1973

Influence of Season and Reproductive Status on Peripheral Plasma Progesterone Levels in the Lactating Bovine.

James Francis Beatty
Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_disstheses

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_disstheses/2515

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoging at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms
300 North Zeib Road
Ann Arbor, Michigan 48106
BEATTY, James Francis, 1942-
INFLUENCE OF SEASON AND REPRODUCTIVE STATUS ON PERIPHERAL PLASMA PROGESTERONE LEVELS IN THE LACTATING BOVINE.

The Louisiana State University and Agricultural and Mechanical College, Ph.D., 1973
Physiology

University Microfilms, A XEROX Company, Ann Arbor, Michigan
INFLUENCE OF SEASON AND REPRODUCTIVE STATUS ON
PERIPHERAL PLASMA PROGESTERONE LEVELS
IN THE LACTATING BOVINE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Dairy Science

by

James Francis Beatty
B.S., University of Southwestern Louisiana, 1965
M.S., Louisiana State University, 1968
December, 1973
ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to his major professor, Dr. J. D. Roussel, for his guidance and counsel throughout the period of graduate study and for his assistance in the preparation of this dissertation.

He would like to express his appreciation to the members of his graduate committee: Dr. J. D. Roussel, Department of Dairy Science; Dr. J. B. Frye, Jr., Head, Department of Dairy Science; Dr. R. A. Teekell, Department of Poultry Science; Drs. T. E. Patrick and L. L. Rusoff, Department of Dairy Science; and Dr. B. R. Farthing, Head, Department of Experimental Statistics.

The provision of laboratory facilities and guidance in performing the assay procedure by Dr. R. A. Teekell is especially appreciated, as is the assistance in accomplishing statistical analysis of the data by Dr. B. R. Farthing.

Appreciation is extended to Dr. J. A. Lee and to Mrs. M. A. Pinero for their help in conducting the laboratory analyses.

The author expresses his deepest appreciation to his wife, Christine, for her encouragement and understanding.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF APPENDIX TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>viii</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. REVIEW OF LITERATURE</td>
<td>4</td>
</tr>
<tr>
<td>A. ADAPTIVE RESPONSES TO CLIMATIC CHANGES</td>
<td>4</td>
</tr>
<tr>
<td>B. PROGESTERONE</td>
<td>24</td>
</tr>
<tr>
<td>III. EXPERIMENTAL PROCEDURE</td>
<td>34</td>
</tr>
<tr>
<td>A. GENERAL OUTLINE</td>
<td>34</td>
</tr>
<tr>
<td>B. SEASONS</td>
<td>36</td>
</tr>
<tr>
<td>C. REPRODUCTIVE STATUS</td>
<td>36</td>
</tr>
<tr>
<td>D. PROGESTERONE ASSAY</td>
<td>36</td>
</tr>
<tr>
<td>E. STATISTICAL ANALYSIS</td>
<td>39</td>
</tr>
<tr>
<td>IV. RESULTS AND DISCUSSION</td>
<td>40</td>
</tr>
<tr>
<td>A. AGE AND PROGESTERONE</td>
<td>40</td>
</tr>
<tr>
<td>B. SEASONS</td>
<td>41</td>
</tr>
<tr>
<td>C. REPRODUCTIVE STATUS</td>
<td>44</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>D. PHOTOPERIOD</td>
<td>46</td>
</tr>
<tr>
<td>E. RELATIONSHIP BETWEEN CORTISOL AND</td>
<td>47</td>
</tr>
<tr>
<td>PROGESTERONE</td>
<td></td>
</tr>
<tr>
<td>V. SUMMARY AND CONCLUSIONS</td>
<td>50</td>
</tr>
<tr>
<td>VI. BIBLIOGRAPHY</td>
<td>54</td>
</tr>
<tr>
<td>VII. APPENDICES</td>
<td>64</td>
</tr>
<tr>
<td>VITA</td>
<td>66</td>
</tr>
</tbody>
</table>
LIST OF TABLES

TABLE

1. Climatic measurements by months and seasons............................................. 35
2. Simple correlations between age, lactation number, progesterone, and cortisol............... 40
3. Effect of temperature-season on peripheral plasma progesterone............................. 41
4. Simple correlations between progesterone level and climatic measurements............... 42
5. Effect of reproductive status on peripheral plasma progesterone............................. 44
LIST OF FIGURES

FIGURE

1. Characteristic pattern of corticoid activity and resistance to stress in the general adaptation syndrome.......................................................... 10

2. Diagrammatic illustration of the effect of constant environmental temperature on milk production and feed consumption of cattle.............. 12
LIST OF APPENDIX TABLES

Table

1. Analysis of variance for effect of season, reproductive status, season X reproductive interaction and lactation number on progesterone . . . 65
ABSTRACT

Plasma progesterone concentrations were determined in 264 blood samples taken from lactating Holsteins over a period of one year in order to investigate the effect of temperature-season on circulating progesterone concentrations. The 264 total samples were obtained by bleeding 18 to 24 animals each month (January to December, 1969) and the samples were assigned temperature-seasons based on climatic conditions prevailing during the 14 days preceding the day the samples were obtained. Temperature season 1 (cool) included the months of December, January, February and March. Season 2 (intermediate) included the months of April, May, October and November; and season 3 (hot), the months of June, July, August and September. The effect of temperature-season on circulating progesterone concentrations was compared with its effect on circulating cortisol levels to determine if the adrenal cortex was involved in any change observed in progesterone concentration.

The effect of reproductive status was evaluated to determine if there were an interaction between season and reproductive status and to investigate the role of progesterone in reproductive malfunctions. Reproductive statuses assigned were: 1) pregnant, 1 to 90 days; 2) pregnant 91 to 180 days; 3) open, cycling and required 3 or less services for conception; 4) open, anestrus; and 5) open, cycling and required 4 or more services for conception.
Experimental animals were maintained under routine housing, feeding and management conditions in order to obtain samples from acclimatized animals. Since photoperiod changed with season, its effect had to be considered.

A highly significant (P< 0.01) relationship between progesterone and animal age (r = -0.57) resulted in these data being age corrected by analysis of covariance. Simple correlations were determined before age corrections were made and in some cases may reflect the effect of age.

Plasma progesterone concentrations in the hot season (4.6 ng/ml) were significantly (P< 0.01) higher than concentrations in the cool and intermediate seasons (3.4 and 3.8 ng/ml, respectively). The simple correlation between plasma progesterone levels and dew point temperature (r = 0.19) was statistically significant (P< 0.01) but the correlation between progesterone concentration and maximum temperature (r = 0.11) was not. Changes in progesterone levels were associated with changes in temperature-humidity-index (r = 0.14; P< 0.05) and minimum temperature (r = 0.16; P< 0.01).

Plasma cortisol concentrations were lower (P<0.01) in the hot season than in the cool and intermediate seasons and suggested the adrenal cortex did not contribute to the increase in progesterone concentrations which occurred in the hot season. A significant (P<0.01) positive correlation (r = 0.45) between cortisol and progesterone was observed.

Reproductive status did not have a statistically significant effect on progesterone levels and the effect of season on progesterone
concentrations was consistent across all reproductive statuses. Plasma progesterone levels in the anestrus animals (3.9 ng/ml) suggested persistent progesterone secretion was responsible for their failure to cycle. Similar progesterone levels were observed in normal (3.5 ng/ml) and repeat breeders (3.6 ng/ml).

Seasonal differences in climatic conditions appeared to be more important than seasonal differences in photoperiod. The cool and intermediate temperature-seasons were considered seasons of equal temperature and different daylength. Progesterone concentrations in these seasons were not significantly different. Progesterone concentrations observed in a hot period (July, August, September) averaged 1.1 ng/ml higher than those observed in a cool period (March, April, May) of equal daylength. This difference is comparatively large but was not tested statistically.
I. INTRODUCTION

Warm-blooded animals (homeotherms) are able to function efficiently in a wide range of environmental temperatures because their internal temperatures are not controlled by the temperature of the external environment. However, this does not mean the homeotherm is completely free of the influences of climate. When climatic conditions change, physiological adjustments must be made in order to maintain homeothermy. It is important that these adjustments be kept in proper perspective in relation to the broader concept of homeostasis. Compensation for one stress may upset other physiological processes and disrupt other equilibria resulting in impaired production and reproduction by the organism.

Losses from reproductive failure constitute one of the greatest losses among female livestock and present evidence indicates the causes are environmental. With the advent of improved management practices, including improved nutrition and disease control, the effects of these environmental factors on reproduction are decreasing. Although the evidence indicates that environmental temperature could be a major reason for low reproductive efficiency in hot climates, little is known of the specific causes of this lowered reproductive efficiency. Environmental temperature affects fertility in the male but it results mainly in lowered fertility, and not in periods of infertility as may be the
case with the female. In addition, the increased use of artificial insemination has tended to alleviate the effects of seasonal fluctuations in fertility in the male. Consequently, reproductive research in the Southern United States has focused increasingly on the magnitude of, and mechanisms involved, in the female in the hot months.

This study was undertaken to determine if adjustments made by lactating dairy cattle for the hot, humid summer conditions common to the Gulf Coast region of the U. S. resulted in a change in the circulating levels of progesterone in these cattle. These hot humid conditions result in lowered reproductive efficiency during this season and are thought to cause a rise in peripheral plasma progesterone.

Since progesterone is one of the hormones controlling reproduction in the bovine, more conclusive information on any connection between heat stress and changes in circulating progesterone levels should lead to a better understanding of the specific physiological mechanisms through which the effect of stress on reproduction is mediated and could suggest steps by which this effect could be alleviated.

Since the adjustments made by the animals in response to changes in the climate might result in different secondary effects for animals in different physiological states a comparison between animals of varying reproductive conditions was included.

No pathway is known through which a change in climate alone would change the progesterone secretion rate by the ovaries. Therefore, any change in circulating progesterone levels as a result of a change in climatic conditions would be presumed to be an effect resulting from a change in release of progesterone from an extra-ovarian source. The adrenal cortex has been suggested as this extra-ovarian
source. Cortisol was assayed in aliquots of the blood samples used for progesterone assay, allowing changes in progesterone levels to be compared with changes in adrenal-cortical secretion rate. Since this investigation was carried out under natural climatic conditions and changes in length of daylight tend to parallel changes in climatic conditions, the effect of this variable must be considered.
II. REVIEW OF LITERATURE

A. ADAPTIVE RESPONSES TO CLIMATIC CHANGES

Hafez (43) stated that a highly complex organism requires a stable temperature in order to function efficiently. A constant body temperature is maintained by adjusting heat loss and heat gain so that the net change in temperature is at or near zero. Heat is gained from the interior as a result of the metabolic processes necessary for maintenance and production (54). Heat is lost by radiation, conduction, convection and evaporation.

1. Physical Responses

In a hot environment the animal increases the blood supply to the surface, thereby increasing the surface temperature (115). This increases heat loss to the environment by radiation, conduction, and convection if the environmental temperature is below body temperature and decreases heat gain from the environment by these processes if environmental temperature is above body temperature (54). Some animals also have the ability to shift heat balance to a higher level with no adverse effects, thereby further increasing surface temperature. The camel can withstand a 6.5 C rise in rectal temperature (66, 114). While radiation, conduction, and convection can be sources of either a loss or a gain in heat, evaporation always results in heat loss and therefore evaporation becomes increasingly important as temperature
increases (54, 58). The animal increases evaporative heat loss by accelerating respiration and sweating rates (54).

In a cold climate the animal decreases the blood flow to the body surface, thereby decreasing the surface temperature and decreasing heat loss to the environment through radiation, conduction, and convection. Respiration rate decreases and sweating ceases in order to reduce evaporative heat loss (118).

Although the amount of heat exchanged between the animal and the environment through the aforementioned processes is dependent on the temperature gradient between the animal and the environment, other factors making up the climatic environment also affect the amount of heat exchange (16). Heat gain from solar radiation may offset heat loss by radiation to the surroundings. Convective exchange is governed largely by both air velocity and relative humidity. Low relative humidity diminishes the effects of both high and low temperatures on the animal (16).

The ideal temperature for most domestic animals is, generally, within the range of 4 to 24 C (40) and the limits of thermoregulation are reached more quickly with temperatures above this range than with temperatures below it (2). The comfort zone for European dairy cattle is -1.1 to 15.6 C (7, 23).

At temperatures equal to or above body temperature evaporation is the only source of heat dissipation (54, 57) and a rise in wet bulb temperature is reported to have twice the effect of a similar rise in dry bulb temperature in heat-stressed cattle (15).

Kibler and Brody (58) reported that evaporative heat loss accounted for 40%, 80%, and 98% of total heat loss at 20 C, 30 C, and 38
C, respectively. Earlier work by these investigators (57) revealed that environmental temperatures above 37.8 C prevented nonevaporative heat loss and resulted in a gain in heat from radiation, convection, and conduction. An increase in respiration rate is the first external physiological response to an increase in ambient temperature (65). Kibler and Brody (59) found respiratory evaporation to be an important method of heat transfer resulting in dissipation of 35% of heat produced by the animal at temperatures between 10 and 43 C. However, Brody (23) stated that this cooling provided by increased respiration rate may be counteracted by the increased heat production from the "work" of panting and that panting developed respirating alkalosis. Kibler (56) attributed the alkalosis caused by thermal stress to decreased feed consumption and metabolism. Ferguson and Dowling (34) reported that the evaporation of moisture released from the apocrine sweat glands of cattle was the main source of heat dissipation in hot environments. Barrada (10) found surface vaporization in Holsteins to be greatly in excess of respiratory evaporation and Weldy and McDowell (111) reported surface evaporation accounted for 70.3% of the total evaporation from Holsteins exposed to 32.2 C. In the study referred to above concerning the percent of total heat dissipation accounted for by vaporization, Kibler and Brody (58) stated that surface evaporation accounted for 30% of the total heat loss at 20 C, 65% at 30 C and 77% at 38 C. McDowell et al. (68) reported increases in surface evaporation to be directly proportional to increases in air temperature and inversely proportional to relative humidity (RH). At 20% relative humidity surface evaporation increased with increasing temperature from 30 to 45 C. At 80% relative humidity, surface evaporation increased
at temperatures from 28.3 - 32.8 C but decreased at 35 C.

2. **Metabolic Responses**

   If adjustments in surface temperature and evaporation rate are not sufficient to maintain heat balance, the animal must do so by adjusting its metabolic rate. Heat stress decreases thyroid secretion rate, feed intake and metabolic rate, thus decreasing internal heat production (2, 54, 72). Heat stress also alters adrenal function (2, 61, 89, 119).

   (a) Thyroid secretion: Studies conducted in the South have shown a 67% reduction in the estimated thyroid secretion rate of lactating cows during the period from April to August (54). Hafez (44) also cited references indicating reduced thyroid secretion rates in hot environments. Mixner et al. (72) reported that thyroxine secretion rate was .165 mg/100 lb body weight/day in the spring and .129 mg/100 lb body weight/day in the fall. Dempsey and Astwood (28) found the thyroid secretion rate to be 9.5 mcg % at 25 C and 1.7 mcg % at 35 C. Thompson et al. (104) reported a decrease in thyroxine secretion under controlled hot conditions. These workers stated that heat production, respiration and rectal temperature rose following initial exposure to heat, then declined on continued exposure and also stated that this decline was possibly brought about by altered thyroid and adrenal cortical function.

   (b) Adrenal secretion: Wilson (119) in a review on evaluation of stressor agents in domestic animals stated that since Selye's (90) 1952 report, adrenal glands from countless experimental animals have been critically analyzed and there was ample evidence to indicate the importance of glucocorticoids in response to stress. Albright and
Alliston (2) in a review of the effects of varying the environment on
the performance of dairy cattle stated that when initial physical reac-
tions (vasomotor, pilomotor, etc.) in response to a climatic change are
not adequate to control body temperature the thermoregulatory centers
of the brain serve to intensify the initial reaction and initiate such
additional actions as metabolic changes. These changes are sequential-
ly controlled with the adrenal medulla generally regarded as being
triggered initially followed by the adrenal cortex and then by the
thyroid (78).

The adrenal medulla can be considered to be composed of post-
ganglionic nerve cells of the sympathetic nervous system lacking axons
(14). Instead of a nerve impulse traveling down the postganglionic
fiber and releasing norepinephrine at the affected cell, epinephrine
and norepinephrine are liberated into the blood. This phenomenon is
responsible for the diffuse or whole body response to sympathetic stim-
ulation. The actions of the autonomic nervous system and its ally the
adrenal medulla are directed toward strengthening an animal's defenses
against the various dangers which beset it, e.g. extremes in tempera-
ture, water deprivation or attack (14).

Reports on adrenal cortical response to climatic stress have
varied considerably due to differences in degree and duration of stress,
prior conditioning of the animal or degree of acclimation, rapidity of
change from pre-experimental to experimental conditions and time of
sampling in relation to the onset of stress. However, if close atten-
tion is paid to the variations in experimental procedure mentioned
above, Selye's (91) report on the patterns of glucocorticoid secretion
by the cortex in relation to stress (Fig. 1) appears highly accurate
when compared with more recent information on adrenal-cortex response
to heat stress in the bovine.

In experiments in which the animals would be considered in the
alarm phase of their reaction to heat stress (Fig. 1), Christison et al.
(25) reported a significant increase in plasma glucocorticoid levels
240 min after abrupt exposure to 42 C and 60% RH, and Wegner et al.
(110) reported increased plasma corticoid levels 12 and 24 h after ex-
posure to 40 to 48 C and 70 to 80% RH.

Alvarez and Johnson (6) studying longer duration exposure to
35 C, noted a rise in glucocorticoids during the first hour of exposure,
a peak after 4 h, and a gradual decrease to values not different from
normal at 48 h. In a second experiment, in which exposure to 35 C was
continued for 24 days and a control group was maintained at 18 C, glu-
cocorticoid levels became lower in the stressed cattle after 12 days
exposure and the 24-day mean for cattle at 35 C was significantly lower
than the mean for cattle at 18 C (6). Stott and Robinson (101) report-
ed a sudden, short-lived increase in plasma cortisol in response to
acute thermal stress and a suppressed level with continued stress.
Collins and Weiner (26) also reported only a transient cortisol eleva-
tion. Guernsey heifers which were monitored for four consecutive es-
trous cycles (2 cycles at 18 C, 55% RH followed by 2 cycles at 33.5 C,
55% RH) had significantly lower plasma cortisol levels in the fourth
estrous cycle than in the first two cycles (1). Bergman (13) reported
a depressed cortisol plasma level and secretion rate as a result of
prolonged exposure to 29 C. Previous work at Louisiana State Universi-
ty, in which lactating Holsteins were taken from 15.5 C, 65% RH to 29.5
C, 65% RH over a period of five weeks (2.8 C/week) and then maintained
Corticoid Activity
Level of Normal Resistance

Alarm Reaction Stage of Resistance Stage of Exhaustion

TIME

Fig. 1. Characteristic pattern of corticoid activity and resistance to stress in the general adaptation syndrome (91).
at 29.5°C for three weeks, resulted in a significant linear decrease in cortisol in the last three periods (61). No clearly defined alarm phase was observed with this gradual exposure to high temperatures but blood samples were taken at the end of each weekly period, therefore the animals may have passed through an alarm phase before being sampled (6, 26). All of the above studies were carried out in climatic control chambers.

(c) Feed intake and body weight change: Body temperature regulation and feed intake regulation are interrelated because heat production changes with changes in the amount of feed consumed (2, 49, 54, 70). Brobeck (22) suggested that animals increase intake to keep warm and decrease it to prevent hyperthermia. Johnson (49) gave a diagrammatic illustration of this concept (Fig. 2). Balch and Campling (8) supported it by altering feeding patterns in goats through artificially induced temperature changes in the hypothalamus. Cooling induced eating in satiated goats while heating inhibited eating in hungry goats.

Johnson et al. (52) suggested a rise in ambient temperatures above 19.3°C had an adverse effect on feed consumption in Holstein cows. Kibler and Brody (57) suggested 21.1°C as the critical temperature. Maust et al. (70) considered temperatures above 27°C to be sufficient to stress lactating animals based on a review of reports in the literature relating temperature conditions to responses of lactating cows (39, 53, 79, 80). Previous work at Louisiana State University revealed a decrease in feed intake and milk production when environmental temperature reached 26.8°C (61). Most of the investigations referred to above were conducted in climatic control chambers at constant temperatures (no diurnal variation).
Fig. 2. Diagrammatic illustration of the effect of constant environmental temperature on milk production and feed consumption of cattle (49).
Under natural climatic conditions or in climatic chamber studies where the conditions are cycled to simulate natural conditions, cattle can withstand maximum temperatures considerably above the critical temperature (27°C), without markedly reducing feed intake, by increasing intake during the cooler night hours (39, 53, 70). However, in this case the minimum temperature becomes an important consideration since it must be below the critical temperature in order for the animal to compensate and it is usually closely related to the duration of temperatures below the critical level.

Again, any other factors which affect the total strain on the animal's homeostatic mechanisms will affect the intake response to the climate. Level of production is important as is stage of lactation (70). High fiber rations also accentuate the effects of high temperatures (20, 39, 80).

Body weight changes parallel feed intake changes in response to climate. Heat stress reduces weight gains in heifers (51, 104) and increases weight losses in high-producing cows (69, 70). Few studies with lactating cows report weight changes because changes in body weight are hard to measure in large lactating animals which are on a full feed and cannot practically be shrunk because of the effect this would have on production. In addition, many trials reported in the literature were of relatively short duration or periods at various temperatures were relatively short (24).

A series of studies on the relationship of seasonal changes in climate to feed utilization and performance of lactating cows has been conducted at Beltsville, Maryland. July - August calvers lost weight rapidly following calving, but after 60 days, their gains paralleled
the average for all cows. Although winter calvers did not show excessive changes in weight following calving, their gains slowed with the onset of summer (67, 69). In June, July and August cows in the early stage of lactation (< 100 days) consumed less energy than cows in the mid (100 to 180 days) or late (180 to 260 days) stages, but were the highest in production indicating they utilized body reserves rapidly to offset the impact of thermal stress (70). Body weight decreased an average of 23 kg in the early lactation group, did not change in the mid-lactation group, and increased in the late lactation group. Although feed consumption was lowest for the early group correlations between climatic measures (maximum temperature, temperature humidity index (THI, etc.) and feed intake, indicated changes in feed consumption in response to day-to-day variations in climatic conditions were greater for the mid-lactation groups. This may be the result of a greater total stress on the early group, causing the temperature, THI, etc. necessary to reduce intake for this group to be lower and resulting in a larger total number of days and hours within days in which intake was affected in the early group as indicated by their higher rectal temperature.

3. **Effects on Production**

As anticipated from the above section on the effects of climatic stress on feed intake, heat stress depresses milk production and this is primarily an indirect effect through nutrient intake (2, 18, 109). Milk production begins to decline at approximately 26.7 C but the precise temperature at which a production drop takes place and the magnitude of the decrease are subject to the modifying effects of other climatic factors and the physiological status of the animal (17, 61). The
willingness of the animal to metabolize body reserves in order to maintain production during periods of reduced intake is important since it may markedly affect the production changes observed with a given change in climate. The metabolism of body fat to partly offset the reduced feed intake caused by heat stress allows the animal to produce a given quantity of milk with a lower internal heat load than would be the case if it did so by maintaining its feed intake. Mobilizing body reserves eliminates the specific dynamic effect (SDE) for that portion of intake which body reserves are replacing. More precisely, it changes the time at which this SDE must be endured to one during which climate is more favorable. Maust et al. (70) reported that cows in the early stage of lactation utilized body reserves rapidly to offset the impact of thermal stress on feed intake and production. Animals in mid-lactation let milk yield bear the brunt of intake decreases in order to maintain body weight, while animals in late lactation increased weight at the expense of production. Consequently, day-to-day fluctuations in climate were greater in the mid- and late lactation groups than in the early group (70). However, the above report does not mean that total lactation yield is affected most in those animals experiencing heat stress in mid- and late lactation or that the effects of heat stress are totally offset by metabolizing body reserves. Maust et al. (70) suspected that total lactation yield might be most severely affected in early lactation animals, as a result of rapid catabolism of body reserves, and Maust and McDowell (69) reported lactation yields were 11% less for July - August calvers than for January - February calvers. Conversion of feed energy directly to milk is more efficient than converting it first to fat and then mobilizing the fat to produce milk (114). In
the report by Maust et al. (70), all animals were exposed to heat stress. Studies involving efforts to reduce the heat load of animals through mechanical cooling, insulation, and etc. have generally shown increased feed intake and production for the treated groups as compared to controls regardless of stage of lactation (24, 84, 85, 87, 116).

As in the case with feed intake and weight change, milk production responses in trials conducted under ambient conditions are smaller than would be expected from constant temperature studies, due to the intermittent rest periods provided by diurnal variations and day-to-day fluctuations in maximum temperature, humidity, etc. (24, 27, 39, 53, 70, 87). Branton and Evans (19) reported seasonal differences in production were much smaller with a drylot feeding regime than with a pasture oriented program. While appetite response to heat stress is immediate, the production response generally is observed two to three days later (70).

4. Effects on Reproduction

The losses from reproductive failure constitute the greatest loss among female livestock and present evidence indicates the causes are environmental (66). With the advent of improved management practices, including improved nutrition and disease control, the effects of these environmental factors on reproduction are decreasing. Although the evidence indicates that environmental temperature could be a major reason for low reproductive efficiency in hot climates, little is known of the specific causes of this lowered reproductive efficiency (66). Environmental temperature affects fertility in the male but it results mainly in lowered fertility, and not in periods of infertility as may be the case with the female. In addition, the increased use of
artificial insemination has tended to alleviate the effects of seasonal fluctuations in fertility in the male. Consequently, reproductive research in the Southern United States has focused increasingly on the mechanisms involved in the decreased reproductive efficiency observed in the female in the hot months.

Investigations in this area have been of two general types: 1) those concerned with changes in the macroenvironment and the effects these changes have on reproductive rates; and 2) those concerned with changes in the microenvironment, as a result of changes in the macroenvironment, and the role which these changes in the microenvironment may have in lowered reproductive efficiency. The macroenvironment is defined here as the environment surrounding the female and the microenvironment as that within the female.

(a) Macroenvironment: In areas where seasonal high temperatures are common, a decrease in fertility accompanies the high ambient temperatures of summer and early fall (2, 54, 75, 76, 77, 99) and the more severe the conditions, the greater the decrease (75, 77). Stott (99) reported a decrease in breeding efficiency in Arizona dairy cattle during the hot summer months and indicated the major variance was contributed by the female. A subsequent report by Stott and Williams (103) cited a low fertilization rate and a high incidence of embryonic mortality as the major factors responsible for the low breeding efficiency observed in the hot, humid season. Poston et al. (77) reported a significant, within herd, correlation (r = 0.30) between monthly fluctuations in calving interval and air temperatures 60 to 120 days postpartum. Calving interval was longest for May calvers which would need to conceive again in July and August, the lowest conception months in order
to maintain an optimum calving interval. Maust et al. (70) reported the calving interval averaged 27 days longer in July - August calvers than in January - February calvers. Florida investigators (41) reported an inverse relationship between average ambient temperature on the day of insemination and fertility. A controlled environment which reduced average degree-hours above 21.1 C improved fertility in the summer months (35). Wiersma and Stott (117) improved breeding efficiency in the summer months 100% by providing cooled shades which kept air temperatures 5.6 to 6.7 C lower than temperatures under the control shades. In contrast to the above reports, Kellegren et al. (55) reported no effect of season on fertility of cows inseminated in Louisiana. However, the summer of the year the trial was conducted was unusually mild. Periods of high temperature and high humidity were infrequent and of short duration.

The major effect of heat stress is apparently exerted at or near the time of mating. Stott and Williams (103) observed a higher than normal rate of embryo mortality prior to 35 days post-mating in the hot months, but no increase in embryo losses later in gestation. Wiersma and Stott (117) used a climatic control chamber to study the time, in relation to mating, at which temperature stress exerted its greatest effect. The first four to six days after breeding appeared to be the critical period. Dunlap and Vincent (31) reported that the conception rate was 48% lower in heifers exposed to 32.2 C for 72 h immediately following breeding, than it was in heifers exposed to 21.1 C for the same period. Tompkins et al. (106) reported an adverse effect on embryo survival in sows which were exposed to high temperatures one to five days after breeding, but observed no effect on embryo survival
when sows were exposed 20 to 25 days post-mating to temperatures sufficient to kill four of the nine sows. While the above reports demonstrate a detrimental effect for exposure to temperature stress one to six days after breeding, Wiersma and Stott (117) also reported that providing ideal climatic conditions during this period did not improve fertility in cows otherwise maintained under ambient summer conditions.

The decreased fertilization rate and increased embryonic mortality observed in heat stressed cattle result in a lower percentage of successful matings. Thermal stress can also result in disturbances in the estrous cycle which further lengthens calving intervals through failure of open animals to be serviced. Gangwar et al. (36) reported longer estrous cycles and decreased duration and intensity of estrus in heat-stressed heifers. The ranges in estrous cycle length were: 19 to 24 days under ambient spring conditions, 19 to 45 days in a control chamber with temperature cycled between 23.9 and 35 C every 24 h, 21 to 26 days under cool conditions in the chamber, and 19 to 36 days under ambient summer conditions. The average durations of the estrus periods for the above listed conditions were: 20, 11, 20 and 14 h, respectively. The incidence of anestrus (estrous cycle longer than 26 days) was 33% in the group stressed in the control chamber. Bond and associates (17) reported heifers became anestrus after five weeks exposure at 32.2 C but re-established normal estrous cycles after 21 weeks exposure. Estrous cycles were disrupted within one week with exposure to 37.8 C. Stott and Williams (103) observed an increase in the number of long estrous cycles (exceeding 26 days) in lactating animals during the months of high ambient temperature and the prolonged cycles were significantly longer than those occurring during the other months. Hollon
and Branton (47) reported an increase in days to first estrus, days to first service, and days open during the hot season in Louisiana.

The effect of heat stress on the duration and intensity of estrus is important in any consideration of the effect of heat stress on the incidence of anestrus. There is some question as to how much of what is reported as anestrus is actually failure to detect estrus periods of shorter duration and decreased intensity. Gangwar et al. (36) emphasized the need for more frequent observation of animals for estrus during periods of high temperature. Poston et al. (77) considered failure to detect estrus to be responsible for part of the seasonal fluctuation observed in reproductive performance.

Nutrition is believed to play a role in seasonal fluctuations in fertility because the quantity and quality of grazing changes with season (14), but the use of stored feeds to insure sufficient amounts of high quality forage year round is thought to alleviate this effect. However, in the high producing dairy cow, the effect of high temperature on appetite may mean that part of the decrease in reproductive efficiency observed in hot seasons is a result of undernutrition, even though sufficient quantities of well-balanced rations are offered to the animal. Studies with beef cattle have shown that excessive weight losses, as a result of failure to provide sufficient energy, are associated with anestrus and low conception rates (107, 120, 121). Hollan and Branton (47) reported conception rates were lower in dairy cattle losing weight than in cattle gaining weight. Accelerated weight losses, anestrus and low conception rates are observed in heat stressed dairy cattle, although decreased appetite and high levels of production, rather than a failure to provide sufficient nutrients, are the reasons
for the insufficient energy intake.

(b) Microenvironment: The investigations referred to above demonstrate the effects of the macroenvironment on reproduction. The question now becomes, by what mechanism does the macroenvironment influence reproduction? What changes take place in the microenvironment, that is, inside the cow, which result in fertilization failure, embryo death, and etc.?

There are three changes that a high macroenvironmental temperature might bring about in the microenvironment (76). The macroenvironment could change the temperature of the microenvironment, its biochemical makeup, or its endocrine balance. Wiersma and Stott (116) reported lower peripheral plasma progesterone levels in cows that had access to cooled shades (5.6 to 6.7 C cooler than control shades) in the summer months. The higher progesterone levels in the control group were attributed to production of progesterone by the adrenal cortex. These workers also reported an increase in circulating progesterone levels in ovariectomized cows exposed to heat stress. Gwadzauskas et al. (41) assayed blood samples taken 14.5 h after insemination over a period of one year and observed a positive relationship between ambient temperatures on the day of insemination and circulating progesterone, but no relationship for adrenal corticoids. There was no association between progesterone levels and fertility. Thwaites (105) found no evidence of increased adrenal activity in heat stressed ewes and his results tend to exclude endocrine functions of the corpus luteum, thyroid and adrenal cortex as mechanisms of embryo mortality in heat stressed ewes. More will be said on the connection between heat stress, glucocorticoid levels and progesterone levels later in this review.
Loe (63) concluded that heat stress and/or stress and proges-
tins apparently cause a change in the number of amino acids in uterine
and oviductal fluids.

The direct effects of temperature on the gametes and the em-
bryo have been investigated extensively by North Carolina workers
(108). Results indicate that heat stress decreases both fertilization
rate and embryo survival, with damage to the embryo occurring before
the eight cell stage (4). Further investigation indicated heat stress
did not affect the ovum prior to fertilization (122), but did affect
the sperm, and that this was a direct effect of increased temperature
(48). Studies involving fertilized ova exposed, in vitro, to two
temperatures (38.5 and 41.0 C) indicated a direct effect of temperature
on the embryo (3, 5). The effect of elevated temperature was less dur-
ing the second cell division than the first (5).

Temperature apparently has some direct effect on reproductive
performance, that is, an effect independent of hormonal and biochemical
changes (108). This does not rule out hormonal and biochemical effects
in addition to the direct effects of temperature (41, 63, 117). The
complexity of the reproductive process and of the animal's reaction to
heat stress, make it unlikely that any one factor or mechanism is res-
ponsible for a major portion of the effects of climatic stress on re-
production.

As with other responses to stress, evaluation of reproductive
response to climatic stress requires strict attention to experimental
procedures and physiological state of the animal. Conception rate is
generally lowest in the late summer but calving interval may be affect-
ed most in the late spring and early summer calvers. In Arizona,
conception rate is lowest in August even though maximum temperatures are higher in June and July, presumably because relative humidity is much higher in August (103).

Nonreturn rates may underestimate the effect of temperature stress on conception rate because of an increase in anestrus and undetected heats. Wiersma and Stott (116) reported that exposure to heat stress during the first four to six days post-insemination decreased pregnancy rates in lactating cows but had no effect in non-lactating cows. Equal increases in rectal temperature in the two groups were obtained by subjecting the non-lactating group to a higher environmental temperature. The effects reported for ambient conditions are generally less than what is observed in climatic control chambers. The more severe the conditions, the greater the effect (75).

5. **Acclimation**

Several reports cited previously have demonstrated the effect that acclimation to temperature stress can have on the response measured (1, 6, 61, 101, 104). Bond et al. (17) reported heifers re-established normal estrous cycles after 21 weeks exposure to 32.2 C. Collins and Weiner (26) indicated opposite changes of certain hormone levels may occur within a given environment as the result of acclimation state. Johnson et al. (50) found little evidence of acclimation in milk production but persistency of lactation was above average and feed intake increased toward normal levels after acclimation. In spite of these and similar reports, Albright and Alliston (2) stated in a recent review that the effects of acclimation on responses to given environmental conditions appear to have received little attention. It is not unusual to encounter reports in which even basic seasonal
acclimation has not been adequately considered in the design of the study or the interpretation of results (2). Any changes in non-climatic environmental factors, such as degree of restraint, milking equipment and procedures or feeding practices must be considered. The animal undergoes an acclimation response to any change in its environment.

Seasonal changes in climatic conditions are gradual over relatively long periods of time and allow the animal to become acclimatized while the changes are being made. Investigations of the physiological changes which are caused by seasonal differences in climate should be made on acclimatized animals.

B. PROGESTERONE

1. Role in Bovine Reproduction

If any generalization can be made about the progestogenic hormone, it is that they are essential for the continuation of reproductive processes in the female mammal: the implantation of the blastocyst in the uterus, the maintenance of the gravid state, the morphological development of the mammary gland, and other phenomena related to gestation and lactation (123). In addition, progesterone may be important as a conditioning agent for normal estrus, can inhibit estrus and affects ovarian function and gonadotropin elaboration (38).

In animals with an estrous cycle, the presence of a functional corpus luteum (CL) and its secretion of progesterone delays the onset of the next estrus period (123). Ovulation and sexual activity are repressed by progesterone during the luteal phase of the cycle. If pregnancy is not accomplished, the CL regresses, progesterone secretion ceases and the animal returns to estrus. Progesterone's ability
to inhibit estrus has led to the use of exogenous progesterone to synchronize estrus. Return to estrus can be delayed in a group of animals with an exogenous progestogenic compound and when the exogenous source is removed the entire group will normally exhibit estrus within seven days (12, 45, 82, 86).

Progesterone or its equivalent activity is absolutely essential for the normal span of pregnancy in all mammalian species studied to date (38, 123), but the necessity for the CL as the source of progesterone in the bovine is in doubt (38). It is generally accepted that the CL is required for the first 200 days of gestation. Pregnancy may continue without the CL between day 200 and 260 but parturition is abnormal, calves are weak and die, and the fetal membranes are retained (33, 97). Extra-ovarian progesterone is responsible for pregnancy maintenance during this period and the adrenal cortex, the placenta, and body fat have been proposed as the sources (33, 38). Stabenfeldt et al. (97) concluded that the cow should be considered a mammal requiring continuous presence of the CL during pregnancy and Erb et al. (33) tended to discount the placenta as an extra-ovarian source of progesterone.

Progesterone is responsible for preparing the uterus to receive the conceptus (blastocyst) (123) but the biochemical changes it brings about in the uterine environment are not well understood. Progesterone induces mucus secretions by the endometrium and underlying stroma essential to implantation of the blastocyst (114), and decreases contractability of the uterus by increasing the membrane potential of the myometrial cells (123). Mills et al. (71) reported a significant correlation \( r = 0.32 \) between circulating progesterone levels and
protein content of uterine fluids. Loe (63) reported that thermal stress and progestins apparently cause a change in the number of amino acids present in uterine and oviductal fluid.

In the interest of simplicity, this discussion on the actions of progesterone was made as if these actions were independent of the influences of other hormones. No hormonal action is completely independent of the action of other hormones. Progesterone causes mucus secretion by an endometrium primed for its influence by the relatively high estrogen levels during estrus (114, 123). The ratio of progesterone to estrogen is often more important than the level of progesterone in the response elicited (114, 123).

2. Levels in the Bovine

The recent development and wide spread acceptance of the competitive protein binding procedure, which allows rapid, relatively simple assaying of progesterone in peripheral plasma samples as small as 0.5 milliliters (ml), has greatly accelerated the progress in determining circulating levels of progesterone (73, 92). The large volumes of plasma (20 to 500 ml) and highly skilled technicians required with previous procedures [primarily gas-liquid chromatography (GLC) and double-isotope-derivitrol (DID)], have been a barrier to data collection (92). Sampling was generally infrequent with small numbers. The DID procedure often resulted in erroneously high estimates due to recovery of tritium not associated with progesterone.

(a) Estrus Cycle: Peripheral plasma progesterone is lowest during a four to six day period centered on the day of estrus, begins to rise three to four days after estrus, reaches a peak 12 to 15 days post estrus and begins to decline about three days prior to the next
estrus. Britt and Ulberg (21) reported circulating progesterone rose from 0.5 ng/ml at estrus to 4.4 ng/ml on day 15, then dropped from 4.2 to 0.5 ng/ml during the three days preceding the next estrus. Most workers (11, 29, 32, 41, 92, 95, 96) agree that the level of progesterone at estrus is less than 1 ng/ml with 6 to 7 ng/ml most common (11, 29, 46, 96).

(b) Pregnancy: Peripheral plasma progesterone levels in pregnant animals are similar to those in open animals for the first 18 days after estrus and a level similar to that attained during the luteal phase of the estrus cycle (4.0 to 8.0 ng/ml) is maintained through the first 100 days (11, 29, 30, 32, 46, 94, 112). There is some disagreement as to whether circulating levels of progesterone during the second 100 days are the equal to or lower than levels during the first 100 days (29, 30, 97). Progesterone increases from 200 days of gestation to 260 days and then begins a slow decline (29, 94, 97). A precipitous decline begins four days prior to parturition and levels at parturition are similar to those during estrus (32, 94, 97).

(c) Normal vs Repeat Breeders: There are few reports in the literature comparing circulating progesterone levels in normal and repeat breeders, presumably because most efforts to date have concentrated on determining the levels during estrous cycles and pregnancy in normal cattle.

Edgerton and Hafs (32) reported nearly identical plasma progesterone levels up to day 11 post-estrus in fertile and infertile cows. Wetteman and Hafs (113) found no difference in circulating progesterone levels of fertile and non-fertile cows during days 2 through 11 after insemination. Circulating progesterone levels on the day of
Estrus were lower in normal cows than in animals which had silent heats, or failed to ovulate or did not develop corpora lutea (81). There were no differences between groups on days 7 and 14 post-estrus. Britt and Ulberg (21) reported peripheral plasma progesterone levels were higher on the day prior to synchronized estrus (3.1 ng/ml) than on the day preceding control estrus (0.5 ng/ml). Conception rates at synchronized estrus are consistently low (12, 45, 82, 86).

Progesterone levels are highly variable between and within cows in similar stages of the estrous cycle and of pregnancy (11, 46, 92, 96, 97).

3. Effect of Stress on Progesterone Level

Wiersma and Stott (117) stated that the symptoms of cows exhibiting infertility during hot weather or when exposed to thermal stress in a heat chamber suggested that progesterone might somehow be involved. Secretion of either excessive or insufficient amounts of progesterone could result in incompatibility of the uterus and the embryo, embryonic mortality, and the prolonged estrous cycles observed in animals bred but not pregnant. These workers (117) reported that lactating cows having access to cooled shades (average temperature 5.6 to 6.7 C cooler than control shades) during the summer months had blood progesterone levels about 10% of those of the control animals. Conception rate for the treated group was double the conception rate of the control group. A subsequent report from the Arizona Station noted an immediate increase in peripheral plasma progesterone levels in response to heat stress (37). Plasma progesterone remained elevated through 30 days of heat stress. The increase was observed in ovariectomized as well as intact animals, indicating the ovaries were not involved.
Stott and Robinson (101) reported that acute thermal stress resulted in a sudden, short-lived, large increase in plasma progesterone concentration while continued stress suppressed the level of the hormone. Abilay and Johnson (1) reported heat stress increased plasma progesterone levels in heifers on day 2 to 19 of the first cycle after exposure and on day 2 to 6 of the second cycle. Lee et al. (61) exposed lactating Holsteins to environmental temperatures which were increased from 15.5 to 29.5 C over a period of five weeks (2.8 C/wk) and maintained at the latter temperature for three weeks. The average peripheral plasma progesterone concentration for the three weeks at 29.5 C was somewhat higher than the concentration at lower temperatures but the difference was not statistically significant (61).

Gwazdauskas et al. (41) investigated the effect of seasonal climatic changes on circulating progesterone levels 14.5 h post-insemination and suggested environmental temperatures on the day of estrus might have had an effect on progesterone levels the day of ovulation and fertilization. Of nine cows with progesterone concentrations over 0.7 ng/ml plasma, eight were sampled when average environmental temperature on the day of insemination was 24.3 or 6.3 C above the mean average temperature for the experiment. A seasonal difference in response to an exogenous progestin has been observed at this station (83). The degree of estrus synchronization attained by feeding melengestrol acetate is less during the hot season than during the cool season (smaller percentage of treated animals exhibit estrus in a given time interval), possibly because removal of the exogenous progestin leaves a relatively high endogenous level (83).
The progesterone response to nutritional stress (underfeeding) appears to be similar to that observed with heat stress. Donaldson et al. (29) reported undernutrition (25% of previous intake) increased circulating progesterone concentrations during the luteal phase of the first cycle after feed restriction but reduced it in later cycles. Undernutrition also increased progesterone levels in mid and late pregnancy. Undernutrition apparently increases progesterone levels only when it is sufficient to cause decreases in body weight.

4. Extra-Ovarian Sources of Progesterone

Wiersma and Stott (117) reported elevated plasma progesterone levels in heat-stressed cattle, some of which were ovarectomized, suggesting that the increased progesterone came from an extra-ovarian source. The adrenal gland was suggested as this source. Progesterone is synthesized in the adrenal cortex as a precursor of the adrenal corticosteroids (114, 117, 123). Balfour et al. (9) reported the concentration of progesterone in adrenal venous blood was 10 to 100 times greater than the concentration in arterial blood collected at the same time. Gomes and Erb (38) in a review on progesterone in bovine reproduction, concluded that the adrenal glands do release progesterone. Adrenocorticotropic (ACTH) injections cause an increase in peripheral plasma progesterone levels (40, 117) and this suggests that any stress could cause an increased release of adrenal progesterone. Gwazdauskas et al. (40) revealed the increase in plasma progesterone occurred between 15 and 30 min after ACTH injection and was comparable to the increase in progesterone concentration expected between days 1 and 5 of the estrous cycle. The progesterone increase was much smaller and of shorter duration than that observed in cortisol concentration (40).
Progesterone returned to basal levels by 2 h post-injection while cortisol was still elevated 4 h post-injection. The investigation did not differentiate the source of progesterone but the increase in progesterone was considered to be of adrenal origin since the adrenal cortex is the primary target organ of ACTH (40). These results suggested the adrenal contributes a significant proportion of plasma progesterone.

Stott and Robinson (101) reported a sudden but short-lived large increase in both plasma cortisol and progesterone in response to acute thermal stress. Constant continued stress suppressed plasma concentrations of both hormones. Conversely, Lee et al. (61) reported a decrease in cortisol levels and no significant change in progesterone concentration as a result of gradual exposure to heat stress. The data did not suggest any relationship between adrenal cortical secretion rate and progesterone concentration. Abilay and Johnson (1) reported plasma progesterone was elevated during the first estrous cycle after exposure to heat stress and on days 2 to 6 of the second cycle. Cortisol was unchanged during the first cycle and decreased during the second. Progesterone and cortisol were positively correlated \( r = 0.65 \) only in the first cycle after exposure to temperature stress. Gwazdauskas et al. (41) reported seasonal changes in environmental temperature increased plasma progesterone levels 14.5 h post-insemination but did not change cortisol concentration. The degree of acclimation may determine whether a relationship between adrenal secretion rate and plasma progesterone concentration is observed in heat stressed animals.

Body fat is another possibility as an extra-ovarian source of progesterone. The effects of climatic stress on reproductive efficiency and plasma progesterone concentrations are similar to the effects of
undernutrition on these variables. The effect of heat stress on body weight change is similar to the effect of undernutrition on weight change because temperature stress decreases feed intake. Circulating progesterone is taken up by fatty tissue and this tends to delay its readsoption into the blood and subsequent metabolism and excretion (123). McCracken (64) reported the progesterone content of body fat of the bovine is five to ten times that in plasma at mid-cycle. Release of progesterone from fatty tissue as a result of metabolism of this tissue for energy could increase circulating progesterone levels. Failure of gonadotropin release, specifically follicle stimulating hormone (FSH) release, has been suggested as the cause of the anestrus and low conception rates associated with insufficient energy intake (102, 120). Stott and Thomas (102) investigated cortisol response to undernutrition and suggested a connection between adrenal corticol secretion and FSH release. However, the differences in reproductive response within a group of underfed heifers were related to differences in weight loss within the group (102). The heifers settling on first service lost less weight prior to breeding than those which did not conceive and had stopped losing weight when they were bred (102). Progesterone is involved in the feedback control of gonadotropin release and differences in progesterone release from body fat could account for the results obtained (114, 123).

5. Effect of Photoperiod

Domestic animals can be divided into three categories based on the effect of photoperiod on their reproduction (42); 1) those that reproduce during the seasons of long daylight, 2) those that reproduce during the seasons of short daylight, and 3) those whose long
domestication has caused their sensitivity to photoperiodic stimulations to degenerate. Cattle are continuous breeders (exhibit estrus and ovulate year round) and are included in the third category but they do exhibit seasonal fluctuations in sexual activity and reproductive efficiency (75). Seasonal differences in photoperiod may account for a proportion of these fluctuations (75, 76).

Both temperature and photoperiod vary with seasons and their relative importance in seasonal differences in reproductive efficiency is apparently influenced by geographic location (75, 76). Photoperiods have been implicated as the important influence in areas of moderate temperature while temperature is considered more important in hot climates (75, 76, 88).

Ortevant et al. (75) concluded that the variations in reproductive activity of cattle (maximum at the beginning of autumn and in spring) are evidence of the existence of an optimal light photoperiodic zone. The optimal photoperiod is believed to be 12 to 13 h. These investigators also concluded that the light photoperiod facilitates the release of gonadotropic hormones; whereas, the dark photoperiod encourages their synthesis.

No report on the effect of photoperiod on plasma progesterone could be found in the literature.
III. EXPERIMENTAL PROCEDURE

A. GENERAL OUTLINE

Peripheral plasma progesterone was assayed in 264 blood samples (jugular vein) taken from lactating Holsteins in the Louisiana State University herd over a period of one year (January to December, 1969). Each month the lactating Holsteins in the herd were grouped according to lactation stage and number. An 18 to 24 cow sample containing proportionate numbers from each group was randomly selected. These animals were bled between 0800 and 0900 hours on a single day during the middle of each month (11 to 19th). Selection of an animal in a previous month did not affect its subsequent selection, therefore, data were collected from individual cows from one to ten times.

Blood samples were collected in 40 ml collection tubes containing three drops of the anticoagulant (dipotassium ethylene diamine tetra-acetate), centrifuged at 886 X G for 20 min, the plasma withdrawn and stored at -4 C until analyses. Rectal temperatures and respiration rates were taken between 0700 and 0800 hours on the day of blood collection.

Animals were maintained under routine management, housing, and milking procedures established for the Louisiana State University dairy herd and had received no treatment for clinical disease symptoms for at least 21 days prior to blood collection. They were given the National
Table 1. Climatic measurements by months and seasons.

<table>
<thead>
<tr>
<th>Temperature-season</th>
<th>Month</th>
<th>Temperature Max (C)</th>
<th>Temperature Min (C)</th>
<th>Dew Point (C)</th>
<th>THI&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Cool)</td>
<td>Dec.</td>
<td>15.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9</td>
<td>6.7</td>
<td>59</td>
</tr>
<tr>
<td>1</td>
<td>Jan.</td>
<td>21.7</td>
<td>6.7</td>
<td>10.0</td>
<td>67</td>
</tr>
<tr>
<td>1</td>
<td>Feb.</td>
<td>17.2</td>
<td>5.6</td>
<td>9.4</td>
<td>62</td>
</tr>
<tr>
<td>1</td>
<td>March</td>
<td>14.4</td>
<td>3.3</td>
<td>6.7</td>
<td>57</td>
</tr>
<tr>
<td>1</td>
<td>Average</td>
<td>17.2</td>
<td>4.9</td>
<td>8.2</td>
<td>61.3</td>
</tr>
<tr>
<td>2 (Intermediate)</td>
<td>Oct.</td>
<td>28.9</td>
<td>16.1</td>
<td>19.4</td>
<td>77</td>
</tr>
<tr>
<td>2</td>
<td>Nov.</td>
<td>23.9</td>
<td>9.4</td>
<td>12.8</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>April</td>
<td>25.6</td>
<td>20.0</td>
<td>25.6</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>May</td>
<td>27.8</td>
<td>15.0</td>
<td>21.7</td>
<td>77</td>
</tr>
<tr>
<td>2</td>
<td>Average</td>
<td>26.6</td>
<td>15.1</td>
<td>19.8</td>
<td>75.0</td>
</tr>
<tr>
<td>3 (Hot)</td>
<td>June</td>
<td>31.7</td>
<td>17.8</td>
<td>18.9</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>July</td>
<td>32.2</td>
<td>23.3</td>
<td>27.2</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>August</td>
<td>34.4</td>
<td>22.2</td>
<td>23.3</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>Sept.</td>
<td>32.2</td>
<td>17.8</td>
<td>23.3</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>Average</td>
<td>32.6</td>
<td>20.3</td>
<td>23.2</td>
<td>82.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average of daily measurements for the 14 days preceding date of blood collection.

<sup>b</sup> THI = 0.72 (dry bulb temperature + wet bulb temperature C) - 40.6.
Research Council's (74) estimated net energy requirements for maintenance and production in a ration consisting of No. 1 leafy alfalfa hay, good corn silage, and a 16% crude protein concentrate. Water and trace mineralized salt in block form also were available ad libitum.

B. SEASONS

The months were grouped into three temperature seasons (cool, intermediate and hot) on the basis of differences in the averages of the maximum temperatures, minimum temperatures, dew point, and temperature-humidity-indices for the 14 days preceding the day the blood samples were collected (Table 1). Temperature-season 1 consisted of the cool months of December, January, February and March. Temperature-season 2 was comprised of the intermediate months of October, November, April and May. Temperature-season 3 consisted of the hot months of June, July, August and September.

C. REPRODUCTIVE STATUS

The effect of reproductive condition on plasma progesterone concentration was determined by dividing the animals into the following reproductive categories: 1) pregnant 1 to 90 days; 2) pregnant 91 to 180 days; 3) open, cycling regularly and required three or less services for conception; 4) open, but not cycling (anestrus); and 5) open, cycling regularly and required four or more services for conception.

D. PROGESTERONE ASSAY

The progesterone concentration of the plasma samples were determined by the competitive protein-binding method of Murphy (73) as modified by Stott (100). The procedure is as follows:
1) Pipet 0.5 ml plasma into a 15 ml conical test tube.

2) Add 3 ml hexane and mix for one minute on a vortex mixer.

3) Remove hexane layer with a disposable pipet into a 15 X 18 mm culture tube. Be careful not to transfer any plasma.

4) Repeat the hexane extraction.

5) Tubes may be covered and temporarily stored under refrigeration at this time if necessary.

6) Evaporate to dryness under nitrogen at 40 - 50 C.

7) Add one ml cold corticosteroid-binding globulin solution (CBG) (in ice bath) to each culture tube. Shake each tube for 10 sec and incubate for 5 min at 45 C.

8) Cool in ice bath for 10 min.

9) Add approximately 40 mg Florasil to the tube and shake 30 sec. Wait 15 sec and add Florasil to next sample, repeating this procedure until all samples are finished.

10) At the same time Florisil is being added to the fifth sample, another technician transfers 500 lambda of the CBG mixture, avoiding any Florisil, into a vial containing 10 ml of scintillation solution.

11) Keeping a 3 sample interval, the two technicians repeat this procedure until all samples have been exposed to Florisil for 2.5 min and 500 lambda CBG removed.

12) Count each vial and compare to standards of 0, 1/2, 1, 2, 3, 4, 5, 6, 8, and 10 ng.

The CBG was prepared using serum from a dog receiving diethylstilbestrol (1 mg/day) to reduce steroid variability (100). The procedure for preparation follows:
1) Place 0.25 mci of corticosterone -1, 2-H\(^3\) into a 25 ml volumetric flask and bring to volume with absolute ethanol. Store in individual glass vials (0.5 ml/vial) in freezer.

2) Place 5 mci corticosterone -1, 2-H\(^3\) (1 vial) in a 100 ml volumetric flask.

3) Add 75 ml PO\(_4\) buffer (0.1 molar KH\(_2\)PO\(_4\) and K\(_2\)HPO\(_4\) in distilled water).

4) Add 2.5 ml dog serum and mix well.

5) Bring to volume with PO\(_4\) buffer and store in dark glass bottle in refrigerator.

The procedure for preparation of scintillation solution for progesterone analysis is as follows:

1) Place 0.3 g POPOP (2, 2' paraphentlene bis 5- phenyloxazole) and 5.0 g PPO (2, 5- diphenyloxazole in a one liter volumetric flask.

2) Bring to volume with toluene and mix well.

3) Add 100 ml Biosolve (Beckman formula BBS-3), mix and store in a dark glass bottle.

Due to variations in concentration of corticosterol binding globulin in dog serum, a new standard curve must be determined each time new CBG is prepared. The most critical step in this procedure is the timing after exposure to Florisil. Eppendorf push-button microliter pipets were used to remove the 500 lambda of CBG mixture in step 10 of the assay procedure in order to improve speed and accuracy.
E. STATISTICAL ANALYSIS

These data were analyzed according to the methods outlined by Steele and Torrie (98) for a 3 X 5 factorial arrangement of treatments (3 seasons and 5 reproductive statuses) in a completely random design. Season was considered a random effect and reproductive status a fixed effect. Repeated observations on a single animal were treated as observations on different experimental units. Least squares procedures were used to correct for unequal numbers of experimental units per treatment. Simple correlations between progesterone and other variables were determined. Analysis of covariance was used to correct treatment means for age differences between treatments.
VI. RESULTS AND DISCUSSION

A. AGE AND PROGESTERONE

The simple correlation between animal age and progesterone concentration (Table 2) was the largest correlation involving progesterone and resulted in these data being adjusted for age differences in the experimental animals. Lactation number, which reflects age, was the variable actually used in making the age corrections. Progesterone concentration decreased an average of 0.8 ng/ml with each successive lactation.

Table 2. Simple correlations between age, lactation number, progesterone, and cortisol.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Lactation No.</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>-0.32**</td>
<td>-0.31**</td>
<td>0.45**</td>
</tr>
<tr>
<td>Progesterone</td>
<td>-0.57**</td>
<td>-0.57**</td>
<td></td>
</tr>
<tr>
<td>Lactation No.</td>
<td>0.96**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** P < 0.01

An effect of age as large as was observed in this investigation is somewhat surprising considering the fact that only sexually mature animals were included. No report of the effect of age on plasma
progesterone concentrations in cattle in the age range involved here could be found in the literature. Any speculation as to the physiological mechanisms involved in this age effect on progesterone or on its physiological significance will have to await further investigation.

All progesterone concentrations reported herein are age adjusted. The simple correlations were determined before age corrections were made and in some cases may be affected by age differences.

B. SEASONS

The purpose of this investigation was to determine whether the hot season resulted in a change in circulating progesterone levels of acclimatized animals. The effect of temperature season on circulating progesterone is shown in Table 3. Progesterone levels were higher in the hot season than in the cool and intermediate seasons. Temperature-season had a significant (P< 0.01) effect on plasma progesterone concentration.

Table 3. Effect of temperature-season on peripheral plasma progesterone.

<table>
<thead>
<tr>
<th>Temperature-seasona</th>
<th>Progesterone (ng/ml)</th>
<th>No. of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Cool</td>
<td>3.4</td>
<td>87</td>
</tr>
<tr>
<td>2 - Intermediate</td>
<td>3.8</td>
<td>90</td>
</tr>
<tr>
<td>3 - Hot</td>
<td>4.6**</td>
<td>87</td>
</tr>
</tbody>
</table>

** (P< 0.01)

a Temperature-season 1 = December, January, February, and March.
Temperature-season 2 = October, November, April, and May.
Temperature-season 3 = June, July, August, and September.

concentration but only as a result of the concentration differences
between the hot season and the average of the cool and intermediate seasons. Plasma progesterone means for seasons 1 (cool) and 2 (intermediate) were not significantly different. The similarity between progesterone levels in seasons 1 and 2 was not unexpected because the temperature differences between the months in the cool and intermediate seasons (Table 1) are primarily fluctuations within a range of temperatures which do not result in any appreciable stress. Maximum temperatures observed during the hot season were consistently and substantially above those required for stress (39, 53, 61, 70, 80).

Simple correlations between progesterone concentrations and climatic measurements are presented in Table 4. These correlations are

Table 4. Simple correlations between progesterone level and climatic measurements.

<table>
<thead>
<tr>
<th>Climatic Measurements</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum temperature</td>
<td>0.11</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>0.16**</td>
</tr>
<tr>
<td>Dew point</td>
<td>0.19**</td>
</tr>
<tr>
<td>Temperature-humidity-index (THI)</td>
<td>0.14*</td>
</tr>
</tbody>
</table>

* (P<0.05)
** (P<0.01)

small and indicate that a relatively small fraction of the variation in progesterone level was associated with changes in climatic conditions. Different progesterone concentrations in open cattle sampled while under similar climatic conditions but in different phases of their respective estrous cycles account for part of this lack of association
and, again, changes in climatic conditions as in seasons 1 and 2, which did not change the total stress on the animal could also contribute.

The lack of a significant correlation between progesterone and maximum temperature may result from the fact that Louisiana summer conditions exert a stress greater than the maximum temperature would indicate because of accompanying high humidity. Maximum temperatures are consistently high and the stress exerted varies with the humidity (39, 53, 54, 61, 79). The simple correlation between dew point and progesterone level was the largest correlation involving a climatic measurement and the correlation between progesterone and THI, which is based on both maximum temperature and dew point, was significant (Table 4). Stott and Williams (103) reported heat stress was greatest in the months of August in Arizona. Maximum temperatures in the adjacent months were higher but humidity was highest in August.

The significant (P< 0.01) correlation between progesterone and minimum temperature was an indication of the relation between minimum temperature and the duration of any rest period provided by diurnal variations in climatic conditions.

Wiersma and Stott (117) and Gwazdauskas et al. (41) have reported natural summer climatic conditions resulted in higher plasma progesterone levels in blood samples taken at or near (14.5 h later) the time of insemination. This investigation included samples taken throughout the estrous cycle as well as samples from pregnant animals. The increase in plasma progesterone concentration observed in these samples was consistent across all reproductive statuses.
C. REPRODUCTIVE STATUS

The effect of reproductive status on plasma progesterone concentration is presented in Table 5. Reproductive status did not have a statistically significant effect on circulating progesterone levels (Appendix table 1).

The progesterone levels in the pregnant animals (Table 5) are within the range (4.0 to 8.0 ng/ml) reported in the literature for animals in the first 200 days of pregnancy (29, 30, 97). The progesterone levels in open, cycling animals (statuses 3 and 5) were expected to be lower than those in pregnant animals because the averages for cycling animals include samples taken near estrus when progesterone concentrations are low. Abilay and Johnson (1) reported the average

<table>
<thead>
<tr>
<th>Reproductive status</th>
<th>Progesterone (ng/ml)</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>3.9</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>3.6</td>
<td>84</td>
</tr>
</tbody>
</table>

1 = Pregnant 1 - 90 days.
2 = Pregnant 91 - 180 days.
3 = Open, cycling regularly and required 3 or less services for conception.
4 = Open but not cycling (anestrus)
5 = Open, cycling regularly and required 4 or more services for conception.
concentration observed during the estrous cycle was 3.6 ng/ml.

Progestosterone values for the normal (status 3) and repeat breeders (status 5) are essentially the same and suggest that if differences in progesterone levels contribute to the repeat breeder problem these differences are either confined to a relatively short period during the estrous cycle, or are important in only a small portion of the repeat breeders. Since the progesterone concentrations in Table 5 are averages of samples taken throughout the estrous cycles of the normal and repeat breeders, these concentrations would tend to underestimate differences in progesterone levels between the two groups which may have occurred for short periods during their estrous cycles. Randel et al. (81) reported plasma progesterone concentrations on the day of estrus were higher in cows with ovarian dysfunction than in normal animals. Concentration of progesterone on days 7 and 14 of the estrous cycle were not different. The complexity of the reproductive process and the numerous conditions which can disrupt make it unlikely that a single factor would account for a large percentage of the reproductive malfunctions which result in repeat breeders.

There are two conditions which might be responsible for the failure of anestrus animals to cycle. First, a failure of gonadotropin release from the pituitary would result in failure of follicle development, ovulation, and corpus luteum development. The lack of a corpus luteum would result in progesterone levels near zero. Second, progesterone might be maintained at a level sufficient to inhibit, through the feedback mechanism, gonadotropin release and the responses this would elicit. In both cases, gonadotropin levels would be low and anestrus would be observed, but in the second case, progesterone would
be the cause of anestrus and progesterone concentrations would be expected to be similar to those observed in pregnant animals. The progesterone level observed in the anestrus animals (Table 5, status 4) indicates progesterone is responsible for their failure to cycle.

D. PHOTOPERIOD

Since both daylength and climatic conditions change with seasons, an effort was made to determine whether photoperiod contributed to the differences in progesterone concentrations observed in different seasons. The progesterone level for the months of March, April, and May were compared with the concentrations in July, August, and September; a comparison of hot and cool periods of equal daylength. Plasma progesterone averaged 3.9 ng/ml in the cool period (March, April, and May) and 5.0 ng/ml in the hot period (July, August, and September).

The 1.1 ng/ml difference in progesterone concentration suggested temperature variation alone could result in large differences in progesterone concentration, however, this difference was not tested statistically. Daylength is increasing in the cool period and decreasing in the hot period but Ortevant et al. (75) considered the length of the photoperiod to be more important than whether it was increasing or decreasing.

Seasons 1 and 2 differ in both daylength and temperature but the temperature differences are not considered important since the temperatures in both seasons are below that required for stress (Table 1). The difference in the photoperiod of these seasons may be responsible for the 0.4 ng/ml difference in the progesterone concentrations for the seasons (Table 3). However, this difference was not
statistically significant.

The above comparisons appear to support the contention that temperature is more important than photoperiod in hot climates (75, 76).

E. RELATIONSHIP BETWEEN CORTISOL AND PROGESTERONE

Reports in the literature have suggested that the increases in circulating progesterone levels observed in heat stressed cattle are contributed by the adrenal cortex as a result of increased adrenal cortical secretion. However, the glucocorticoids secreted by the cortex are calorogenic and a decrease in their output appears logical in animals attempting to decrease their internal heat load (27, 60). Cortisol concentrations were determined in aliquots of the blood samples used for progesterone assay in this investigation allowing the effect of season on progesterone concentrations to be compared with its effect on adrenal-cortical secretion rate. Cortisol levels were lower (P<0.01) in the hot season than in the cool and intermediate seasons indicating adrenal-cortical output was lower in the hot season. As noted above, progesterone levels were higher in the hot season than in the cool and intermediate seasons (Table 3). An earlier investigation at Louisiana State University also indicated that the adrenal cortex is not responsible for increased progesterone levels in acclimatized, heat-stressed cattle (27).

Although a negative correlation between cortisol and progesterone might be as a result of the opposite effect of temperature-season on these variables, the correlation obtained was large and positive (r = 0.45). The simple correlations were calculated before age corrections were made and a similar effect of age on both cortisol and
progesterone (Table 2) probably contributed to the association between cortisol and progesterone. In addition, sudden, large deviations in climatic conditions may have caused alarm reactions, abnormally high cortisol secretion by the adrenal-cortex, and substantial progesterone release from the adrenal-cortex. This apparently happened in the month of February. The mean maximum temperature for the 14 days preceding bleeding was 17.2°C but the maximum temperature jumped to 26.0°C four days prior to bleeding, remained at that level for two additional days, and dropped to 17.2°C on the day prior to bleeding. Cortisol and progesterone concentrations in February averaged 90.4 and 5.1 ng/ml plasma, respectively. Cortisol and progesterone concentrations averaged 42.3 and 3.4 ng/ml plasma, respectively in the cool season. In a previous investigation in which all animals were in the second lactation and were maintained under artificial climatic conditions which precluded sudden large fluctuations in these conditions, the correlation between cortisol and progesterone was not significant.

These data do not dispute the theory that the adrenal cortex can be, and probably is in certain situations, an extra-ovarian source of progesterone (40, 61, 117). However, they do indicate that a different extra-ovarian source must be responsible for increased progesterone levels in acclimatized, heat-stressed cattle. The adrenal output, as measured by circulating cortisol level, was lower in the hot months than in the cool months in the cattle in this study. Similar results have been obtained in investigations conducted in climatic control chambers (6, 61). The theory that heat stress increases adrenal-cortical output appears incorrect when monitoring is continued beyond the initial alarm stage for cattle stressed by artificial means and, as was
the case in this investigation, when they are under natural climatic conditions and temperatures increase gradually over a long period of time. Decreased glucocorticoid output is compatible with an effort on the part of the animal to decrease its internal heat load. Therefore, the theory that increased circulating levels of progesterone observed during hot seasons are a result of release of progesterone from the adrenal cortex appears incorrect.

The extra-ovarian source could be body fat, although there was no evidence of this. McCracken (64) has reported high levels of progesterone in body fat. The phenomenon of cattle suffering substantial weight losses failing to cycle is well known both in dairy cattle and in beef cattle (12, 120, 121). Accelerated and more sustained weight losses are a common problem for high producing dairy cattle exposed to heat stress, primarily as a result of reduced feed intake. The continuous metabolism of fat reserves by an animal trying to maintain its production in the face of insufficient intake of feed may result in release of progesterone in these reserves into the bloodstream. The inhibition of estrus by progesterone is well documented, as is the lowered conception at synchronized estrus in cattle synchronized with exogenous progesterone. Failure of cattle to exhibit estrus and lowered conception rates are common problems in hot environments. The "fat" theory is essentially pure speculation in the absence of any direct evidence to support it. However, the indirect evidence is sufficient to warrant further investigation.
V. SUMMARY AND CONCLUSIONS

This investigation was conducted to determine whether the hot, humid summer conditions common to the Gulf Coast region of the United States resulted in a change in peripheral plasma progesterone levels in lactating dairy cattle. A comparison of the effect of these conditions on circulating progesterone concentrations with their effect on circulating cortisol levels was included to determine if the adrenal-cortex was involved in any change observed in progesterone concentrations. A comparison of data from animals grouped according to their reproductive condition and efficiency was included to determine if the effect of season on plasma progesterone concentrations was changed by the reproductive status of the animal and to investigate the role of progesterone in reproductive malfunctions.

Plasma progesterone concentrations were determined in 264 jugular vein blood samples taken over a period of one calendar year from lactating Holsteins maintained under routine housing, feeding, and management conditions. The 264 total samples were obtained by bleeding 18 to 24 cows each month. The year was divided into cool, intermediate, and hot temperature-seasons. Each sample was assigned a temperature-season based on climatic conditions prevailing during the 14 days preceding the date the animal was bled and a status based on the reproductive condition of the animal when bled and the number of services
required for conception. The reproductive statuses assigned were:
1) pregnant 1 to 90 days; 2) pregnant 91 to 180 days; 3) open, cycling and required 3 or less services for conception; 4) open, anestrus; and 5) open, cycling and required 4 or more services for conception. Since photoperiod changed with season, the effect, if any of this variable on progesterone concentration had to be considered.

Mean plasma progesterone concentrations in blood samples obtained during the cool, intermediate, and hot temperature-seasons were 3.4, 3.8, and 4.6 ng/ml, respectively. Progesterone concentrations during the hot season were significantly higher ($P < 0.01$) than the concentrations during the cool and intermediate seasons whose means did not differ significantly. The simple correlation between plasma progesterone levels and dew point temperature ($r = 0.19$) was statistically significant ($P < 0.01$), while the smaller correlation between progesterone concentration and maximum temperature ($r = 0.11$) was not, indicating that when temperatures are consistently and substantially above levels required for stress, the stress exerted varies with the humidity. The correlation between circulating progesterone level and THI ($r = 0.14$) was statistically significant ($P < 0.05$). The significant ($P < 0.01$) correlation between minimum temperature and progesterone concentration ($r = 0.16$) is indicative of the relation between minimum temperature and the duration of any rest period provided by diurnal variations in climatic conditions during the hot season.

Plasma cortisol concentrations were lower in the hot season than in the cool and intermediate seasons, indicating adrenal-cortical output was lower during the hot season. Although the effect of temperature-season on cortisol levels was opposite its effect on
progesterone concentrations, a significant (P<0.01) positive correlation (r = 0.45) between progesterone concentration and cortisol concentration was observed. A similar effect of age on the two variables appeared to be partly responsible for the positive association between them. Sampling of some animals in the alarm phase of the general adaptation syndrome may also have contributed.

Mean plasma progesterone concentration was highest (4.5 ng/ml) in cattle in the first trimester of pregnancy. Mean progesterone concentrations in animals in the second trimester of pregnancy (4.0 ng/ml) were intermediate. The lowest levels were observed in the open cycling animals (3.5 ng/ml in normal breeders and 3.6 ng/ml in repeat breeders). The season by reproductive status interaction was not statistically significant.

The contribution of photoperiod to the differences in progesterone levels observed between seasons was evaluated by comparing progesterone concentrations in a hot period (July, August, September) to concentrations in a cool period (March, April, May) of equal day-length. Plasma progesterone levels averaged 5.0 ng/ml in the hot period and 3.9 ng/ml in the cool period, suggesting temperature difference alone could result in large differences in progesterone concentration. In addition, seasons 1 and 2 were considered seasons of equal temperature and different day-length. The climatic conditions are different but the climatic stress they exert on the animal is not. Progesterone concentrations in seasons 1 and 2 were not significantly different.

The correlation between age and progesterone (r = -0.57) was the largest correlation involving progesterone and resulted in these data being corrected for the effect of age.
The data summarized above appear to support the following conclusions:

1) Ambient climatic conditions common during the hot season in the Gulf Coast region result in an increase in peripheral plasma progesterone levels in lactating dairy cattle.

2) The adrenal cortex does not contribute to this increase in acclimatized cattle.

3) The effect of temperature-season on plasma progesterone concentrations was not changed by the reproductive status of the animal.

4) If abnormal progesterone concentrations contribute to the repeat breeder problem, these abnormal concentrations prevail for a short period during the estrous cycle and/or are a factor in a small percentage of repeat breeders.

5) Anestrus was primarily the result of persistent progesterone secretion rather than failure of gonadotropin release.

6) Progesterone decreases with increasing age in lactating animals.

7) Additional research will be required to determine the physiological mechanism responsible for the increase in peripheral plasma progesterone concentrations during the hot seasons and the contribution of this increase to the reduced reproductive efficiency observed in these seasons.
VI. BIBLIOGRAPHY


VII. APPENDICES
### Appendix Table 1. Analysis of variance for effects of season, reproductive status, season X reproductive interaction and lactation number on progesterone.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Partial sums of squares</th>
<th>F value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>2</td>
<td>45.190</td>
<td>4.87</td>
<td>0.0086</td>
</tr>
<tr>
<td>Reproductive status</td>
<td>4</td>
<td>21.668</td>
<td>1.16</td>
<td>0.3250</td>
</tr>
<tr>
<td>Season X reproductive status</td>
<td>8</td>
<td>29.579</td>
<td>0.79</td>
<td>0.6069</td>
</tr>
<tr>
<td>Lactation No.</td>
<td>1</td>
<td>579.467</td>
<td>124.94</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>248</td>
<td>1150.096</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
VITA

James Francis Beatty was born March 30, 1942 in Baton Rouge, Louisiana. He graduated from St. Joseph's High School, New Roads, Louisiana in May 1960.

In September of 1960, he entered the University of Southwestern Louisiana and in May of 1965, he received a Bachelor of Science degree in Animal Science.

He entered the Louisiana State University graduate school in September of 1965 and received the degree of Master of Science in Reproductive Physiology in the Department of Dairy Science in August, 1968. The title of his M.S. thesis was "The Effects of Extenders and Freezing Methods on Motility of Bovine Spermatozoa".

In June, 1968 he began work toward the degree of Doctor of Philosophy in Reproductive Physiology in the Department of Dairy Science. During this period of graduate study he was elected to associate membership in the Society of Sigma Xi.

He served as a research associate in the Department of Dairy Science from December, 1967 to August, 1970 at which time he resigned to concentrate on course requirements for his Ph. D. On July 1, 1971 he was employed as an assistant dairyman at the North Mississippi Branch Experiment Station and presently holds that position.