trt 1 and Trt 2 and remained unchanged in control gilts after an initial increase from d

Table 5. Total plasma cortisol, % unbound cortisol (UB-C), % CBG-C bound cortisol (CBG-C), % albumin Bound cortisol (Alb-C), CBG binding capacity (CBG-BC) in Trt 1 (5mg/kg HA;n=4); Trt 2 (2.5 mg/kg HA; n=6); Trt 3 (Control; n=5) on d 9-13 of pregnancy†.

| Item | Day of Pregnancy 9 | 11 | 13 | 20 |
|---|---|---|---|---|
| **Cortisol (ng/ml)** | | | | |
| Trt 1 | $18.6^{a} \pm 8$ | $41.2^{b} \pm 8$ | $73.1^{c} \pm 8$ | $42.4^{b} \pm 8$ |
| Trt 2 | $39.3^{a} \pm 7$ | $56.7^{b} \pm 8$ | $66.5^{c} \pm 8$ | $46.4^{b} \pm 9$ |
| Trt 3 | $42.0 \pm 8$ | $38.2 \pm 8$ | $36.0 \pm 8$ | $45.2 \pm 8$ |
| | NS | NS | ** | NS |
| **UB-C (%)** | | | | |
| Trt 1 | $10.0^{a} \pm 2$ | $12.5^{a} \pm 2$ | $20.14^{b} \pm 2$ | $16.6^{b} \pm 2$ |
| Trt 2 | $10.9^{a} \pm 1$ | $12.0^{a} \pm 2$ | $16.90^{b} \pm 2$ | $15.9^{b} \pm 2$ |
| Trt 3 | $10.2 \pm 2$ | $13.3 \pm 2$ | $13.01 \pm 1$ | $13.2 \pm 2$ |
| | NS | NS | NS | NS |
| **CBG-C (%)** | | | | |
| Trt 1 | $65.3^{a} \pm 9$ | $50.2^{a} \pm 8$ | $40.6^{a} \pm 11$ | $23.0^{b} \pm 11$ |
| Trt 2 | $51.7^{a} \pm 7$ | $44.7^{a} \pm 8$ | $43.6^{a} \pm 9$ | $24.2^{b} \pm 9$ |
| Trt 3 | $61.9 \pm 9$ | $50.9 \pm 9$ | $58.3 \pm 8$ | $41.8 \pm 8$ |
| | NS | NS | NS | NS |
| **AlB-C (%)** | | | | |
| Trt 1 | $24.7^{a} \pm 7$ | $37.2^{a} \pm 6$ | $37.9^{a} \pm 8$ | $61.5^{b} \pm 8$ |
| Trt 2 | $37.4^{a} \pm 5$ | $43.2^{a} \pm 6$ | $43.0^{a} \pm 7$ | $62.5^{b} \pm 7$ |
| Trt 3 | $29.2 \pm 7$ | $38.6 \pm 7$ | $33.6 \pm 6$ | $45.0 \pm 6$ |
| | NS | NS | NS | NS |
| **CBG-BC (pmol/ml)** | | | | |
| Trt 1 | $26 \pm 11$ | - | $16 \pm 13$ | $7 \pm 16$ |
| Trt 2 | $42 \pm 11$ | - | $32 \pm 16$ | $22 \pm 22$ |
| Trt 3 | $42 \pm 13$ | - | $27 \pm 11$ | $37 \pm 13$ |

† Data presented as least squares means ± SEM.

abc Numbers with different superscripts in the same row are different (P< .001).

** Trt 1 and Trt 2 are significantly different (P < .01) from Trt 3.

NS Not significant

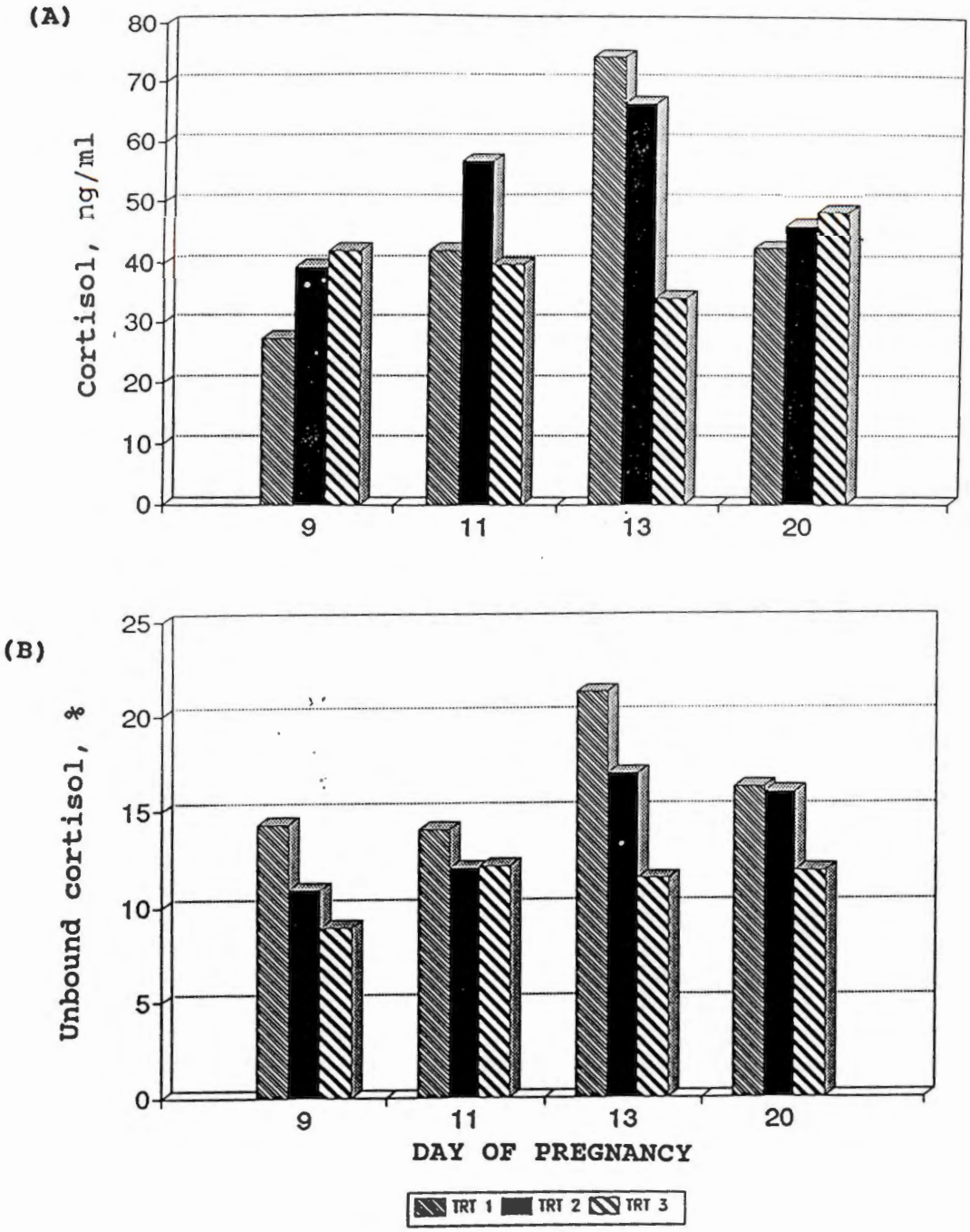


Figure 4. Total plasma cortisol (A) and percent unbound cortisol (B) in pregnant gilts following twice daily administration of 5 mg/kg hydrocortisone acetate (HA; Trti), 2.5 mg/kg HA (Trt2), or sesame oil (Control; Trt 3) on d 9-13 of pregnancy.

9 to 11 (Figure 4).

Percentage CBG and Albumin Bound Cortisol

Overall percentage of cortisol bound to CBG (CBG-C) was not different ($p>.05$) as a result of the imposed treatments (Table 5). All gilts responded similarly over days sampled. The percentage of CBG-C decreased in all animals from d 9-20. In Trt 1 and Trt 2 CBG-C values were lowest on day 20 (23 % and 24 % respectively ) compared to control gilts (41.8 %) (Figure 5). The percentage of albumin bound cortisol (Alb-C), which was highly correlated ( $r = -.96$) to CBG-C values, was not different ($p>.05$) between treatments (Table 5). However, there was a significant ($p<.01$) day effect in all treatments from days 9-20 (Figure 5). In Trt 1 and Trt 2 Alb-C values were highest on day 20 (61.5 % and 62.5 % respectively) compared to control gilts (45 %).

CBG Binding Capacity

The CBG-BC was not affected ($p>.05$) by treatment regardless of day sampled (Table 5). Although not significant CBG-BC concentrations declined across days in all animals (Figure 6).

Plasma Progesterone

Treatment had no effect ($p>.05$) on plasma progesterone concentrations (Table 6). Plasma progesterone

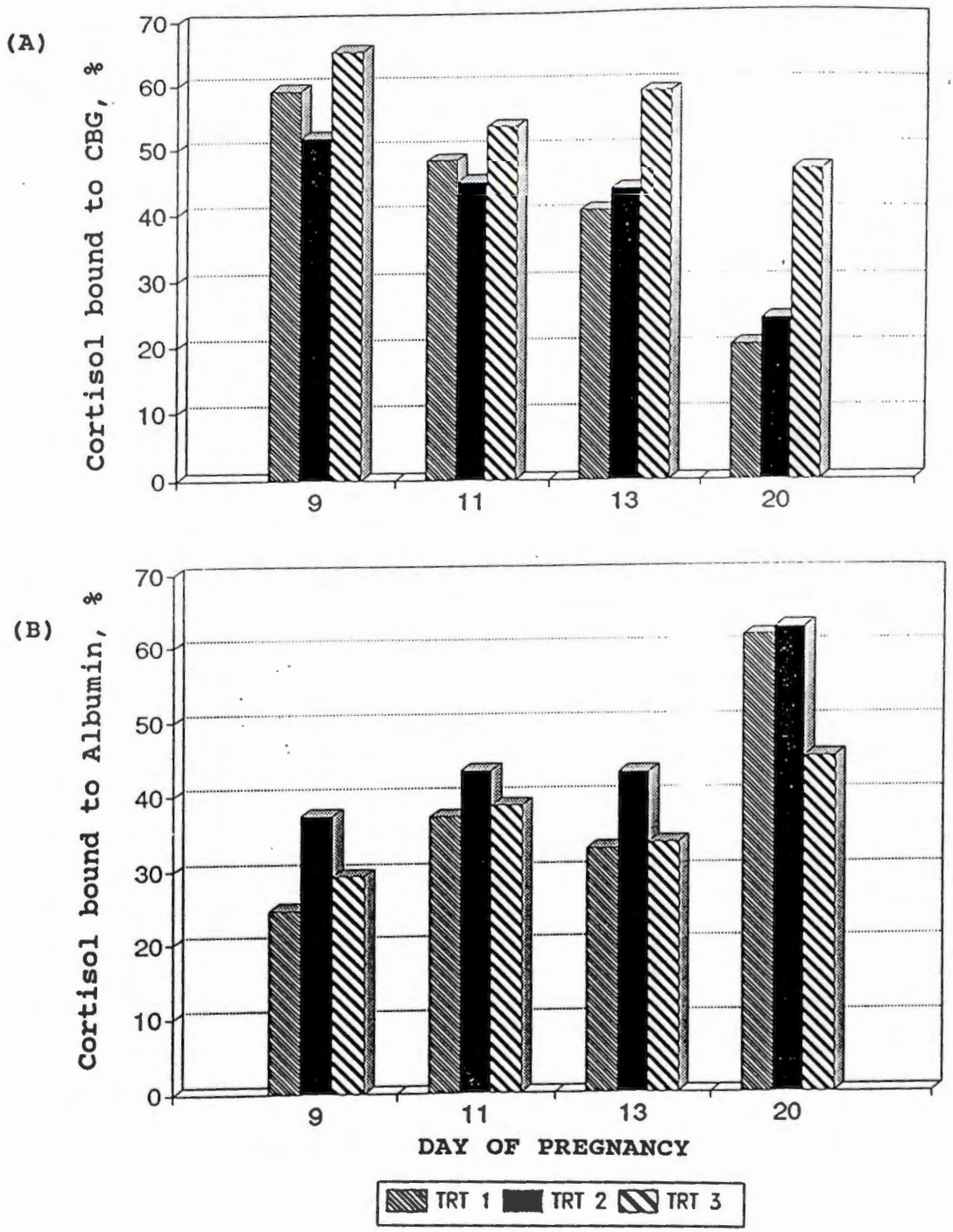


Figure 5. Percent cortisol bound to CBG (A) and percent cortisol bound to albumin in pregnant gilts following twice daily treatment of 5 mg/kg hydrocortisone acetate (HA; Trt1), 2.5 mg/kg HA (Trt2), or sesame oil (Control; Trt 3) on d 9-13 of pregnancy.

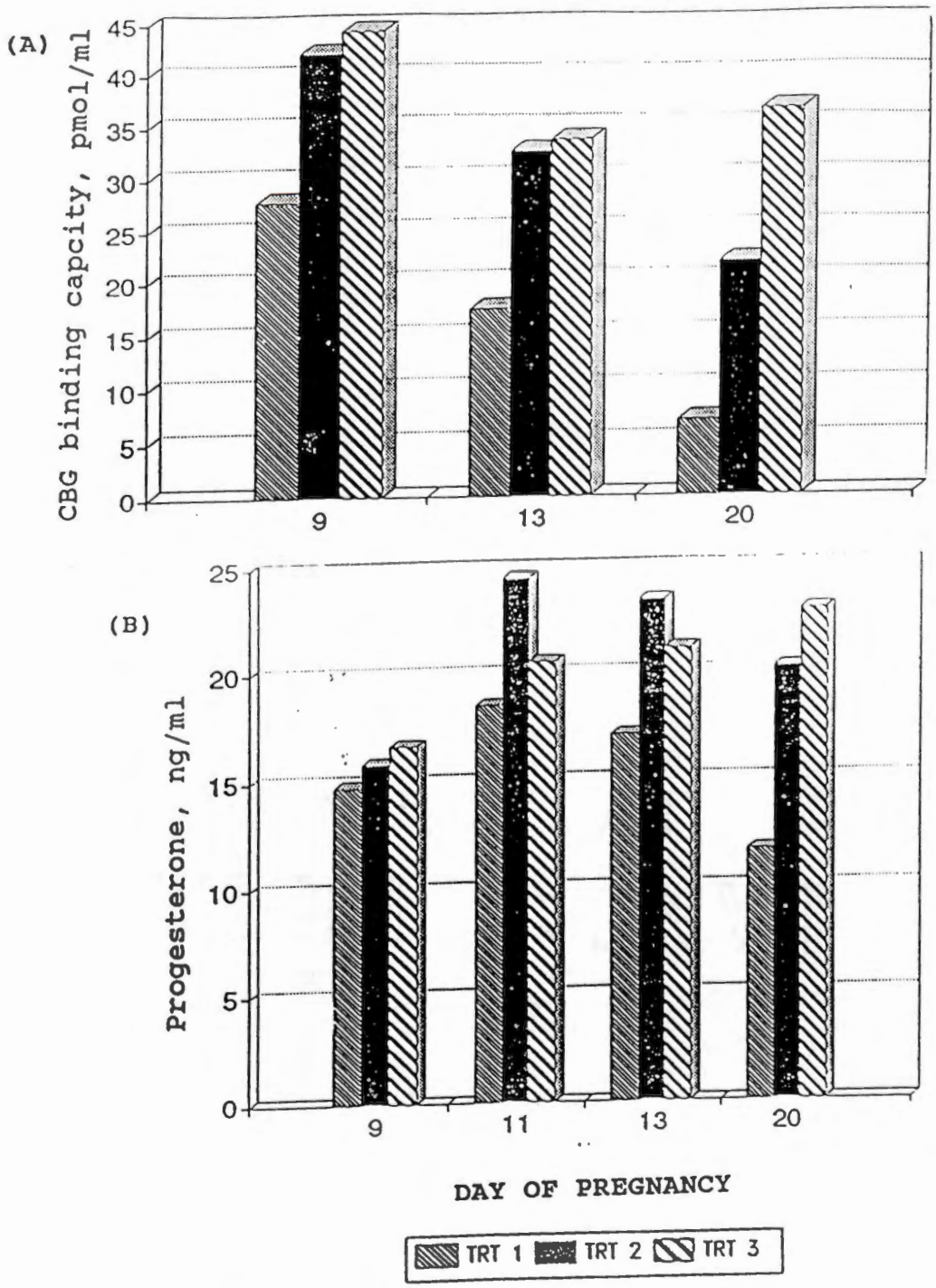


Figure 6. CBG binding capacity (A) and progesterone levels in pregnant gilts following twice daily administration of 5 mg/kg hydrocortisone acetate (HA; Trt1), 2.5 mg/kg HA (Trt2), or sesame oil (Control; Trt 3) on d 9-13 of pregnancy.

Table 6. Plasma progesterone ($P_4$) and total plasma protein (Prot) concentration in gilts treated with 5 mg/kg bodyweight (BW) hydrocortisone acetate (HA; Trt 1;n=4), 2.5 mg/kg BW HA (Trt 2;n=6), or sesame oil (control; Trt 3;n=5) on d 9-13 of pregnancy†.

| | Day of Pregnancy | | | |
|---|---|---|---|---|
| Item | 9 | 11 | 13 | 20 |
| **$P_4$ (ng/ml)** | | | | |
| Trt 1 | $15^a \pm 3$ | $21^b \pm 3$ | $19^b \pm 3$ | $17^c \pm 3$ |
| Trt 2 | $16^a \pm 3$ | $24^b \pm 3$ | $23^b \pm 3$ | $20^b \pm 3$ |
| Trt 3 | $16^a \pm 3$ | $21^b \pm 3$ | $20^b \pm 3$ | $24^b \pm 3$ |
| | NS | NS | NS | NS |
| **Prot (mg/ml)** | | | | |
| Trt 1 | $3.8 \pm 2.0$ | $4.1 \pm 2.0$ | $6.4 \pm 2.0$ | $6.0 \pm 2.0$ |
| Trt 2 | $3.2 \pm 1.8$ | $5.0 \pm 2.3$ | $9.8 \pm 1.8$ | $4.1 \pm 2.3$ |
| Trt 3 | $5.4 \pm 2.3$ | $7.7 \pm 2.3$ | $4.2 \pm 2.0$ | $8.2 \pm 2.0$ |
| | NS | NS | NS | NS |

† Data presented as least squares means ± SEM.

abc Numbers with different subscripts are different within rows ($p<.05$).

NS - Not significant

concentrations increased ($p<.05$) from day 9 to 11 in all animals and remained unchanged thereafter (Figure 6).

## Total Plasma Proteins

Total plasma proteins were not different ($p>.05$) between treatments or days of pregnancy (Table 6) in any group. Protein values ranged from 3 mg/ml on d 9 to a high of 10 mg/ml on d 13.

## Corpora Lutea Number and Weight

Total and individual ovary CL numbers and weight were not different ($p>.05$) between treatments as measured on d 46$\pm$2 of pregnancy (Table 7).

## Fetal Number, Weight, and Length

Fetal number was not different ($p>.05$) between treatments (Table 7). The overall average number of fetuses per gilt was 8. Fetal weight was different ($p<.05$) between treatments. Fetal weight for Trt 2 animals averaged 23.7 g compared to Trt 1 and control gilts (27.4 and 26.3 g respectively). Fetal lengths were not different ($p>.05$) between treatments and the average fetal length was 72.3 mm.

## Placental Weight, Allantoic and Amniotic Fluid Volumes

Placental weight in Trt 1 tended to be higher ($P<.1$) than Trt 2 and control gilts. Trt 1 placental weights were

highest (89.6 g) compared to Trt 2 and control animals (81.9 and 77.9 g, respectively). Allantoic fluid volume per fetus in Trt 2 gilts (27.5 ml) was less ($p<.001$) than that found in Trt 1 (84.2 ml) and control gilts (71.4 ml). Amniotic fluid volumes were similarly affected ($p<.05$) by treatment (Table 7). Fetuses from gilts in Trt 2 had 23.5 ml of amniotic fluid compared to fetuses of in Trt 1 and control gilts (27.2 and 26 ml respectively).

Table 7. Corpora lutea number, CL weight, fetal number, fetal weight, fetal length, placental weight (Plac-Wgt), Amniotic (Amn Flu) and Allantoic fluid (All Flu) volume in gilts treated with 5 mg/kg bodyweight (BW) hydrocortisone acetate (HA; Trt 1; n=4), 2.5 mg/kg BW HA (Trt 2;n=6), or sesame oil (control; Trt 3;n=5) on d 9-13 of pregnancy†.

| Item | Day 46± 2 of Pregnancy Left Horn | Right Horn |
|---|---|---|
| **CL No.** | | |
| Trt 1 | 6 ± 1 | 8 ± 1 |
| Trt 2 | 7 ± 1 | 7 ± 1 |
| Trt 3 | 8 ± 1 | 6 ± 1 |
| **CL Wgt (g)** | | |
| Trt 1 | 3.5 ± .6 | 3.1 ± .7 |
| Trt 2 | 3.3 ± .6 | 3.4 ± .7 |
| Trt 3 | 3.1 ± .6 | 3.8 ± .7 |
| **Fetal No.** | | |
| Trt 1 | 3.5 ± .4 | 4.1 ± .4 |
| Trt 2 | 3.7 ± .4 | 3.6 ± .4 |
| Trt 3 | 3.7 ± .4 | 3.7 ± .4 |
| **Fetal Wgt (g)** | | |
| Trt 1 | 28 ± 1 | 27 ± 1 |
| Trt 2 | 24 ± 2** | 23 ± 1** |
| Trt 3 | 26 ± 2 | 26 ± 1 |
| **Fetal Lgth (mm)** | | |
| Trt 1 | 74 ± 2 | 73 ± 2 |
| Trt 2 | 71 ± 2 | 71 ± 2 |
| Trt 3 | 73 ± 2 | 72 ± 2 |
| **PLAC Wgt (g)** | | |
| Trt 1 | 95 ± 6 | 85 ± 6 |
| Trt 2 | 83 ± 5 | 80 ± 5 |
| Trt 3 | 80 ± 5 | 76 ± 5 |
| **ALL FLU (ml)** | | |
| Trt 1 | 87 ± 13 | 81 ± 13 |
| Trt 2 | 28 ± 12** | 27 ± 12** |
| Trt 3 | 68 ± 11 | 74 ± 11 |
| **AMN FLU (ml)** | | |
| Trt 1 | 29 ± 1.4 | 25.2 ± 1.4 |
| Trt 2 | 23 ± 1.4* | 23.8 ± 1.4* |
| Trt 3 | 26 ± 1.2 | 25.8 ± 1.2 |

† Data presented as least squares means ± SEM.
* Trt 2 is different ($p<.05$) from Trt 1 and Trt 3 gilts.
*** Trt 2 is different ($p<.01$) from Trt 1 and Trt 2 gilts.

# CHAPTER V

# DISCUSSION

## EXPERIMENT ONE

The results obtained from this experiment confirm that multiple injections of estradiol will prolong the lifespan of the corpora lutea. Estradiol benzoate treatment from d 11-15 of the estrous cycle has been reported to extend the luteal function in swine for 70-300 d (Bazer et al., 1982; Geisert et al., 1987).

Plasma cortisol (total, % unbound, % CBG bound, and % albumin bound) levels have not been documented previously in pseudopregnant gilts. Total plasma cortisol levels averaged 30.2 ng/ml on d 11 and 34.5 ng/ml on d 21 in all pseudopregnant gilts. Administration of hydrocortisone acetate (HA; 600 mg) twice daily on d 21-30 of pseudopregnancy was effective in raising total plasma cortisol levels. HA-treated gilts averaged 295 $\pm$ 30 ng/ml of cortisol compared to 35 $\pm$ 9 ng/ml in control gilts on d 31. Barb and coworkers (1982) reported elevated cortisol levels in response to twice daily injections of HA (250 mg) for the first 12 d of the estrous cycle in gilts. In their study HA treated gilts had 60 ng/ml of cortisol compared to 20 ng/ml in control gilts.

Unbound cortisol levels were not affected by treatment. This would imply that the animal was not under a chronic stress based on previous studies (Kattesh et al., 1980; Barnett et al., 1981a, b, 1983; Hemsworth et al., 1987) in which unbound cortisol levels were elevated and maximum corticosteroid binding capacity (MCBC) or corticosteroid binding capacity (CBC) remained constant or decreased. The percentage of cortisol bound to CBG or albumin was not different between treatments. It is possible that the large amount of cortisol found in the plasma of HA-treated gilts kept the CBG from being totally denatured during heating in the distribution assay (Burton and Westphal, 1972; Hammond et al, 1990 ). If this were the case then the percentage of unbound cortisol would also have been affected, since a decrease in the percent CBG-C would result in an increase in UB-C.

The binding capacity of CBG (as an indirect measure of CBG concentration) for cortisol was affected ($p<.001$) by HA treatment. The binding capacity decreased to $7 \pm 6$ pmol/ml compared to $39 \pm 6$ pmol/ml in control gilts following 10 days of HA treatment, indicating that CBG was decreased in HA-treated gilts. This assay is probably a better measure of changes in CBG. Hammond and coworkers (1990) showed that there was no difference between CBG-BC and a direct measure for CBG as measured by radioimmunoassay in humans.

Total uterine cortisol levels were higher ($p<.001$) in

HA-treated gilts compared to control gilts, indicating that cortisol, especially when elevated, can be transported into the uterine lumen. The cortisol found in the uterus is biologically active based upon an examination of percent cortisol distribution in uterine flushings in our laboratory. Parrott et al. (1989) found that cortisol in pig saliva is also unbound and biologically active. The absence of CBG in the uterine flushings and saliva (Parrott et al., 1989) of swine suggest that CBG cannot pass across the cellular membranes into other bodily fluids. The mechanism that prevents CBG from entering the uterus or salivary glands is not known. The function of free cortisol in the uterus is not known. Rabin and coworkers (1990) found that glucocorticoids inhibited estrogen stimulated uterine growth, and decreased uterine estradiol receptor concentration but did not affect the estrogen induction of the progesterone receptors in rats. Uterine size was not measured in the present study. However, the elevated levels of cortisol in the uterine flushings may have a similar affect on estrogen receptor numbers and uterine growth in swine like that found in rats.

Plasma progesterone ($P_4$) concentrations have not been previously documented in pseudopregnant gilts treated with hydrocortisone acetate. The $P_4$ levels for control gilts were similar to concentrations found in other studies (Frank et al., 1977; Zieick et al., 1986; King and Rajamahendran,

1988). Interestingly, plasma $P_4$ levels were reduced ($p<.01$) as a result of the HA treatment. Although LH levels were not measured, the lower $P_4$ levels on day 31 may have been caused by a decrease in LH secretion due to the elevated cortisol in HA-treated gilts. Barb and coworkers (1982) showed that cortisol was capable of suppressing the preovulatory surge of LH in prepuberal gilts. In pseudopregnant rats subjected to restraint stress from d 1-6 of pseudopregnancy corticosterone levels were elevated and $P_4$ levels were lower ($p<.05$) compared to control animals (Moorehead and Gala, 1989).

Corpora lutea $P_4$ concentrations were not different between treatments, but uterine flush $P_4$ levels were lower ($p<.001$) in HA-treated gilts (d 31), suggesting that elevated cortisol levels in HA-treated gilts could have directly affected the secretion of $P_4$ from the ovary. Levels of $P_4$ in the uterine flush mimic the decrease in plasma $P_4$ levels in HA treated gilts.

Total plasma proteins were not affected by treatment. These results suggest that HA treatment had no effect on liver protein production. Our results are similar to Barnett and coworkers (1984) in which protein levels were not affected by a chronic stress.

Uterine protein levels were affected ($p<.001$) by HA-treatment. Uterine protein values for control gilts were similar to results reported by Geisert and coworkers (1987).

They reported uterine protein values of 25.4 mg/ml on day 14 of pseudopregnancy, which is comparable to that seen on day 31 of pseudopregnancy for control gilts (21.4 mg/ml) in the present study. HA-treated gilts had 8.2 mg/ml of total proteins in the uterine flush. The lower protein levels in the uterus of HA-treated gilts could reflect a decrease in progesterone induced proteins, resulting from the lower plasma and uterine $P_4$ levels. Since plasma and uterine $P_4$ levels were decreased due to HA-treatment, it is possible that the progesterone induced proteins could account for the decrease in total uterine proteins. In pregnant gilts total uterine proteins were not affected by heat stress (37°C) on d 8-16 of pregnancy (Wetterman et al., 1988). They reported an average of 3.5 mg/ml of total protein in the uterine flushings of all gilts. Hembree (1983) reported no difference in uterine proteins of cycling gilts administered HA on d 8-13 of the estrous cycle compared to control gilts.

The number of CL were not affected by HA-treatment but, the CL tended ($p<.1$) to weigh less in HA-treated gilts (2.0 g) compared to control gilts (2.7 g). The lower CL weights in HA-treated gilts could account for the decreased $P_4$ concentrations found in the plasma and uterine flush.

The high cortisol concentrations (295$\pm$30 ng/ml) observed in HA-treated gilts and the decrease in CBG-BC levels would suggest the animals were under a chronic stress. If the time period for administration of HA would

have been lengthened, $P_4$ levels may have decreased to a level low enough to cause the animal to cycle, since levels below 5 ng/ml indicate regression of the CL in sows (Coryn et al., 1979). A study in which HA was administered for 15-20 days during pseudopregnancy might reveal the length of time an animal must be exposed to a chronic stress before reproduction is affected. Pseudopregnant gilts provide a good model for studies on the affects of stress on a pregnant animal without interference from the conceptus.

## EXPERIMENT TWO

The elevated cortisol levels on d 13 indicate that 5 mg/kg HA (Trt 1) and 2.5 mg/kg HA (Trt 2) administered twice daily from d 9-13 sufficiently increased cortisol levels similar to that seen in animals representative of a stress response. Total cortisol levels in control gilts averaged 39.3 ng/ml across d 9-20 and were similar to results reported by Kattesh and coworkers (1980) in which pregnant gilts averaged 30.5 ng/ml on d 42 of pregnancy.

The percentage of unbound cortisol was not affected by treatment. These results differ from previous results in which pregnant gilts housed in tethers (restraint strap) for 46 days post breeding had higher overall free cortisol levels compared to control gilts (Barnett et al., 1985). It is possible that the 5 day regimen of HA treatment was

insufficient to simulate a chronic stress response as reflected by the percentage free cortisol.

The percentage of CBG bound cortisol (CBG-C) or albumin bound cortisol (Alb-C) was not affected by treatment. The % CBG-C decreased in Trt 1 and Trt 2 from d 9 (65%±9 and 51%±7, respectively) to d 20 (23%±11 and 42%±9 respectively). Control gilts were not different from d 9 (61%±9) to d 20 (42%±8). The percentage of Alb-C was highly correlated (r=-.96) to CBG-C, and as CBG-C decreased Alb-C increased inversely from d 9-20. Sitteri et al. (1982) showed that since albumin has a low affinity for cortisol, albumin bound cortisol can be considered free cortisol. In the present study cortisol bound to albumin increased as CBG bound cortisol decreased. Therefore, considering that the combination of unbound and albumin bound cortisol is essentially biologically active cortisol then an increase in plasma free cortisol did occur in the present study as a result of HA administration.

The CBG binding capacity (CBG-BC) in Trt 1 and Trt 2 was lower on d 20 (7±16 and 18±22 pmol/ml respectively) compared to d 9 (26±11 and 42±11 respectively) of pregnancy. Control values for CBG-BC were relatively constant across days, ranging from 42±13 on d 9 to 37±13 pmol/ml on d 20. The large variation among animals within treatment could explain why a significant difference between treatments was not detected.

Plasma progesterone ($P_4$) levels were not affected by treatment, but were affected by pregnancy. Plasma $P_4$ levels agree with values reported by Zeicik and coworkers (1986) and King and Rajanahedran (1988). In beef cows heat stress (37·C) on d 8-16 of pregnancy had no affect on $P_4$ levels (Biggers et al., 1987). In pregnant ewes heat stress (40·C) for 10 wks starting on d 60 lowered $P_4$ levels (Bell et al., 1989). Thus duration of stress or HA administration (d 9-13) may be a determining factor in its affect on $P_4$ levels.

Total plasma proteins were not different between treatment and are similar to results reported by Barnett and coworkers (1985).

Fetal number was not affected by treatment, but fetal weight was significantly ($p<.001$) affected by HA in Trt 2 (2.5 mg/kg HA). Average fetal weight was 23.8±1.2 g in Trt 2 compared to 27.5±1.2 g in Trt 1 (5 mg/kg HA) and 26.4±1.2 g in control gilts. In fetal sheep a decrease in body weight was correlated with a decrease in placental weight when the dam was under a prolonged heat stress (40 ·C) between d 64-136 of pregnancy (Bell et al., 1989). In the present study placental weight was 83±5 g in Trt 2 compared to 80±5 g in control gilts. In Trt 2 (2.5 mg/kg HA) allantoic and amniotic fluid volumes were lower ($p<.001$ and $P<.05$ respectively) compared to Trt 1 (5 mg/kg HA) and control gilts. The decrease in fluid volumes could account for the lower fetal weights in Trt 2 gilts. The difference

in Trt 2 compared to Trt 1 and control gilts cannot be explained from this study.

The time period in which treatments were administered, day 9-13 of pregnancy, may not be long enough to affect reproduction. Until day 10 the CL appears to be unaffected by exogenous prostaglandin ($PGF_2\alpha$) (Bazer and Thatcher, 1977), possibly the effects of HA are also hindered.

# CHAPTER VI

# SUMMARY

## EXPERIMENT ONE

Eleven crossbred gilts exhibiting a normal estrous cycle (18-23 d) were administered estradiol valerate (5 mg) twice daily on d 11-15 to induce pseudopregnancy. Gilts were randomly selected to receive either 5 mg/kg body weight (avg. BW =120 kg) of hydrocortisone acetate (HA) in sesame oil (5ml) or sesame oil (5 ml;control) subcutaneously twice daily on d 21-30 of pseudopregnancy. Blood samples (20 ml) were collected on d 11, 21 and 31 prior to treatment administration. Uterine flushings were obtained on the day following the last day of treatment.

Total plasma cortisol levels were elevated ($p<.001$) due to 10 d of HA treatment compared to control animals. Percent distribution of cortisol; % unbound cortisol (UB-C), % CBG bound cortisol (CBG-C), and albumin bound cortisol (Alb-C) was not affected by treatment. CBG binding capacity (CBG-BC) was lower ($p<.001$) in HA treated gilts compared to control gilts on d 31 (7.4 vs. 38.7 pmol/ml, respectively). The decrease in CBG-BC indicated that the amount of CBG decreased due to treatment. Plasma $P_4$ levels were decreased ($p<.01$) due to HA treatment (8.9 ng/ml) compared to control gilts (17.8 ng/ml). Total plasma protein and albumin

concentrations were not different between treatments.

Cortisol concentrations in the uterine flushings of HA treated gilts was higher ($p<.001$) compared to control animals. Cortisol in the uterine flushings is unbound, and therefore biologically active. This suggests that CBG is cannot be transported across the uterus. $P_4$ levels were significantly ($p<.001$) decreased in HA treated gilts (2.0 ng/ml) compared to control gilts (6.8 ng/ml). Protein concentrations in the uterine flush of HA treated gilts were lower ($p<.001$) compared to control gilts. Albumin concentrations in the uterine flush were not affected by treatment. CL number and $P_4$ concentrations were similar between treatments. CL weight was significantly ($p<.01$) lower in HA treated gilts. These results indicate that elevated cortisol levels can alter hormone and protein concentrations related to pregnancy. The pseudopregnant animal is a good model to use when trying to understand the affects of stress without the interference of the fetuses.

## EXPERIMENT TWO

Eighteen crossbred gilts exhibiting a normal estrous cycle (18-23 d) were naturally bred by a mature boar on the first and second day of estrus. Gilts were randomly assigned to receive HA (5 mg/kg BW; Trt 1), HA (2.5 mg/kg BW; Trt 2), or sesame oil (control) twice daily on d 9-13 of pregnancy. Blood samples were collected on d 9, 11, 13, and

20 of pregnancy. On d 46 gilts were slaughtered and reproductive tract were collected. Number, Weight, and crown rump length of fetuses was measured and recorded.

Total plasma cortisol levels were increased ($p<.05$) in Trt 1 and Trt 2 groups compared to control gilts. Percent distribution; % UB-C, % CBG-C, and % Alb-C was not significantly ($p>.05$) different among all animals sampled.

CL number and weight were similar in all animals sampled. Fetal number, length, and placental weight were not different due to HA treatment compared to control gilts. Fetal weight was lower ($p<.05$) in Trt 2 (2.5 mg/kg HA) compared to Trt 1 5 mg/kg HA) and control gilts. Allantoic and amniotic fluid volumes were lower ($p<.001$ and $p<.05$, respectively) in Trt 2 gilts compared to Trt 1 and control gilts. The results from this study indicate that elevated cortisol levels can affect certain parameters associated with pregnancy.

# LITERATURE CITED

## LITERATURE CITED


Andrews, R. V. 1977. Influence of the adrenal gland on gonadal function. In: J. A. Thomas and R. L. Singhal (Ed.), Regulatory Mechanisms Affecting Gonadal Hormone Action. University Park Press, Baltimore. Pp. 197-215.

Arnold, J. D., H. G. Kattesh, T. T. Chen, and R. L. Murphree. 1982. Adrenocorticotropin administration during early gestation on conceptus development in swine. Theriogenology. 17: 475-484.

Babin, D. S., E. O. Johnson, D. D. Brandon, C. Liapi, and G. P. Chrousos. 1990. Glucocorticoids inhibit estradiol-mediated uterine growth: possible role of the uterine estradiol receptor. Biol. Reprod. 42:74-80.

Baldwin, B. A. and D. B. Stevens 1973. The effect of conditioned behavior and environmental factors on plasma corticosteroid levels in pigs. Physio. Behav. 10:267-274.

Baldwin, D. M. 1979. The effects of glucocorticoids on estrogen dependent luteinizing hormone release in the ovariectomized rat and on gonadotropin secretion in the intact female rat. Endocrinology. 105:120-128.

Barb C. R., R. R. Kraeling, G. B. Rampacek, E. S. Fonda, and T. E. Kiser. 1982. Inhibition of ovulation and LH secretion in the gilt after treatment with ACTH or hydrocortisone. J. Reprod. Fert. 64:85-92.

Barnett, J. L., G. M. Cronin, and C. G. Winfield. 1981a. The effects of individual and group penning of pigs on total and free corticosteroids and the maximum binding capacity. Gen. Comp. Endocrinol., 44:219-225.

Barnett, J. L., G. M. Cronin, C. G. Winfield, and A. M. Dewar. 1984. The welfare of adult pigs: the effects of five housing treatments on behaviour, plasma corticosteroids and injuries. Appl. Anim. Behaviour Sci. 12:209-232.

Barnett J. L., C. G. Winfield, G. M. Cronin, and A. W. Makin. 1981b. Effects of photoperiod and feeding on plasma corticosteroid concentrations and maximum corticosteroid-binding capacity in pigs. Aust. J. Biol. Sci. 34:577-585.

Barnett, J. L., P. H. Hemsworth, and A. M. Hand. 1982. Effects of chronic stress on some blood parameters in the pig. App. Anim. Behav. Sci. 9:273-277.

Barnett, J. L., C. G. Winfield, G. M. Cronin, P. H. Hemsworth, and A. M. Dewar. 1985 The effect of individual and group housing on behavioral and physiological responses related to the welfare of pigs. App. Anim. Behav. Sci. 14:149-161.

Bazer, F. W., R. D. Geisert, W. W. Thatcher, and R. M. Roberts. 1982. The establishment and maintenance of pregnancy. In D. J. A. Cole and G. R. Foxcroft (editors), Control of Pig Reproduction. Butterworths, London. p. 227-252.

Bazer, F. W. and W. W. Thatcher. 1977. Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin by the uterine endometrium. Prostaglandins. 14:397-401.

Behrens S. and N. Parvizi. 1988. Endogenous opiods and their effects on reproduction. Tierarztl-Prax. 16:213-217.

Bell, A. W., B. W. McBride, R. Slepetis, R. J. Early, and W. B. Currie. 1989. Chronic heat stress and prenatal development in sheep: I. Conceptus growth and maternal plasma hormones and metabolites. J. Anim. Sci. 67:3289-3299.

Biggers, B. G., R. D. Geisert, R. P. Wettemann, and D. S. Buchanan. 1987. Effect of heat stress on early embryonic development in the beef cow. J Anim Sci. 64:1512-1518.

Burton, R. M. and U. Westphal. 1972. Steroid hormone- binding proteins in blood plasma. Metabolism. 21:253-276.

Christenson, R. K. 1980. Environmental influences on the postpartum animal. J. Anim. Sci. (suppl. 2) 51:53-67.

Coryn, M., J. Spincemaille and M. Vandeplasssche. 1979. Estrous cycle, pregnancy and parturition in the mouse, cow and sow: Progesterones and estrogens. Annls. Endocr. 40:511-512.

Coubrough, R. 1985. Stress and fertility. A review. Onderstepoort J. Vet. Res. 52:153-156.

Dalin, A. M., L. Nyberg, and L. Eliasson. 1988. The effect of transportation/relocation on cortisol, CBG and induction of puberty in gilts with delayed puberty. Acta. Vet. Scand. 29:207-217.

Dantzer, R., H. W. Gonyou, S. E. Curtis, and K. W. Kelly. 1987. Changes in serum cortisol reveal functional differences in frustration-induced chain chewing in pigs. Physiol. Behavior. 39:775-777.

Dobson, H. 1987. Effect of transport stress on luteinizing hormone released by GnRH in dairy cows. Acta Endocrinol. 115:63-70.

Dreiling, C. E., F. S. Carman, and D. E. Brown. 1990. Maternal endocrine and fetal metabolic response to heat stress. J. Dairy Sci. 74:312-327.

England, D. C. and D. T. Spurr. 1969. Litter size of swine confine during gestation. J. Anim. Sci. 28:220-225.

Feldmann, D., C. E. Mondon, J. A. Horner, and J. N. Weiser. 1979. Glucocorticoid and estrogen regulation of corticosteroid-binding globulin production by rat liver. Amer. J. Physiol. 237:E493-E499.

Fischer, H. E., F. W. Bazer, and M. J. Fields. 1985. Steroid metabolism by endometrial and conceptus tissues during early pregnancy and pseudopregnancy in gilts. J Reprod. Fert. 75:69-78.

Flint, A.F., R. D. Burton, J. E. Gadsby, R. B. Heap, and E. L. Sheldrick. 1984. Embryonic steroid synthesis and luteal oxytocin production: controlling mechanisms for the maternal recognition of pregnancy. J. Steroid. Biochem. 19:973-978.

Flowers, B., T. C. Cantley, M. J. Martin, and B. N. Day. 1988. Effect of elevated ambient temperatures on puberty in gilts. J. Anim. Sci. 67:779-784.

Flukerson, W. J. and B. Y. Tang. 1979. Ultradian and circadian rhythms in the plasma concentration of cortisol in sheep. J. Endocrinol. 81:135-140.

Frank, M., Bazer, F. W., Thatcher, W. W., and Wilcox, C. J. 1977. A study of prostaglandin F2A as a luteolysin in swine: III. Effects of estradiol valerate on prostaglandin F,progestins, estrone and estradiol concentrations in utero-ovarian vein of non-pregnant gilts. Prostaglandins 14:1183-1196.

Fuquay, J. W. 1981. Heat stress as it effects animal reproduction. J. Anim. Sci. 52: 164-174.

Geisert, R. D., M. T. Zavy, R. P. Wettemann, and B. G. Biggers. 1987. Length of pseudopregnancy and pattern of uterine protein as influenced by time and duration of oestrogen administration in the pig. Reprod. Fert. 79:163-172.

Geisert, R. D., M. T. Zavy, R. J. Moffatt, R. M. Blair, and T. Yellin. 1990. Embryonic steroids and the establishment of pregnancy in pigs. J. Reprod. Fert. Suppl. 40:293-305.

Geisert, R. D., R. H. Renegar, W. W. Thatcher, R. M. Roberts, and F. W. Bazer. 1982. Establishment of pregnancy in the pig. I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol. Repro. 27:925-939.

Goodnight, J. W., J. P. Sall and W. S. Sarle, 1982. GLM procedure. In: Alice A. Ray (Ed.), SAS User's Guide: Statistics. SAS Institute Inc., Cary, North Carolina. p. 139.

Gwazdauskas, F. C. 1985. Effects of climate on reproduction on cattle. J. Dairy Sci. 68:1568-1578.

Hammond, G. L. and P. A. Lahteenmaki. 1983. A versatile method for the determination of serum cortisol binding globulin and sex hormone binding globulin binding capactites. Clinica Chimica Acta. 132:101-110.

Hammond, G. L., J. A. Nisker, L. A. Jones and P. K. Siiteri. 1980. Estimation of the percentage of free steroid in undiluted serum by centrifugal ultrafiltration-dialysis. J. Biol. Chem. 255:5023-5026.

Hembre, J. R. 1983. Plasma cortisol and progesterone concentrations, uterine proteins and corpora lutea LH/hCG binding characteristics in gilts following adrenocorticotropin or hydrocortisone acetate administration during diestrus. M. S. Thesis. The University of Tennessee.

Hemsworth, P. H., J. L. Barnett, and C. Hansen. 1987. The influence of inconsistent handling by humans on the behaviour, growth and corticosteroids of young pigs. Appl. Anim. Behav. Sci. 17:245-252.

Hennessy, D. P. and P. Williamson. 1983. The effects of stress and ACTH administration in hormone profiles, oestrus and ovulation in pigs. Theriogenology. 20:13-26.

Herbein, J. H., R. J. Martin, L. C. Griel, and J. F. Kavanaugh. 1977. Serum hormones in the perinatal pig and the effect of exogenous insulin on blood sugars. Growth. 41:277-283.

Hoagland, T. A. and R. P. Wettemann. 1984. Influence of elevated ambient temperature after breeding on plasma corticoids, estradiol and progesterone in gilts. Theriogenology. 22:15-24.

Howarth, Jr. and H. W. Hawk. 1968. Effect of hydrocortisone on embryonic survival in sheep. J. Anim. Sci. 27:117-121.

Johnson, L. W., 1989. Llama reproduction. Vet. Clin. North. Amer. Food Anim. Prac. 5:159-182.

Juniewicz, P. E. and B. H. Johnson. 1981. Influence of adrenal steroids upon testosterone secretion by the boar testis. Biol. Reprod. 29:464-471.

Kaplan, N. M. 1988. The adrenal glands. In: Text Book of Endocrine Physiology. J. E. Griffin and S. R. Ojeda eds., Oxford University press, New York. pp. 245-272.

Kattesh, H. G., S. F. Charles, G. A. Baumbach, and B. E. Gillespie. 1990. Plasma cortisol distribution in the pig from birth to six weeks of age. Bio. Neonate. 58:220-226.

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. 50:897-905.

King, G. J and R. Rajamahendran. 1988. Comparison of plasma progesterone profiles in cyclic, pregnant, pseudopregnant and hysterectomized pigs between 8 and 27 days after oestrus. J. Endocr. 119:111-116.

Li, P. S. and W. C. Wagner. 1983. In vivo and invitro studies on the effect of adrenocorticotropic hormone or cortisol on the pituitary response to gonadotropin releasing hormone. Biology Reprod. 29:25-37.

Liptrap, R. M. 1970. Effect of corticotropins and corticosteroids on oestrus, ovulation, and oestrogen secretion in the sow. J. Endocrinology. 47:197-205.

Liptrap, R. M. 1973 Oestrogen secretion by sows with induced cystic ovarian follicles. Res. Vet. Sci. 15:215-219.

MacLusky, N. J., F. Naffolm, and C. Levanth. 1988. Immunocytochemical evidence for direct synaptic connections between corticotropin-releasing factor (CRF) and gonadotropin-releasing factor (GnRH)-containing neurons in the preoptic area of the rat brain. Brain Res. 439:391-396.

McCarthy, J. L., R. C. Corely, and M. X. Zarrow. 1960. Diurnal rhythm in plasma corticosterone and lack of diurnal rhythm in plasma compound F-like material in the rat. Proc. Soc. Exp. Bio. Med. 104:787-791.

McCauley, I. and P. E. Hartmann. 1984. Changes in piglet leucocytes, б-lymphocytes and plasma cortisol from birth to three weeks after weaning. Res. Vet. Sci. 37:234-241.

Moberg, G. P. 1976. Effect of environment and management stress on reproduction in dairy cows. J. Dairy Sci. 59:1618-1624.

Moberg, G. P. 1985. Influence of stress on reproduction: measure of well being. In: Animal Stress. G. P. Moberg, ed. Am. Physiol. Soc., Bethesda, MD. p. 245-268.

Moberg, G. P., 1987. Influence of the adrenal axis upon the gonads. In: Oxford Reviews of Reproductive Biology. J. Clarke, ed. Oxford Univ. Press, Oxford England. p. 456-496.

Moberg, G. P., 1990. How behavioral stress disrupts the endocrine control of reproduction in domestic animals. J. Dairy Sci. 74:304-311.

Moberg, G. P., D. P. Stoebel, and R. Cook. 1981. Effect of dexamethasone on the oestrogen-induced release of luteinizing hormone in the anoestrous ewe. J. Endocrinol. 90:221-225.

Morehead, M. H. and R. R. Gala. 1989. The restraint stress-induced decrease of the nocturnal prolactin surge and the physiology of pseudopregnancy and pregnancy in the rat. Life Sci. 45:207-215.

Munck, A., P. L. Guyre, and N. J. Holbrook. 1984 Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Rev. 5:25-44.

Murray, F. A., F. W. Bazer, H. D. Wallace and A. C. Warnick. 1972. Quantitative and qualitative variation in the secretion of protein by the porcine uterus during the estrous cycle. Biol. Reprod. 7:314-320.

Naylor, A. M., D. W. Porter and D. W. Lincoln. 1990. Central administration of corticotropin-releasing factor in the sheep: effects on secretion of gonadotropins, prolactin and cortisol. J. Endocrinol. 124:117-124.

Olster, D. H. and M. Ferin. 1987. Corticotropin-releasing hormone inhibits gonadotropin secretion in the ovariectomized Rhesus monkey. J. Clin. Endocrinol. Metab. 65:262-266.

Omtvedt, I. T., R. E. Nelson , R. 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. 32:312-317.

Parrott, R. F, B. H. Misson, and B. A. Baldwin. 1989. Salivary cortisol in pigs following adrenocorticotrophic hormone stimulation: comparison with plasma levels. Br. Vet. J. 145:362-366.

Perry, J. S. and I. W. Rowlands. 1962. Early pregnancy in the pig. J. Reprod. Fert. 4:175-188.

Petraglia, F., S. Sutton, W. Vale, and C. Rivier. 1986. Opioids act centrally to modulate stress-induced decrease in luteinizing hormone in the rat. Endocrinology. 119: 2445-2450.

Prezkop, F., E. Stupnicka, E. Wolinska-Witort, K. Mateusiak, B. Sadowski, and E. Domanski. 1985. Changes in circadian rhythm and suppression of the plasma cortisol level after prolonged stress in the sheep. Acta Endocrinolgica. 110:540-545.

Rabin, D. S., E. O. Johnson, D .D. Brandon, C. Liapi, and G. P. Chrousos. 1990. Glucocorticoids inhibit estradiol-mediated uterine growth: possible role of the uterine estradiol receptor. Biol. Reprod. 42:74-80.

Rajeev, J., D. Zwickler, C. S. Hollander, H. Brand, A. Saperstein, B. Hutchinson, C. Brown, and T. Audhya. 1990. Corticotropin-releasing factor modulates the immune response to stress in the rat. Endocrinol. 128:1329-1336.

Rivier, C. and W. Vale. 1984. Influence of corticotropin-releasing factor on reproductive functions in the rat. Endocrinology. 114:914-918.

Rivier C., J. Rivier, and W. Vale. 1986. Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. Science. 231:607-609.

Roberts, R. M. and F. W. Bazer. 1988. The functions of uterine secretions. J. Reprod. Fert. 82:875-892.

Robertson, H. A. and G. J. King. 1974. Plasma concentrations of progesterone, oestrone, oestradiol-17B, and of estrone sulphate in the pig at implantation, during pregnancy and parturition. J. Reprod. Fert. 40:133-141.

Sanson, W. K. 1988. The anterior pituitary. In: Text Book of Endocrine physiology. J. E. Griffin and S. R. Ojeda, eds. Oxford University Press. Oxford, New York.

Selye, H. 1956. Stress of Life. McGraw-Hill Book Co., NY.

Siiteri, P. K., J. T. Murai, G. L. Hammond, J. A. Nieker, W. J. Raymoure and R. W. Kuhn. 1982. The serum transport of steroid hormones. Rec. Prog. Horm. Res. 38:457-471.

Stoebel, D. P. and G. P. Moberg. 1979. Effect of ACTH and cortisol on estrus behavior and the luteinizing hormone surge in the cow. Fed. Proc. Fed. Amer. Soc. Exp. Biol. 38:1254 (abs).

Stone, B. A. and R. F. Seamark. 1985. Steroid hormones in uterine washings and in plasma of gilts between days 9 and 15 after oestrus and between days 9 and 15 after coitus. J. Reprod. Fert. 75:209-221.

Suter, D. E. and N. B. Schwartz. 1985. Effects of glucocorticoids on secretion of luteinizing hormone and follicle stimulating hormone by female rat pituitary cells in vitro. Endocrinol. 117:849-854.

Swenson, M. J. 1984. Physiological properties and cellular and chemical constituents of blood. In: Dukes' Phisiology of Domestic Animals. Comstock Publishing Associates. Ithica, New York.

Tait, J. F., and S. Burnstein. 1964. In vivo studies of steroid dynamics in man. In: The Hormones. G. Pincus, K. V. Thimann, and E. B. Astwood eds., Academic Press, New York. Vol 5, pp. 441-457

Tompkins, E. C., C. J. Heidenreich and M. Stob. 1967. Effect of post-breeding thermal stress on embryonic mortality in swine. J. Anim. Sci. 26:377-380.

Vreeburg, J . T., W. J. DeGreef, M. P. Doms, P. VanWouw, and R. F. Weber. 1984. Effects of adrenocorticotropin and corticosterone on the negative feedback action of testosterone in the adult male rat. Endocrinol. 115:997-983.

Weisak, T. 1989. Effect of pregnancy, injection of oestradiol benzoate or hCG on steroid concentration and release by pig luteal cells. J. Repro. Fert. 86:247-254.

Westphal, U. 1983. Corticosteroid binding globulin. Molec. Cell. Biochem. 55:145-160.

Wettemann, R. P., F. W. Bazer, W. W. Thatcher, D. Canton, and R. M. Roberts. 1988. Conceptus development, uterine response, blood gases and endocrine function of gilts exposed to increased ambient temperature during early pregnancy. Therigenology. 30:57-74.

Wildt, D. E., G. D. Reigle and W. R. Dukelow. 1975. Physiological temperature response and embryonic mortality in stressed swine. Am. J. Physiol. 229:1471-1475.

Wise D. A. and N. L. Eldred. 1986. Maternal social stress disrupts reproduction of hamsters drinking high-caloric fluids. Pharmaco Biochem Behavior. 25:449-456.

Yousef, M. K. 1985. Stress Physiology of Livestock. Vol. I and II. CRC Press, Inc., Florida.

Ziecik, A., T. Doboszynska, and L. Dusza. 1986. Concentrations of LH, prolactin, and progesterone in early-pregnant and oestradiol-treated pigs. Anim. Reprod. Sci. 10:215-224.

# APPENDICES

# APPENDIX A

## PROCEDURE FOR DETERMINATION OF PERCENT DISTRIBUTION OF BOUND AND UNBOUND CORTISOL IN PLASMA

## Procedure for Determination of Percent Distribution of Bound and Unbound Cortisol in Plasma

I. HANDLING OF PLASMA

A. Store at -20·C, and minimize thawing and freezing.

B. Plasma should be centrifuged before each assay, any precipitate should be discarded.

II. MEMBRANE PREPARATION

A. Dialysis tubing (Fisher Scientific) spectra/por, with a molecular weight cut off of 12,000-14,000 daltons. Size:25 mm X 15.9 mm diameter. 50 ft./roll.

B. Boil sections (1-2 ft.) of dialysis tubing in 95% ethanol in the hood for 30 to 45 minutes. Rinse tubing extensively with distilled water to remove ethanol.

C. Boil membrane twice for 15-20 minutes in a 2 to 3 L solution containing 5-10 mg of disodium EDTA and 5-10 g of sodium carbonate, rinse with distilled water between boiling.

D. Wash membrane extensively in distilled water to remove the disodium carbonate and EDTA. Take time to make sure EDTA is completely washed off (EDTA can interfere with the assay results).

E. Store membrane in a 0.02% sodium azide (0.1g/500 ml) solution at 4·C for up to 1 month.

III. CAPSULE PREPARATION

A. Rinse dialysis tubing in distilled water before each assay to remove any EDTA residue and sodium azide.

B. Cut dialysis tubing into small squares and fit over the fire polished end of a glass capsule and held in place by rubber band made from latex tubing (pimeline industries, Inc., 1/3" x 1/32").

E. Store Capsules in a 0.02% sodium azide solution for no more than 7 days prior to assay.

IV. CLEANING GLASS CAPSULES FOR REUSE

A. Remove membrane and rubber bands and rinse capsules, once in distilled water, and twice in methanol.

B. Heat capsules in a .01 N solution of sodium hydroxide for 2 hours.

C. Pour off 0.01 N sodium hydroxide and rinse twice with 0.01 N hydrochloric acid.

D. Rinse once with distilled water and twice with methanol.

E. Take an aliquot of the methanol and count in a Beta counter to see if there is any residual radioactivity. If radioactivity is present, then rinse with methanol again.

V. EXPERIMENTAL PROCEDURE

A. In preparation for an assay of 10 samples:

1. In 10 disposable test tubes add [1,2- $^{3}H$] cortisol (3 x $10^{5}$ dpm) (Sigma Diagnostics, St. Louis,MO ) dissolved in ethanol.
2. Evaporate ethanol, and add 5 ul of [$^{14}C$] glucose (12 x $10^{3}$ dpm) to each test tube.
3. Add 500 ul of plasma to each tube, Vortex.
4. Tubes are then incubated at 37·C for 30 minutes. Vortex, and incubate for an additional 30 minutes at room temperature.

B. Preparation of scintillation vials for plasma equilibrated with radiolabled cortisol and glucose.

1. Scintillation vials labeled 1-10 are prepared by numbering them 1a 1b, 2a 2b, etc.
2. Add 3 paper filter discs to each vial (dia. 13 mm; whatman No. 1)
3. Insert isodialysis capsules into the labeled scintillation vials.
4. Pipette duplicate aliquots (200 ul) of the plasma incubations into the isodialysis capsules.
5. The vials are then centrifuged at 3000 x g for 1 hour at 37·C.

6. A second set of scintillation vials are prepared by labeling then 1ap 1bp, 2ap 2bp, etc. One filter disc is added to each scintillation vial.
7. After centrifugation, isodialysis capsules are carefully removed from the scintillation vials and 30 ul of the remaining ultrafiltrate is pipetted off and into the respective scintillation vial ( 1ap 1bp, etc.).
8. Scintillation vials should then be arranged in order: 1a 1b 1ap 1bp, 2a 2b 2ap 2bp, etc.
9. Add 350 ul of distilled water to each scintillation vial, vortex.
10. Add 3.5 ml of scintillation fluid (scintiverse II), vortex and count on a beta scintillation counter, on the $^{14}C/^{3}H$ channel.

C. Determination of the percent distribution of bound cortisol

1. Prior to the assay, heat 600 ul of plasma samples at 60°C for 1 hour in a water bath.
2. Proceed with the assay as described for the determination of plasma free cortisol.

# APPENDIX B

# PROCEDURE FOR DETERMINATION OF CBG BINDING CAPACITY IN PLASMA

## Procedure for Determination of Plasma CBG Binding Capacity

I. Handling of Plasma

A. Store at -20°C, and minimize thawing and freezing.

B. Plasma should be centrifuged before each assay, any precipitate should be discarded.

II. Preparation of phosphate buffered saline (PBS)

A. To 1 liter of distilled water add:

| | |
|---|---|
| 5.4 g of $NaH_2PO_4 \cdot H_2O$ | 14.2 g $Na_2HPO_4$ |
| 9.0 g of NaCl | 1.0 g of gelatin |
| 1.0 g of sodium azide | |

B. Dissolve gelatin by heating and add sodium azide when cool.

C. Use a PBS/10 i.e. 1 in 10 dilution of above in distilled water.

III. Preparation of dextran coated charcoal (D.C.C.) in PBS/10

A. Add to 1 liter of PBS/10 buffer:

| | |
|---|---|
| 2.5 g "Norit GSX" | 0.25 g Dextran |

B. Stir for at least 4 hours at 40°C. Store at 4°C.

IV. (1,2-$^3$H) cortisol solution (Sigma Chemical, St. Louis, MO) for CBG assay

A. Purified $^3$H cortisol (100 pmol) (25,000 cpm; .053 uCI) in ethanol is pipetted into a glass vial. Dry

under $N_2$ and redissolve in 10 ml of PBS/10 to give 1 pmol $^3$H- cortisol /100 ul.

V. Cortisol solution for CBG assay

A. Make up a $10^{-3}$ M solution of cortisol in ethanol. Take 20 ul and dry under $N_2$ in a glass vial. Redissolve in 10 ml of PBS/10 to give 200 pmol/100 ul.

VI. Assay procedure

A. Take 500 ul of plasma and add 500 ul of PBS/10.

B. Add 1 ml of D.C.C. in PBS/10 (i.e. 1 in 4 dilution).

C. Incubate for at least 15 minutes at room temperature.

D. Centrifuge at 1500g for 10 minutes at 4°C.

E. CBG incubation mixture

1. Supernatant from (D) above is diluted 1 in 5 in PBS/10 (i.e 1:20 dilution).

2. To 2 of these tubes (A) add:

A. 100 ul of 10 pmol $^3$H-cortisol/ml solution

B. 100 ul of PBS/10 buffer.

3. To the other tube (B) add:

A. 100 ul of 10 pmol $^3$H-cortisol/ml solution.

B. 100 ul of 2nmol cortisol/ml solution.

F. Incubate at room temperature for 1 hour.

G. Place in ice/water bath for 10 minutes.

H. Add 500 ul of D.C.C in PBS/10 at 0°C to all tubes (use a repeat pipetter) and incubate for <u>exactly</u> 2.5 minutes in ice/water bath.

I. Centrifuge at 1500g for 10 minutes at 4°C.

J. Decant supernatant into scintillation vial.

K. Add 3.5 ml Scintiverse II scintillation fluid and count for 2 minutes

VII. Specific bound cpm can be calculated for each sample by subtracting the background cpm (tube B) from the mean of the total bound c.p.m. (tube A) values.

Specific bound $^{3}H$-cortisol (pmol/ml in undiluted serum) can then be calculated from the specific bound cpm using the following equation:

$$\text{Specific bound steroid (pmol/ml)} = \text{specific bound (c.p.m.)} \times \frac{100}{E} \times \frac{1}{2.2 \times 10} \times \frac{1}{A} \times \frac{1}{DF} \times D \times 10^{*}$$

Where: E= counting efficiency (25%)

A= specific activity of tracer in uCi/pmol (.053uCI)

DF= decay factor for $^{3}H$ (negligible)

D= dilution factor (20)

* multiplied by 10 to give pmol/ml.

# APPENDIX C

# PROCEDURE FOR QUANTIFICATION OF TOTAL PLASMA PROTEIN CONCENTRATION

## Procedure for Quantification of Total Plasma and Uterine Protein Concentration

I. Reagents

A. Coomassie Blue G-250 protein Reagent (Peirce Chemical

Company, Rockford, IL)

B. Standard BSA reagent (bovine albumin Fractain V,

Peirce Chemical Company, Rockford, IL)

II. Standard curve preparation

200 mg/dl = 100 ul std. BSA + 0 ul distilled water (DI water)

100 mg/dl = 50 ul std. BSA + 50 ul DI water

50 mg/dl = 25 ul std. BSA + 75 ul DI water

25 mg/dl = 12.5 ul std. BSA + 87.5 ul DI water

12.5mg/dl = 6.25 ul std. BSA + 93.75 ul DI water

III. Assay Procedure

A. Prepare standard curve in duplicate in 12 x 75 mm test tubes.

B. Prepare proper dilution ratios for all samples in labeled 12 x 75 mm test tubes.

C. Pipette 50 ul of unknown plasma dilutions (duplicate) into labeled tubes. Add 2.5 ml of protein assay reagent to each tube.

IV. Preparation of Spectrophotometer

A. Set spectrophotometer wavelength to 595 nm

B. Blank machine with deionized water in both holders.

C. Ready for samples, leave water blank in back holder.

V. Calculations

| | | | | |
|---|---|---|---|---|
| ( plasma sample absorbance - blank absorbance) | x | dilution factor | = | net absorbance of unknown sample |

Protein concentrations in mg/dl were then derived by plotting

the net absorbance against the standard curve developed within the assay.

# APPENDIX D

# PROCEDURE FOR QUANTIFICATION OF ALBUMIN IN PLASMA

## Procedure for Quantification of Albumin in Plasma

I. Sample Preparation

A. Label tubes

1. reagent blank
2. standard
3. control
4. samples

II. Experimental Procedures

A. Pipet 1.0 ml of albumin reagent [BCG] (Sigma Diagnostics, St. Louis, MO.) into each tube.

B. Add 10 ul of deionized water, protein standard solution (5 g/ dl), control and unknown samples to appropriately labeled tubes. Mix well by gentle inversion.

C. Read absorbance (A) at ambient temperature (18-26 C) at 628 nm.

III. Calculations

$$\text{Albumin conc. (g/dl)} = \frac{\text{A sample} - \text{A blank}}{\text{A standard A blank}} \times 5 \text{ g/dl}$$

# VITA

Michael J. Behrens, son of Paul and Nancy Behrens, was born on May 2, 1967 and raised in Memphis, Tennessee. He graduated from Christian Brothers High School in May 1985.

In September 1985 Michael began his undergraduate studies at The University of Tennessee at Martin. He resieved the degree of Bachelor of Science in Agriculture in May 1989. Michael then entered the graduate program in Animal Science at The University of Tennessee, Knoxville in August 1989 and was awarded the degree of Master of Science in August 1991.