HIGH CLIMATIC TEMPERATURE EFFECTS ON PERIPHERAL BLOOD PROGESTERONE AND CORTISOL LEVELS AND REPRODUCTIVE EFFICIENCY IN THE BOVINE

by

Brannick Lester Riggs

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STATEMENT BY AUTHOR

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SIGNED: Brannick L. Riggs

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Durward G. Stott

G. H. STOTT
Dairy Science Department Head

Nov 7, 1969
Date

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TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Plasma progesterone and cortisol levels were determined in dairy cattle in the central Arizona area during the high temperature, low fertility months of the year. These levels were compared with the levels in cattle during the cooler part of the year when conception rates are highest. The purpose of this comparison is to study the levels of these two hormones during seasonal fluctuations in breeding efficiency.

Progesterone levels are significantly lower in dairy cattle during the hot part of the year. Cortisol levels show no change. Low progesterone levels suggest an involvement of this hormone in low breeding efficiency during seasonal high temperatures.

INTRODUCTION

Low breeding efficiency during the hot summer months has hampered the dairy industry in Arizona since it first began. The cause of this lower efficiency has been of interest for many years.

Studies have eliminated the bull as being the prime factor and have indicated that the cow responds to the seasonal high temperatures in such a way as to impair the normal reproductive process. Recent research indicates that the embryo is lost at an early stage.

The direct effect of higher body temperature on the sperm, the ovum, and the embryo, as well as the change in thyroid activity have been studied, and it appears that these factors are not the main cause of the observed effects.

Most evidence points to an endocrine involvement. Recently an extremely sensitive method for measuring steroid hormone levels has provided a possible tool for studying the endocrine reaction to heat stress.

The following experiment was designed to compare the progestin and cortisol levels in dairy cattle exposed to high seasonal temperature with that in animals during the cool part of the year.

REVIEW OF LITERATURE

Low breeding efficiency is one response to high seasonal temperatures which causes problems in domestic animals in desert regions (2, 3, 19, 21, 22, 29, 37, 51, 52, 54, 56, 60). The mechanism of this response has been the subject of many investigations and has been studied from various approaches.

Since it is known that temperature is critical to sperm viability (1, 60), the problem was first thought to be due to the effect of high temperature on the semen. It was felt that the sperm were either affected in the male reproductive tract or in the female tract since both sexes show increased body temperatures during high ambient temperatures. Dutt, Ellington, and Carlton (20) proposed that the elevated body temperature of the ewe may affect the sperm motility or its ability to penetrate the zona pellucida.

More recent work has indicated that the infertility is not due to the male alone. Tests and comparisons by Williams (64) and by Stott (56) with bulls demonstrate that although semen is adversely affected by high temperature, it does not impair fertility to the extent observed during seasonal high temperatures. From these studies and others it has been concluded that the major problem in cattle is with the cow (20, 59).

With the evidence of the aforementioned studies plus the use of artificial insemination in dairy cattle, studies have now been directed toward finding the cause of low breeding efficiency in the cow.

It was first suspected that the direct effect of the elevated body temperature of the female was the factor affecting normal reproduction processes (18). Interesting experiments with ovum transplants in other species have indicated other possibilities (3, 67).

Viability of ova transferred from donor ewes maintained at 70° or 90°F. to mated recipients maintained at 70°F. was not adversely affected. However, ova transferred from animals at 70°F. to animals at 90°F. showed a definite deleterious effect (67). From these studies it was concluded that in the ewe there are some adverse effects on the embryo before it reaches the uterus, but the main effect is the incompatible environment within the uterus (3, 67).

Experiments using controlled environment chambers have shown that the damage occurs prior to implantation in both sheep and cattle. Once the embryo has implanted, temperature has very little effect in causing embryonic death (3, 18, 19, 20, 28, 55, 60).

Although cooling rams or ewes has increased breeding efficiency in one study (2), experiments with cows have shown that cooling the cow on the day she comes in heat and up to six days after she is bred does not result in the expected improvement in conception (62). This rules out a direct effect on the sperm, ovum, or early conceptus as being the cause of lowered fertility and points to a possible involvement of the endocrine system. The apparent long term effect of the high temperature indicates that a physiological response has occurred. The logical system to be involved in this response is the endocrine system.

Studies have been conducted to determine the extent of endocrine involvement. One of the areas which has been studied is the action of

the thyroid gland during high temperature (7, 9, 15, 35, 39, 45, 48, 49, 64). Brow-Grant and coworker's classic method to study the activity of the thyroid and thyroxine release (10) has provided a tool with which this interaction can be measured.

Brody and Frankenbach (9) found that thyroidectomized heifers were completely undeveloped sexually at 40 months. Lennon and Mixner (39) also found a negative correlation between levels of protein bound iodine and rate of conception in cattle. These two findings indicate a possible interaction of thyroxine with reproductive functions.

An inverse relationship between temperature and thyroid activity has been seen in cattle and sheep (7, 15, 35, 45). During periods of high temperature, release of thyroxine is decreased.

Work in sheep (48, 49) has shown that injections of thyroxine increase breeding efficiency. Ryle proposes in this work that thyroxine deficiency causes lower fertility either because the trophoblast enlarges too slowly to stimulate proper endometrium response for implantation or because disproportionate growth in the conceptus causes death.

Williams and Stott (65) found that thyroidectomized cows showed excellent conception rates (80% first service) as compared to normal (17-31%) under the same climatic conditions. This indicates that in cattle at least, the effect of high temperature on reproduction is not mediated through the thyroid.

One of the first endocrine glands that becomes involved in a stress situation is the adrenal. It has been found that the adrenals react in accordance with the type of stress. Collins and Weiner (15) have reveiwed some of the endocrinological interactions during heat

exposure. They stated, as have others (6, 25, 46, 50), that during acute heat stress the adrenal output is drastically increased, but in gradual increases of temperatures over longer periods of time the output is actually diminished. The high temperature chambers used in many experiments stimulate an acute stress reaction while the gradual build up to high seasonal temperatures is a more chronic stress situation. One must be cautious then, in comparing studies using the temperature chamber with those conducted during seasonal high temperatures.

Lactating animals are apparently more subject to stress and their breeding efficiency is affected to a greater degree than non-lactating animals (8, 29, 46). Lactation makes greater metabolic demands and the increased feed intake and increased metabolism cause a greater heat load to be produced within the animal's body. Since hormones are involved in lactation, there is also a possibility that this endocrine involvement might be partially responsible for the low breeding efficiency.

It has been suggested (15, 23, 47, 61) that the infertility of heat stressed animals is due to an inhibition of gonadotrophin output due to a shift in pituitary hormone production to adrenocorticotropic hormone (ACTH). Most workers conclude though, that ACTH increases embryonic mortality through its action on the adrenals (23, 47, 61).

The steroid hormones of the adrenal cortex are of particular interest because of their relationship to the gonadal steroids. Of particular interest is adrenal progesterone.

Progesterone was first isolated from the adrenals in 1938 (5). Since then it has been confirmed that adrenal tissue does produce and secrete progesterone (4, 30, 31).

Progesterone has been implicated with seasonal breeding problems because of the observation of embryonic loss, silent heats, longer interestrual periods, and a longer time from onset of estrus to ovulation in animals exposed to high temperatures (20, 64). Moody (41) found an increase in adrenal tissue progesterone concentration in cows during acute exposure to high ambient temperature and associated this high level with the embryonic degeneration observed. Numerous studies have been conducted to determine if progesterone decreases or increases embryonic mortality (13, 14, 33, 36, 38, 40, 43, 53, 55, 59, 63, 66).

Several mechanisms have been suggested for the action of progesterone in promoting infertility (8, 13, 14, 16, 17). Kim and Foreman (38) found that sufficient supplies of progesterone maintained both normal mitotic activity in the trophoblast and normal contractions of the uterus in spayed rats, and conversely, that an insufficient supply would not maintain this development.

Chang (13, 14) found that the main effect of progestational compounds was a disturbance of sperm capacitation and transport causing degeneration of the eggs and expulsion of them from the uterus of rabbits.

If insufficient levels are present, the endometrium will not be prepared to receive the embryo and death of the embryo will occur (60). If excess amounts are present, it has been found to inhibit cleavage,

possibly by limiting the supply of proteins and amino acids by aggregating on the protective coating of the egg (16).

Progesterone is known to influence the rate of transport of sperm and ova through the oviduct (13, 14, 17, 44). Chang (13, 14) reports that progesterone increases the rate of transport in rats, while Hunter (33) found in the hamster that progesterone diminishes transport rates. Both agree that interference in transport rate does interfere with normal fertilization and development. These opposing reports indicate that an imbalance of progesterone in either direction may cause problems in breeding efficiency.

Some of these same effects have also been seen in cows. Johnson, Ross, and Fourt (36) report that administration of 500 mg. of a slow dispersing progesterone to normal cows and heifers at the time of breeding resulted in 72.2% efficiency as compared to 53.9% in the control group. Wiltbank et al. (66) also found that progesterone injections three days after estrus slightly increased the percent survival in cows of low fertility.

Others claim a detrimental effect from progesterone treatment (13, 14, 33, 43, 53, 55, 59, 63). Slack et al. (53) found that progesterone administered at the time of breeding caused efficiency to drop 20.7%. Stott and Williams (58) found that progesterone injections during times of high ambient temperatures actually increased embryonic death when given on the day the cow was bred. There was no apparent difference when given ten days post-breeding.

Borges (8) observed a migration of the embryo from the uterine horn on the side where ovulation occurred to the opposite horn in

cattle that had been exposed to high temperatures. He suggested that this was due to a localized incompatibility which further indicates an endocrine involvement in the problem of infertility.

Since cortisone has been shown to interrupt pregnancy in mice and rabbits (47), and cortisol injections have interrupted pregnancy in sheep (32), there is a question of the role of the other adrenal steroids in heat exposure infertility.

Knowledge of the action of steroids has been slow in coming forth because of the lack of methods sensitive enough to measure them in the blood. The more modern and sophisticated techniques of fluorometry and spectrophotometry lack specificity. Gas-liquid chromatography, although much more sensitive, is still not satisfactory. The method is subject to interference from contamination and is long and involved (26).

Murphy (42) has recently proposed a method for measuring steroids based on the affinity of a serum protein, transcortin, for steroid molecules. The transcortin is saturated with a labeled steroid. The steroid which has been extracted from the sample is added and a displacement equillibrium is reached. The unbound steroid molecules are then removed. The labeled steroid that remains bound is determined and compared to standards run through the same procedure.

Based on this method, Johansson, Neill, and Knobil (34) have devised a rapid method for progestin determination. Using non-polar solvents, non-polar progestins are extracted without extracting other steroids. They are then analyzed by the same protein binding procedure.

Hagerman and Williams (27) have compared this method with the double isotope derivative method and have found it very comparable. The

advantage of the protein binding method is the simplicity and speed with which a large number of samples can be routinely assayed.

With this method it is now feasible to run experiments to determine plasma steroid levels in animals during periods of exposure to high environmental temperature and compare them to normal levels. The present study was designed to compare the levels of progesterone and cortisol and to try to determine their role in infertility due to high ambient temperature.

EXPERIMENTAL PROCEDURE

Breeding records indicate that breeding efficiency correlates with seasonal high and low temperatures in Arizona (56). The highest efficiency occurs in April and May when temperature and humidity are within the comfort zone of cattle. High temperature and humidity in July and August result in a seasonal low breeding efficiency.

In the present study, blood samples were collected in August, at the time of reported lowest breeding efficiency and again in May, the season of reported highest breeding efficiency, for the determination of plasma hormones. The samples were taken on the day the animals were bred to minimize the fluctuation in hormone levels due to the estrous cycle. Four months later the actual pregnancies resulting from this breeding were checked and recorded. Health records of the cows were also checked and samples from any with reproductive abnormalities such as cystic ovaries, infected uterus, etc., were discarded.

Holstein cows from three large commercial dairies in the central Arizona area were used for this experiment. Samples were taken from first service cows only. Venous blood samples were collected into heparinized containers. All samples were collected over a two day period for both May and August. The samples were immediately placed on ice to inhibit enzymatic breakdown of the hormones and were centrifuged within two hours of collection. The plasma was removed and stored at -10°C. until it was analyzed.

Cortisol levels were determined by the competitive protein binding method (CPB) of Murphy (42). The objective was to determine if there was an involvement of cortisol in seasonal breeding infertility and also as a monitor of adrenal activity.

Total progestins were measured by the CPB method as modified by Johansson, Neill, and Knobil (34). This method does not use thin layer chromatography (TLC) to separate progesterone from the other steroids, but instead utilizes the ability of non-polar solvents to extract non-polar progestins without extracting other steroids. The only steroids extracted with non-polar solvents that participate to any significant degree in the displacement are progesterone and 17alpha-hydroxypregn-4-ene-3,20-dione.

To determine what percent of the total progestins was actually progesterone, TLC was used to check every tenth sample. Since more than 95% of the progestins appeared to be progesterone, it is felt that the comparisons of levels of total progestins gives an accurate indication of progesterone levels.

In adapting these procedures for use with bovine plasma, several problems were encountered which were overcome by the following modifications:

(1) The counting of radioactivity in samples that had been exposed to the fluorescent lights in the laboratory was very erratic. It was learned that this was due to a reaction of the wave length of light emitted by the fluorescent bulbs with peroxides in the dioxane scintillation fluid. This problem was overcome by the use of toluene in place of dioxane since it is

not affected by light. Biosolv BBS-3 from Beckman Instruments was used to make the aqueous corticosteroid binding globulin (CBG) solution soluble in the toluene.

(2) The use of florisil as an adsorbant changes the pH of the CBG solution from 6.8 to 9.1. At a pH this high the binding properties of transcortin become very unstable. This caused significant variation in the counts of radioactivity of the standards and the samples. The use of a phosphate buffer reduced the variation to an acceptable level. The buffer consisted of 0.1M mono-sodium phosphate and 0.1M disodium phosphate in equal proportions. Once the CBG solution was made up, it was kept refrigerated until it was incubated with the extracted steroid.

Several other precautionary measures were also taken to reduce variation and to make an internal check on the procedure. To eliminate variability caused by contamination from glassware, it was soaked in sodium dichromate-sulfuric acid glass cleaning solution overnight. It was then rinsed with water, boiled in soapy water for 30 minutes and rinsed thoroughly in hot water and then in distilled water.

All samples were run in duplicate and any with a difference of 5% or more in counts per minute (cpm) were run again. Most duplicates showed less than 2% variation in cpm.

Recovery was determined on every tenth sample for progesterone. This was done by adding C^{14}-progesterone to the same amount of plasma used in the analysis and extracting it in the usual manner into a scintillation vial. The extract was dried down, scintillation fluid added, and the radioactivity counted and compared to the same amount of

C^{14}-progesterone put directly into vials. Since this is the only step in the analysis where some progesterone might be lost, it is felt that this would compensate for the loss. As it turned out, the recovery was so high (96±1%) that no adjustments were made to the amounts as analyzed.

The 90±2% recovery of cortisol, determined in the same manner, is comparable to that reported by Murphy (42), and was consistent and high enough that no correction for loss was made for it either.

The data for both hormones were statistically analyzed by means of a one-way analysis of variance with an F ratio to compare the mean levels in August and May (24). Calculations were also made within each month to compare hormone levels in animals that settled with the levels in those that did not.

RESULTS AND DISCUSSION

The results of the seasonal study of plasma progestin and cortisol concentrations related to breeding efficiency in dairy cattle are summarized in Tables I and II.

TABLE I

NON-RETURN RATES AND AVERAGE SEASONAL PLASMA PROGESTIN LEVELS

	AUGUST	MAY
Number of cows sampled	38	32
Percent non-return	26	66
Progestins (Ng/ml)		
Average of all cows[a]	.76 (.89)[b]	1.24 (.62)
Average of pregnant cows	.78 (1.10)	1.39 (1.10)
Average of non-pregnant cows	.76 (.80)	.95 (1.00)

a=p .05
b=(standard deviation

Progestin levels in August were significantly (p .05) lower than those in May. In comparing the levels in the animals that conceived with the levels in the animals that did not, there was no significant difference during either month.

These differences suggest that low plasma progestin levels could be involved in the seasonal breeding efficiency in the cow. Several other studies lend support to this supposition.

It is well known that progesterone stimulates proliferation of the endometrium in preparation for pregnancy, and conversely, this development will not occur if sufficient progesterone is not present (24). Under these circumstances the uterus is not compatable with the conceptus and normal pregnancy cannot continue.

Progesterone also affects the rate of passage of the ovum and sperm through the oviduct (13, 14, 17, 44). If the rate of passage of either or both is altered, it is possible that they will not unite at a time when they are capable of fertilization. If fertilization occurs, the ovum may pass too rapidly or too slowly down the oviduct to reach the uterus at a time when it is prepared to receive the conceptus.

Borges (8) found, upon slaughtering cattle that had been exposed to high ambient temperatures, that the conceptus had migrated from the horn on the side where ovulation had occurred to the opposite horn. This is suggestive that either the uterus was not adequately prepared for implantation because of a hormonal imbalance or the rate of transport was affected so that the conceptus reached the uterus too soon.

A number of physiological mechanisms could cause the low levels of plasma progestins observed in the present study. Moody (41) and Glenn (25) found, in cattle exposed to high temperature in a controlled environment chamber, that the immediate response is an increase in adrenal progesterone. Stott (57) has found that cortisol follows the same pattern. These high levels of steroid hormones could cause a decrease in pituitary output of gonadotrophins and ACTH which would cause the depression of ovarian as well as adrenal output of progesterone.

A reduction in adrenal output has been shown in cattle as well as other species when they have been exposed to high temperatures over a long period of time (6, 15, 25, 46, 50). The mechanism of this reduction in activity has not been elucidated. However, this suggests a decrease in ACTH secretion from the pituitary. The same pattern is seen in thyroid activity (7, 15, 35, 46) indicating a decrease in pituitary thyroid stimulation hormone (TSH) secretion. It follows that if ACTH and TSH secretion is reduced, release of the other pituitary hormones such as the gonadotrophins may also be reduced. If this supposition is correct, ACTH would not stimulate adrenal progesterone production nor would the corpus luteum be maintained by luteinizing hormone (LH) to produce sufficient levels of progesterone to maintain pregnancy.

The plasma cortisol levels do not show the statistical difference seen in the progestin levels. These data are summarized in table II.

TABLE II

NON-RETURN RATES AND AVERAGE SEASONAL PLASMA CORTISOL LEVELS

	AUGUST	MAY
Number of cows sampled	38	32
Percent non-return	26	66
Cortisol (Ng/ml)		
Average of all cows	18.8 (12.7)[a]	18.2 (11.3)
Average of pregnant cows	24.2 (10.3)	17.3 (8.7)
Average of non-pregnant cows	16.9 (13.0)	19.8 (15.2)

a=(standard deviation)

There is no statistical difference between the August and May cortisol levels nor between levels in pregnant and non-pregnant cows in either month. With this limited data, no conclusions were made concerning the involvement of cortisol in seasonal breeding efficiency fluctuations.

It was surprising to find that the cortisol levels and progestin levels did not show the same trends. However, there are two ways in which this might be explained. There is a possible involvement of luteal tissue in heat exposure which would alter the progesterone output independently of cortisol. Or, if the role of progesterone in cortisol synthesis is a very minor one as has been proposed (11, 12), production of the two steroids might be independent of each other. By using labeled steroids, Cameron, Beynon, and Griffiths (11) and Cameron and Griffiths (12) found that only 7% of the cortisol and cortisone is formed from pregnenolone via progesterone. The major pathway of cortisol and cortisone synthesis involves pregnenolone conversion to 17alpha-hydroxypregnenolone instead of to progesterone. If this is true, then adrenal cortisol and adrenal progesterone production rates would be independent of each other.

This also leads to speculation on the action of pituitary hormones on tissue other than their accepted target organs. Adrenal progesterone production may be influenced by LH, or luteal steroid production by ACTH. This leads to the possibility of more than adrenal steroid secretion being involved in the hormonal imbalance causing low seasonal breeding efficiency.

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