

THE EFFECT OF HIGH AMBIENT TEMPERATURES ON  
PROGESTERONE CONCENTRATIONS IN THE CORPUS  
LUTEUM AND ADRENALS OF THE BOVINE

by

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## ABSTRACT OF THESIS

# THE EFFECT OF HIGH AMBIENT TEMPERATURE ON PROGESTERONE CONCENTRATIONS IN THE CORPUS LUTEUM AND ADRENALS OF THE BOVINE

Corpus luteum weights, adrenal gland weights, luteal tissue progestin concentrations, and adrenal progesterone concentrations were compared in forty-five heifers and cows that were divided into four groups. Twenty-two were placed in a controlled temperature chamber and their body temperature elevated (heat-treated) on day one (day animal was bred). Eleven were slaughtered on day 14 and eleven on day 32. Twenty-three control animals were bred, with eleven slaughtered on day 14 and twelve on day 32. Each of the four groups was divided into subgroups as determined by the presence of normal embryo, dead or degenerate embryo, or no embryo.

No statistical differences were detected between subgroups for either the corpora lutea or adrenal weights.

Statistical differences in total progestin concentration of the luteal tissue were observed in the following 32-day subgroups: control

nonpregnant versus control pregnant, control nonpregnant versus heat-treated nonpregnant with the highest average level of progesterone in the control nonpregnant group.

In the four 32-day nonpregnant heat-treated animals no detectable amount of 20-B-ol was present.

Limited data on adrenal progesterone found levels ranging from 6.7 mcg to 34.9 mcg in control animals and from 36.9 mcg to 71.6 mcg in heat-treated animals.

Evidence suggests heat stress does increase the production of adrenal progesterone, and the possibility of an adrenal relationship to lowered breeding efficiency may exist in areas where high ambient temperatures are common.

## INTRODUCTION

Failure to reproduce in dairy cattle is undoubtedly due to a number of causes, which include among them poor quality semen, genetic influence, improper nutrition, hormonal imbalance, disease, parasites, and climatic conditions. Exact dollar losses due to reproductive failure are extremely difficult to estimate. However, it is generally believed that reproductive failure costs the American dairyman and beef producer more than a quarter of a billion dollars annually.

The climatic effect on reproductive efficiency is of considerable economic importance in Arizona and many of the Southern states. In the Salt River Valley, which contains over 80% of the dairying in Arizona, breeding efficiency during the summer months drops considerably, especially in the Holstein and Guernsey breeds.

In Arizona 50-60 percent of the dairy cows are bred artificially to bulls that have been tested for high fertility. Many of them are bred with semen produced in areas of cooler summer temperatures. For these reasons it seems reasonable to conclude that abnormalities in the female are responsible for a large portion of the mid-summer seasonal breeding difficulty.

Research workers have suggested that the decline in breeding efficiency during the mid-summer months is not the failure of the sperm to fertilize the ovum, but the failure of the embryo to live longer than 35-40 days. Thus, the question arises, what is the exact physiological abnormality that causes this early embryonic death?

The present study was designed to investigate the possibility of a hormonal imbalance due to elevated body temperatures during the time of estrus and shortly thereafter. The quantitative aspects of progesterin production in the corpus luteum and adrenals in relation to embryonic death during the first month of pregnancy offers a unique mechanism to explore this hypothesis.

Unfortunately experiments of this type are difficult to conduct because of the problem of handling large animals in sufficient numbers to produce statistical differences. There is also the problem of the steroid chemistry which is indeed tricky even with the employment of the advanced techniques which have been developed in the last four years. However, experiments of this nature do offer hope in elucidating the physiological causes of abnormal breeding efficiency.

## REVIEW OF LITERATURE

### Seasonal Variation in Breeding Efficiency

Seasonal variation in breeding efficiency of dairy cattle has been reported by numerous authors (6, 8, 17, 20, 38, 40, 41, 45, 48, 59, 60, 68, 69, 73, 75, 80, 91, 93, 94, 97, 98). Lowered breeding efficiency coincides with high ambient temperature in areas where high summer temperatures are common (17, 38, 41, 45, 48, 60, 80, 91, 92, 97). Stott and Williams (93) demonstrated that in Arizona lowered breeding efficiency corresponds not only to the high temperature season but also particularly to the period of highest humidity during this season. In areas of cooler summer temperatures, lowered breeding efficiency correlates with the season of the shortest periods of daylight (8, 40, 48, 68, 69).

Considerable work has been published on the bull, ram, rabbit, and rat in an attempt to elucidate the male's contribution to lowered seasonal breeding efficiency (9, 14, 16, 20, 29, 32, 33, 37, 38, 40, 45, 48, 59, 65, 72, 94). There is general agreement that high ambient temperatures are detrimental to the breeding efficiency of the male in all species that have been studied (16, 20, 37, 59, 65, 67, 72, 98).

Data on the female's part in seasonal breeding difficulty is limited for the cow (36, 75, 91, 92). However, considerable work has been done with the ewe (3, 4, 5, 26, 27, 29, 30, 31, 54, 77, 78, 104), rat and mouse (1, 10, 21, 57, 66, 70), and rabbit (19, 81).

### Early Embryo Mortality

Dutt (28), working with sheep exposed to high temperatures, found no significant difference in the number of ova fertilized, but did find a higher incidence of abnormal ova. By varying the day that the ewe was exposed to the heat treatment he determined that the zygote of the sheep is most susceptible to damage by high ambient temperature while it is in the oviduct.

Ryle (77), working with sheep that were acclimated to heat, reports 36% pregnant in the hot room at 25 days versus 75% pregnant in the control yard. Daily injection of progesterone and vitamin A appeared to have no effect on embryo survival. Of considerable interest is the apparent effect of thyroxine injections which resulted in 90% pregnant at 25 days for the hot-room ewes and 92% pregnant with live embryos for the control-yard ewes.

From this observation, Ryle suggests that hypothyroidism, which is known to be prevalent in hot climates (15, 54, 62, 97), has a detrimental effect on reproductive performance. Two possible

explanations based on the apparent increase in embryo weight by thyroxine injections are offered. First, in the absence of sufficient maternal thyroid hormone, the trophoblast may enlarge too slowly to give proper endometrial response to maintain the embryo, or, second, improper growth of the conceptus may cause direct embryonic death.

This was in contrast with the work with dairy cattle of Spielman et al. (84) and Williams (101) in which they both concluded that breeding efficiency was not reduced by thyroidectomy.

Alliston and Ulberg (5) by the use of ova-transfer technique have illustrated the inability of normal fertilized ova from temperature-stressed ewes to develop in the uterus of a non-stressed ewe. They suggest that the speed the embryo travels through the oviduct may be increased in the case of the stressed ewe and the embryonic mortality may be due to "immature" embryos reaching the uterus. This is in agreement with Dutt (28) that the critical period is the time when the fertilized ova is in the oviduct.

Shah (81) pointed out that high temperatures did interfere with pregnancy in rabbits, but concluded that the heat was adversely affecting the maternal tissue and not the embryo itself.

Research workers using rats and mice as the experimental animal have found that an elevated temperature interferes with the

estrous cycle (21) as well as pregnancy (42, 43, 57). Aldred et al. (1) observed that progesterone treatment prior to heat treatment reduced the embryo resorption rate. McLaren and Michie (66) obtained the lowest per cent successful fertilized-ovum transfers when the donor was 2-1/2 days and the recipients 3-1/2 days past coitus, which gives strength to the view of Alliston and Ulberg (5) that the fertilized ovum reaches the uterus too early.

Cameron (19), using a hot-water bath to raise the body temperature 6-7°F of 72-to-80 hour-pregnant rabbits, reports 17 out of 18 matings failed to produce litters.

With the development of artificial insemination and tested fertility of bulls, attention has been drawn to early embryo mortality in cattle. Hansel (51) reports that efficient cows have a fertilization rate of 96%, but a subsequent 10% embryo mortality loss. Tanabe and Casida (96) in a study of reproductive failures in cows of known low fertility found an embryonic mortality of 39.2% during the first 34 days of pregnancy. A later study with dairy heifers of low fertility by Tanabe and Almquist (95) showed a fertilization rate of 66.7%, but also found a subsequent high embryonic death of 54.1% within the first month of pregnancy. Olds (71) estimates embryo mortality accountable for up to 65% of all fertilizations with repeat breeder cows.

Bearden et al. (13) reported that nearly all of the loss in breeding efficiency when high-fertility semen was used in artificial insemination resulted from embryo mortality. He calculated this mortality to be 10.5%, as he found 96.6% of the cows carrying fertilized ovum at 3 days compared to 86.1% carrying normal embryos at 33 days. In the case of cows bred to bulls of low fertility, 76.9% had fertilized ovum at day 3, and only 57.7% had normal embryos at day 33. Thus when compared with cows bred to bulls of known high fertility, the fertilization rate was 19.7% lower, and the embryo mortality rate was 9.1% higher.

Hawk et al. (52) studied embryonic mortality in low fertility cows and determined the wastage between day 16 and day 34 post-breeding. Fifty-eight percent had normal embryos at day 16, while only 28% were normal at day 34, for an estimated embryonic death of 51.7%.

Working with beef heifers, Laing (61) reported a 25% embryonic death loss for the first 25 days of pregnancy. With dairy cows, Fosgate and Smith (44) found a 6.38% fetal mortality between pregnancy check at 34-50 days and parturition.

In an attempt to better understand the causes of embryo mortality, considerable work has been carried out with several farm animal species (7, 22, 24, 25, 39, 49, 53, 58, 74, 79, 83, 85, 86, 92, 102, 103),

and laboratory experimental species (43, 55, 99, 100) to determine what hormones are needed to prevent embryo wastage.

Day et al. (24), working with ovariectomized gilts, found that estradiol benzoate alone would not maintain pregnancy. Progesterone alone did maintain the embryo, but optimum conditions were obtained in the uterus by a combination of estradiol benzoate and progesterone. In a later study with intact gilts, Day et al. (25) reported that 100 mg progesterone caproate and 50 mcg of estradiol benzoate per 100 lbs. body weight on the 11th day after mating failed to show statistical improvement in embryo survival. In an attempt to reduce the normal incidence of embryo mortality in intact gilts, Haines et al. (49) found that progesterone injections failed to produce more live embryos. This is in agreement with the work of Sammelwitz (79) with rats and swine, who reported that higher dosages of progesterone appeared to be detrimental to embryo survival.

Considerable work has been done with progesterone therapy in dairy cattle with widely varying results. Johnson et al. (58) administered 100 mg progesterone on day 2, 3, 4, 6, and 9 and found that the treated group had a breeding efficiency 28% better than the controls. Dawson (23) reported increased conception in repeat breeder cows. Wiltbank et al. (102) working with cows of known lowered fertility found no significant differences in breeding efficiency

when 50 mg progesterone per day was injected. An extensive study of progesterone treatment in relation to time of insemination and its effect on breeding efficiency with dairy cows by Slack et al. (83) revealed that progesterone given near the time of insemination was detrimental to breeding efficiency. When the treatment was delayed to 10 or 14 days after insemination, the detrimental effect was not as obvious, but there was no indication of any beneficial effect. These results were in good agreement with Stott and Williams (92) where, though no statistical differences were found, 500 mg progesterone injected at the time of insemination appeared to decrease breeding efficiency while a similar injection at 10 days post-insemination seemed to have little effect.

Attempts have been made to determine the amount of progesterone needed to maintain pregnancy in dairy cattle. Raeside and Turner (74) removed the corpus luteum from pregnant heifers at 44 and 48 days and injected 25 mg progesterone. As these heifers aborted, they concluded that this was insufficient to maintain pregnancy. Staples and Hansel (86), using the unique technique of inhibition of the corpus luteum by oxytocin injection, suggested that approximately 100 mcg progesterone are required in the corpus luteum at day 15 to have an uterine situation favorable for embryo survival.

Research workers agree that the major source of progesterone in the early phases of pregnancy is the corpus luteum (39, 46, 47, 89). However, the fact that the adrenal glands secrete significant amounts of progesterone has been established (11, 12, 47, 89).

Research with intact and adrenalectomized rats indicates a definite adrenal ovarian relation under normal as well as heat-treated conditions (42, 43, 99, 100). Thompson et al. (97) found a highly significant increase in blood steroid levels when dairy heifers were exposed to high ambient temperatures.

Velardo (99) observed that ACTH injections reduced litter size and produced a larger number of stillbirths. ACTH, given on the day of mating and for 6 subsequent days, showed the greatest reduction in litter size. This agrees well with the observation of Dutt et al. (31) with heat-treated sheep where the detrimental effect was much less if the ewes were exposed to heat 8 days after breeding.

Velardo (99) also observed that adrenalectomized rats maintained on cortical implants had no reduced litter sizes when ACTH was administered and offered this as additional support that there are numerous interactions among hormones of the adrenal cortex and ovaries. In strong support of Velardo's work is the work of Fernandez (43) with adrenalectomized heat-treated rats. Fernandez's work illustrated that cortical-implanted adrenalectomized rats could

maintain normal embryo development even under heat-stress conditions where intact rats were observed to have embryo degeneration. Robson and Sharaf (76) found that ACTH injection interrupted pregnancy in both rats and mice, which gives additional support to this work.

### Progesterone Determination

Considerable progress has been made in the past 13 years in the development of chemical methods to determine progesterone concentration (18, 34, 35, 64, 82, 85, 90). The chemical techniques have provided methods to detect lower concentration of progesterone than the bio-assay methods (56, 85).

At present, the most common chemical techniques of progesterone determination employ hot alcohol extraction, column chromatography, counter-current distribution, paper chromatography, ultra-violet absorption at  $240\text{ m}\mu$  and the use of progesterone  $4\text{-C}^{14}$  (85, 90).

## EXPERIMENTAL PROCEDURE

### General

Forty-five heifers and cows, mostly of the Holstein breed, were bred and then assigned to one of four groups. Twenty-two were placed in a controlled temperature chamber where they were exposed to elevated temperatures (heat-treated) on day one (day animal was bred). Eleven of these were slaughtered on day 14, and the other eleven were left to be slaughtered on day 32. In the 32-day group, if animals returned to estrus before the slaughter date, they were rebred, and the heat treatment on day one was repeated. Twenty-three control heifers were bred, eleven of which were slaughtered on day 14, and the other twelve left for slaughter on day 32. Heifers in the 32-day control group that returned to estrus before the slaughter date were rebred until they went the 32 days without showing estrus.

The heifers were checked for estrus at least twice daily. Animals in estrus in the morning were bred in the late afternoon, while animals observed in estrus in late afternoon were bred the following morning. An animal was considered at the start of estrus when she would first stand to be mounted by another animal.

Freshly collected semen and some frozen semen was used to breed the heifers. Semen was checked for motility, and only semen from bulls of known high fertility was used.

Heifers that were in the heat-treated groups were put in a temperature-and-humidity-controlled room, and temperature and humidity were regulated so that the animal's body temperature would rise 2° to 5°F. After a twenty-four-hour exposure to elevated ambient temperatures, the animal was allowed to run loose in the open corral.

Animals that were in the 32-day groups were palpated every two to three days starting at about the 17th day. This allowed detection of silent estrus and assured that cows found without embryos at slaughter had either lost their embryo or had a long estrous cycle.

When the animals were slaughtered, the corpus luteum and adrenal glands were weighed to the nearest one-hundredth of a gram. In the case of cystic corpora lutea two weights were taken, the gross weight (tissue plus fluid) and a net weight (tissue minus fluid).

Two samples of about one gram each to be used for progesterone assay were taken from each corpus luteum. These fresh sample weights were recorded along with the total weight of the corpus luteum. Samples were then placed in individual vials which contained 95% ethanol and were immediately placed on Dry Ice. A small sample

of the luteal tissue was also placed in Bouin's solution to be used in future histological studies.

After weighing the adrenal glands, a sample for progesterone assay of about seven grams was taken and placed in a vial which contained 95% ethanol. This was then placed on Dry Ice to insure rapid refrigeration. A small sample of the adrenal gland was also fixed in Bouin's solution for later histological studies.

Embryos recovered were grossly observed for abnormalities. The four major groups (control 14 day, control 32 day, heat-treated 14 day, and heat-treated 32 day) were divided into the following subgroups: (1) normal embryos, (2) dead or degenerate embryos, and (3) no embryos. Corpus luteum weights, adrenal weights, and the progesterin concentrations in adrenals and corpora lutea were compared.

For simplicity the following abbreviations are used to designate subgroups:

- |                            |   |
|----------------------------|---|
| C-14-D <sub>ER</sub>       | Control slaughtered day 14 after bred -<br>embryo recovered.                    |
| C-14-D <sub>NER</sub>      | Control slaughtered day 14 after bred -<br>no embryo recovered.                 |
| H. T. -14-D <sub>NER</sub> | Heat treated day bred - slaughtered day 14<br>after bred - no embryo recovered. |

C-32-D<sub>ER</sub> Control slaughtered day 32 after bred -  
embryo recovered.

C-32-D<sub>NER</sub> Control slaughtered day 32 after bred -  
no embryo recovered.

C-32-D<sub>ED</sub> Control slaughtered day 32 after bred -  
embryo found dead.

H. T. -32-D<sub>ER</sub> Heat treated day bred - slaughtered day  
32 after bred - embryo recovered.

H. T. -32-D<sub>NER</sub> Heat treated day bred - slaughtered day  
32 after bred - no embryo recovered.

#### Progesterone Determination

The general procedure for the progesterone determination follows that outlined by Staples and Hansel (86), and, where major changes were made, special acknowledgment is given. The same procedure was used for both the corpora lutea and adrenals.

The pre-weighed samples of the corpus luteum were removed from the freezer and cut into fine pieces: these were placed in a tissue homogenizer. At this time, 0.01 microcuries of progesterone 4-C<sup>14</sup> was added to the sample. The 95% ethanol from the sample vial and two 5-ml 95% ethanol rinses were placed in the homogenizer. The tissue was then ground completely. The contents were poured

into a 200-ml R. B. boiling flask, and 100 ml 95% ethanol was used to rinse the residue in the homogenizer into the boiling flask.

The flask was placed on a condenser, and the ground tissue was refluxed for 2 hours at 77°C. After removal from the condenser, the flask was allowed to stand for ten minutes to let the tissue residue settle. About 100 ml of the hot ethanol extract was filtered through a Waterman No. 1 filter paper into a 250-ml R. B. boiling flask. A 50-ml portion of 95% ethanol was added to the tissue residue flask, and this was put back on the condenser and allowed to reflux an additional hour. The tissue-residue flask was then removed from the condenser, and the complete contents of the flask filtered into the flask that contained the first portion of the filtrate. Two 10-ml aliquots of 95% ethanol were used to rinse the residue flask.

The ethanol extraction, under reduced pressure, was placed on a 70°C hot-water bath and evaporated to dryness. The dried sample was placed on an alumina absorption column and chromatographed. Column chromatography was similar to the procedure used by Staples (85), with the exception that 100 ml burets with glass plugs were used and an additional 50 ml of chloroform was collected following the 25% chlorohexane collection.

Samples were dried again in the previously described manner and then partitioned by a normal pentane and 70% methanol system.

This was accomplished by using two 60-ml separatory funnels, the second of which had 30 ml of normal pentane in it. Thirty ml of n-pentane was used to rinse the dried sample from the flask to the number one separatory funnel. The system was then subjected to nine transfers of 10 ml 70% methanol, and the methanol was collected in a 200-ml R. B. flask and dried down.

The dried partitioned sample was rinsed from the R. B. flask by means of three 1-ml pipettings and placed in a 3-1/2 ml tube. The sample was dried and concentrated in the bottom of the tube by rinsing down the sides of the tube with aliquots of 0.2 ml absolute methanol.

Descending paper chromatography was then used to further purify the progesterone samples. Absolute methanol was used to spot the samples on 2.5 x 56.8 cm strips of Waterman No. 1 filter paper that is suitable for chromatography. With each set of samples a blank spotted with absolute methanol only and a standard sample of 20 mcg of progesterone were also run. A standard 12 x 24 inch chromatography jar with rack and tray was used in an air-conditioned room where the temperature was maintained at 70°F. The chromatography jar was set up for a two-phase (80% methanol and hexane) system as described by Bush (18). After allowing the samples to equilibrate for at least 12 hours, they were run by the addition of hexane (equilibrated with 80% methanol) to the solvent tray.

The chromatographed strips were removed from the jar and air dried. This was followed by photography of the paper chromatograms by ultra-violet light (Mineralight, Ultra-Violet Products, Inc., San Gabriel, California) exposure on Ansco Scona Reflex Type C semi-matte light-weight photographic paper.

The progestin spots on the paper strips were located by use of the photographs. Elution was completed by hanging the paper containing the progesterone on a 23-gauge hypodermic needle that was attached to a 10-ml syringe. Ten ml of absolute methanol was placed in the syringe and allowed to drip through the paper. The elutant was caught in a 15-ml centrifuge tube and was dried down under reduced pressure. Care was taken to concentrate the sample in the bottom of the tube.

A model D. U. Beckman spectrophotometer was used to measure absorption at 220, 230, 240, 250, and 260  $m\mu$ . Samples that did not show the characteristic progesterone absorption curve, with maximum absorption at 240  $m\mu$ , were rechromatographed and then re-read.

Each sample was read against a reference blank which was eluted from the absolute methanol chromatogram. The sample readings were then corrected by the use of the Allen's Correction Factor (2).

An example of an Allen's corrected reading sample:

Wave length (m $\mu$ )	220	240	260
Optical density of unknown in 2 ml absolute methanol	.205	.535	.180

$$\begin{aligned}
 \text{A.C.O.D. } 240 &= \text{O.D. } 240 - \frac{\text{O.D. } 220 + \text{O.D. } 260}{2} \\
 &= .535 - \frac{.205 + .180}{2} \\
 &= .535 - .193 \\
 &= .342
 \end{aligned}$$

$$\text{Corrected for dilution} = 2(.342) = .684$$

$$\text{A.C.O.D. } 240 = \text{Allen's corrected optical density at } 240 \text{ m}\mu$$

$$\text{O.D.} = \text{Optical density}$$

Samples were dried and then transferred to planchets, and the progesterone 4-C<sup>14</sup> was counted on a Model 186 gas-flow Nuclear-Chicago Counter. The percent recovery was determined and used to correct the progesterone values for loss in the extraction and purification systems.

#### Solvents Used

Because of the sensitivity of the progesterone determinations to almost any type of contaminant, all solvents were redistilled before use.

95% Ethanol	Redistilled
Absolute Methanol	Mallinckrodt, anhydrous acetone free, analytical reagent, redistilled
Normal Pentane	Phillips 66, pure grade, redistilled
Chloroform	Baker, technical grade, redistilled
Hexane	Phillips 66, high purity, redistilled

### Statistical Analysis

The Student's t test (88) was used to evaluate differences in the corpora lutea weights, adrenal weights, and luteal progesterone concentration. Statistical analysis was made between heat-treated subgroups and control subgroups (heat-treated pregnant versus control pregnant, and heat-treated nonpregnant versus control nonpregnant). Additional comparisons were made within each of the primary groups (pregnant versus nonpregnant, dead or degenerate embryos versus nonpregnant, and dead or degenerate embryos versus pregnant).

The calculations for the metabolite 20-B-ol included only those cows with detectable amounts present.

## RESULTS

### Corpus Luteum and Adrenal Weights

No significant differences in corpora lutea weights or adrenal gland weights (Tables 1, 2, 3, and 9) were found in comparison between the 14-day control pregnant versus control nonpregnant and heat-treated nonpregnant versus control nonpregnant.

There was a cystic corpus luteum found in the study. This was in the heat-treated 14-day nonpregnant group. Total gross weight was 49.85 gms and the net weight (tissue minus fluid weight) was 12.30 gms (Table 3).

Comparisons of corpora lutea weights between the subgroups in the 32-day control group also failed to produce significant differences, even though two of these animals had dead embryos when slaughtered (Tables 4, 5, and 9).

There appeared to be a trend toward heavier corpora lutea in the 32-day heat-treated nonpregnant animals, but the difference was not significant when tested at the ( $P < 0.05$ ) level (Tables 6, 7, and 9).

Subgroup comparison between control 32-day animals and heat-treated 32-day animals found no differences in weights of either corpora lutea or adrenal glands.

#### Luteal Tissue Progestins Concentrations

Statistical analysis of the 14<sup>1</sup>-day groups for progesterone,  $\Delta^4$ -pregnene-20-B-ol-3-one (20-B-ol) and total progestins concentration of the luteal tissue on a microgram and microgram per gram of luteal tissue did not show significant differences ( Tables 1, 2, 3 , and 9). In the 14-day control group 2 out of 11 (18.2%) did not have detectable amounts of 20-B-ol, while in the 14-day heat-treated group 3 out of 11 (27.3%) failed to produce detectable amounts of the metabolite.

Statistical analysis of the subgroups within the 32-day controls (Tables 4, 5, and 9) found a higher total concentration of progestins ( $P < 0.05$ ) in the subgroup where no embryos were recovered as compared to the subgroup where normal embryos were recovered. When progestins on a mcg/gm of luteal tissue were compared between these two subgroups, a highly significant ( $P < 0.01$ ) higher concentration of progestins was found in the nonpregnant subgroup. Comparisons of progestin concentration in the subgroup with dead embryos with the normal-embryo and nonpregnant subgroups revealed no differences.

Comparisons between the subgroups in the 32-day heat-treated group (Tables 6, 7, and 9) found no difference in the progesterone concentration. However, 20-B-ol was not detected in any of the four animals that were not pregnant when slaughtered. Of the seven pregnant animals only one failed to have detectable amounts of 20-B-ol.

When the 32-day control subgroups were compared to the similar subgroups in the 32-day heat-treated group (Table 9), statistical differences ( $P < 0.01$ ) were found between total luteal progestin concentrations for the nonpregnant control subgroup and the nonpregnant heat-treated subgroup. The differences are accounted for by the lack of detectable amounts of 20-B-ol in the heat-treated nonpregnant subgroup.

#### Adrenal Progesterone

At least one adrenal gland was analyzed for progesterone in each of the subgroups. Because of the small numbers involved, statistical analyzation was not attempted, but the values are reported (Table 8).

Some progesterone was detected in each of the adrenal glands analyzed, but no 20-B-ol was detected. Progesterone concentrations in the heat-treated subgroups ranged from 36.9 mcg to 71.6 mcg, while in the control subgroups progesterone concentration ranged

from 6.7 mcg to 34.9 mcg. Concentration in mcg/gm of adrenal tissue ranged from 1.8 to 5.0 for heat-treated subgroups and from 0.4 to 1.9 for control subgroups.

## DISCUSSION

Though no statistical differences were detected in corpora lutea weights, adrenal weights, or in the progesterin concentration in the luteal tissue of the control 14-day and heat-treated 14-day animals, in the present study the adrenal gland progesterone concentration did show differences. Heat-treated animals in every subgroup had on the average about twice the progesterone concentration as the corresponding control subgroup.

Velardo (99) found that ACTH injections administered to intact rats reduced litter size, while similar injections to adrenalectomized cortical-implanted rats did not. This is evidence that the adrenal gland does affect embryo survival. Additional support to this is the work of Fernandez-Cano (42, 43), using heat-stressed rats, where a higher embryo survival rate was found in adrenalectomized rats, than in intact rats. From this he concluded that increased adrenal activity during stress conditions seems to have a definite effect on embryo survival.

The limited adrenal data in this study suggests that progesterone concentration is increased by heat treatment. Several theories can be

logically built from this observation that would give possible explanation of breeding difficulties in hot climatic areas.

One theory is that progesterone in small quantities at the time of estrus seems to speed up the time of ovulation, and shortens the duration of estrus [Hansel et al. (50)]. This explanation gives two possible reasons for lowered fertility during times of high ambient temperatures: (a) shortened duration of estrus results in failure of observation of estrus, (b) the time of ovulation is hastened, resulting in immature fertilized ovum reaching the uterus too early [Alliston et al. (3)], resulting in embryonic death.

A study by Williams (101) of ovulation time with cows in central Arizona during the first two weeks in September found no evidence of abnormally early ovulation. However, he did observe that the cows were very inactive during the heat of the day. If we assume that the increased level of progesterone observed in the adrenal gland in this study is released to the blood (97), we could conjecture a shortened duration of estrus as observed by Hansel et al. (50). Correlating the inactivity of cows during the heat of the day with the possibility of a shortened duration of estrus, the failure of detection of estrus seems plausible.

Statistical differences were found in the corpora lutea progesterone concentration between the 32-day nonpregnant control

subgroup and the 32-day pregnant control subgroup. Apparently the 32-day nonpregnant control cows had persistent corpora lutea. This could be a possible explanation for the observed higher progesterone content, or possibly, as only two animals were in this subgroup, the observed difference was due to chance. There were progestin concentrations of individual animals in other groups that were higher than either of these two animals.

The failure of the four cows in the 32-day heat-treated, nonpregnant group to have detectable amounts of 20-B-ol is difficult to explain, as 8 out of 11 (72.7%) of the 14-day heat-treated nonpregnant animals did have detectable amounts of the metabolite. However, since only four animals were in the 32-day subgroup, chance could be a possible explanation. Another explanation could be that the heat treatment caused a slow build-up of adrenal cortico-hormones that could block the action of progesterone and therefore change the metabolism in the luteal cell. Velardo (100) found that adrenal cortico-hormones and ACTH do block the action of progesterone in the rat.

## SUMMARY

Corpus luteum weights, adrenal weights, luteal progesterin concentration, and adrenal progesterone concentrations were compared in forty-five heifers and cows that were divided into four main groups: heat-treated on the day bred and slaughtered on day 14 or day 32, and controls slaughtered on day 14 or day 32. Each of the four groups was divided into subgroups as determined by the presence of normal embryo, dead or degenerate embryo, and no embryo.

Corpora lutea and adrenal weight for 14-day and 32-day control and heat-treated cows revealed no statistical differences.

Progesterone, 20-B-ol, and total progestins concentration were the same for control and heat-treated 14-day animals.

The 32-day nonpregnant controls had a statistically higher total progesterone concentration than the pregnant controls. The two control animals that had dead embryos had higher total progestin levels than the pregnant controls, but these were not statistically significant.

Comparison of the 32-day control and heat-treated nonpregnant subgroups revealed a statistically higher progesterone concentration

in the control, basically due to the lack of detectable 20-B-ol in the heat-treated subgroup.

Limited adrenal progesterone data showed that 14-day and 32-day heat-treated animals ranged from 36.9 to 71.6 mcg of progesterone, while control animals in these groups ranged from 6.7 to 34.9 mcg.

Evidence suggests an increased progesterone production by the adrenals under stress conditions.

APPENDIX

TABLE 1. -- Corpus luteum weights, adrenal weights, and luteal tissue progesterin concentrations for nonpregnant control cows slaughtered 14 days post insemination.

Sub Group	Cow Number	Corpus Luteum Weight in gms	Cow Weight in Pounds	Adrenal Weight in gms	Luteal Tissue Progesterone		Luteal Tissue 20-B-ol		Luteal Tissue Progestins	
					mcg	mcg/gm	mcg	mcg/gm	mcg	mcg/gm
NER	H-49	7.85	998	19.29	257.5	32.8	150.7	19.2	407.5	51.9
NER	H-72	5.91	630	15.12	147.2	24.9	79.8	13.5	227.0	38.4
NER	H-85	4.41	610	14.14	168.0	38.1	68.8	15.6	236.8	53.7
NER	H-41	7.69	1186	23.85	196.9	25.6	N. D.	N. D.	196.9	25.6
NER	H-74	4.34	678		268.2	61.8	78.9	18.2	347.1	80.0
NER	H-89	7.45		11.88	356.8	47.9	92.4	12.4	449.2	60.3
NER	H-45	3.74	880	18.35	201.2	53.8	22.8	6.1	224.0	60.0
NER	H-55	5.87	1010	18.19	259.5	44.2	N. D.	N. D.	259.5	44.2
NER	H-93	4.44	500	11.99	132.8	29.9	70.2	15.8	203.0	45.7
n		9	8	8	9	9	7	7	9	9
$\bar{X}$		5.74	812	16.60	220.9	39.9	80.5	14.4	283.4	51.1
$s^2$		2.59		16.74	5061.43	167.3	1440.83	19.1	8793.33	236.85
$\frac{s}{\bar{X}}$		.54		1.44	23.71	4.31	14.35	1.65	31.26	5.13

N. D. - Not detectable.

APPENDIX

TABLE 2. --Corpus luteum weights, adrenal weights, and luteal tissue progesterin concentrations for pregnant control cows slaughtered 14 days post insemination.

Sub Group	Cow Number	Corpus Luteum	Cow Weight	Adrenal Weight	Luteal Tissue Progesterone		Luteal Tissue 20-B-ol		Luteal Tissue Progestins	
		Weight in gms	in Pounds	in gms	mcg	mcg/gm	mcg	mcg/gm	mcg	mcg/gm
ER	H-56	5.55	770	16.64	89.9	16.2	67.2	12.1	157.1	28.3
ER	H-97	5.14	634	32.20	323.8	63.0	47.3	9.2	371.1	72.2
n		2	2	2	2	2	2	2	2	2
$\bar{X}$		5.35	702	24.42	206.9	39.6	57.3	10.7	264.1	50.3
$s^2$		.08		121.06	27,354.6	1095.12	198.0	4.21	22,898.0	963.60
$\frac{s^2}{X}$		.2		7.78	116.95	23.40	9.95	1.45	107.0	21.95

APPENDIX

TABLE 3. -- Corpus luteum weights, adrenal weights, and luteal tissue progesterin concentrations for nonpregnant cows heat-treated day inseminated, slaughtered 14 days post insemination.

Sub Group	Cow Number	Corpus Luteum Weight in gms	Cow Weight in Pounds	Adrenal Weight in gms	Luteal Tissue Progesterone		Luteal Tissue 20-B-ol		Luteal Tissue Progesterins	
					mcg	mcg/gm	mcg	mcg/gm	mcg	mcg/gm
NER	H-61	8.88	740	13.91	191.8	21.6	68.4	7.7	260.2	29.3
NER	H-66	5.63	760		228.6	40.6	33.8	6.0	262.4	46.6
NER	H-37	5.65	1236	28.18	167.2	29.6	101.1	17.9	268.3	47.5
NER	H-33	5.33 49.85*	1496	22.44	202.0	37.9	117.8	22.1	319.8	60.0
NER	H-48	12.30	1340	27.87	165.8	16.0	N. D.	N. D.	165.8	16.0
NER	H-51	5.74	1160	19.20	239.4	41.7	N. D.	N. D.	239.4	41.7
NER	H-53	5.73	1260	19.04	120.3	20.9	51.5	9.0	171.8	29.9
NER	H-34	5.84	1240	27.87	165.8	28.4	N. D.	N. D.	165.8	28.4
NER	H-38	4.24	1300	18.45	287.0	67.7	54.7	12.9	341.4	80.6
NER	H-39	4.17	1180	14.15	137.2	32.9	73.4	17.6	210.6	50.5
NER	H-50	6.13	1664	11.08	215.8	35.2	98.7	16.1	314.5	51.3
n		11	11	10	11	11	8	8	11	11
$\bar{X}$		6.33	1216	20.20	192.8	33.9	74.9	13.7	247.3	43.8
$s^2$		5.41		39.01	2340.25	195.63	826.08	32.45	3985.64	315.72
$\frac{s}{\bar{X}}$		.7		1.98	14.59	4.22	10.16	2.01	19.04	5.36

\*Cystic C. L., luteal tissue 12.30 gms.

N. D. - Not detectable.

APPENDIX

TABLE 4. --Corpus luteum weights, adrenal weights and luteal progesterin concentrations for pregnant control cows slaughtered 32 days post insemination.

Sub Group	Cow Number	Corpus Luteum	Cow Weight	Adrenal Weight	Luteal Tissue Progesterone		Luteal Tissue 20-B-ol		Luteal Tissue Progestins	
		Weight in gms	in Pounds	in gms	mcg	mcg/gm	mcg	mcg/gm	mcg	mcg/gm
ER	H-78	7.03	874	13.74	429.5	61.1	113.8	16.2	543.3	77.3
ER	H-80	7.11	1016		404.6	56.9	144.3	20.3	548.9	77.2
ER	H-90	5.12	820	13.75	330.2	64.5	197.6	38.6	527.8	103.1
ER	H-94	8.23	670	20.49	260.1	31.6	128.4	15.6	388.5	47.2
ER	H-95	5.33	760	16.50	196.1	36.8	N.D.	N.D.	196.1	36.8
ER	H-96	5.09	590	18.64	226.5	44.5	73.9	21.1	300.4	59.0
ER	H-91	3.27	614		151.4	46.3	49.4	15.1	200.8	61.4
ER	H-77	7.36	796	14.62	225.9	30.7	78.8	10.7	304.7	41.4
n		8	8	6	8	8	7	7	8	8
$\bar{X}$		6.07	768	16.29	278.0	46.6	112.3	19.7	376.3	62.9
$s^2$		2.65		7.79	10,018.13	173.58	2519.13	81.79	22,134.36	490.76
$s_{\bar{X}}$		.57		1.14	35.39	4.66	18.97	3.42	52.60	7.83

N. D. - Not detectable.

APPENDIX

TABLE 5.--Corpus luteum weights, adrenal weights, and luteal progesterin concentrations for non-pregnant and degenerate embryos in control cows that were slaughtered 32 days post insemination.

Sub Group	Cow Number	Corpus Luteum Weight in gms	Cow Weight in Pounds	Adrenal Weight in gms	Luteal Tissue Progesterone		Luteal Tissue 20-B-ol		Luteal Tissue Progestins	
					mcg	mcg/gm	mcg	mcg/gm	mcg	mcg/gm
NER	H-69	6.19	888	25.74	590.5	95.4	74.4	12.5	664.9	107.4
NER	H-79	5.15	530	19.66	300.8	58.4	231.8	45.0	532.6	103.4
n		2	2	2	2	2	2	2	2	2
$\bar{X}$		5.67	709	22.70	445.7	76.9	153.1	28.8	598.8	105.4
$s^2$		.55		18.48	41,963.04	684.50	12387.38	528.12	8751.64	8.0
$s_{\bar{X}}$		.52		3.04	144.85	18.5	78.7	16.25	66.15	2.0
Embryo Death	H-92	4.57		15.72	334.1	73.1	232.6	50.9	566.7	124.0
Embryo Death	H-99	6.51	640	18.39	276.0	42.4	107.4	16.5	383.4	58.9

TABLE 5--Continued

Sub Group	Cow Number	Corpus	Cow	Adrenal	Luteal Tissue		Luteal Tissue		Luteal Tissue	
		Luteum Weight in gms	Weight in Pounds	Weight in gms	Progesterone mcg	mcg/gm	20-B-ol mcg	mcg/gm	Progestins mcg	mcg/gm
n		2	1	2	2	2	2	2	2	2
$\bar{X}$		5.54	640	17.06	305.1	57.8	170.0	33.7	475.1	91.5
$s^2$		1.89		3.56	1687.80	471.24	7837.52	591.68	16,799.45	2119.0
$s-\bar{X}$		.97		1.34	29.05	15.35	62.60	17.20	91.65	32.55

APPENDIX

TABLE 6. -- Corpus luteum weights, adrenal weights, and luteal progesterin concentrations for pregnant cows heat treated day inseminated, slaughtered 32 days after insemination

Sub Group	Cow Number	Corpus Luteum	Cow Weight	Adrenal Weight	Luteal Tissue Progesterone		Luteal Tissue 20-B-ol		Luteal Tissue Progesterins	
		Weight in gms	in Pounds	in gms	mcg	mcg/gm	mcg	mcg/gm	mcg	mcg/gm
ER	H-88	5.18	740	14.61	132.6	25.6	83.4	16.0	216.0	41.7
ER	H-64	6.60	792	15.39	307.0	46.5	92.4	14.0	399.4	60.5
ER	H-82	4.85	592	11.56	177.5	36.6	45.1	9.3	222.6	45.9
ER	H-32	6.98	1150	19.33	138.9	19.9	N.D.	N.D.	138.9	19.9
ER	H-59	5.75		14.31	257.6	44.8	77.6	13.5	335.2	58.3
ER	H-60	5.51	644	13.24	194.5	35.3	80.9	14.7	275.4	50.0
ER	H-58	6.79	620	12.03	367.3	54.1	307.6	45.3	674.9	99.4
n		7	6	7	7	7	6	6	7	7
$\bar{X}$		5.95	756	14.4	225.1	37.5	114.5	18.8	323.2	53.7
$s^2$		.70		6.73	7862.20	144.48	9210.03	173.66	31,251.14	586.14
$\frac{s}{\bar{X}}$		.32		.98	33.51	4.54	39.18	5.38	66.82	9.15

## APPENDIX

TABLE 7.--Corpus luteum weights, adrenal weights, and luteal progesterin concentrations for non-pregnant cows heat treated day inseminated, slaughtered 32 days post insemination.

Sub Group	Cow Number	Corpus Luteum	Cow Weight	Adrenal Weight	Luteal Tissue Progesterone		Luteal Tissue 20-B-ol		Luteal Tissue Progestins	
		Weight in gms	in Pounds	in gms	mcg	mcg/gm	mcg	mcg/gm	mcg	mcg/gm
NER	H-47	7.53	1030	21.50	144.6	19.2	N. D.	N. D.	144.6	19.2
NER	H-40	6.89	1044	24.33	176.4	25.6	N. D.	N. D.	176.4	25.6
NER	H-43	5.21	1646	29.49	293.8	56.4	N. D.	N. D.	293.8	56.4
NER	H-31	6.71		36.12	315.7	47.1	N. D.	N. D.	315.7	47.1
n		4	3	4	4	4	0	0	4	4
$\bar{X}$		6.59	1240	27.86	232.6	37.1			232.6	37.1
$s^2$		.96		41.26	7184.50	308.38			7184.50	308.38
$\frac{s}{\bar{X}}$		.49		3.21	42.38	8.78			42.38	8.78

N. D. - Not detectable.

## APPENDIX

TABLE 8. --Adrenal progesterone concentration.

Group	Cow	mcg	mcg/gm
H. S. -14-D <sub>NER</sub>	H-34	50.2	1.8
	H-66	40.9	2.6
	$\bar{X}$	45.6	2.2
C-14-D <sub>NER</sub>	H-72	21.2	1.4
	H-85	22.6	1.6
	$\bar{X}$	21.9	1.5
C-14-D <sub>ER</sub>	H-56	6.7	.4
H. S. -32-D <sub>NER</sub>	H-47	40.6	1.9
H. S. -32-D <sub>ER</sub>	H-59	71.6	5.0
	H-64	36.9	2.4
	$\bar{X}$	54.3	3.7
C-32-D <sub>ED</sub>	H-99	34.9	1.9
	H-92	26.7	1.7
	$\bar{X}$	30.8	1.8
C-32-D <sub>ER</sub>	H-90	23.4	1.7

APPENDIX

TABLE 9. -- Subgroup averages for corpus luteum weights, adrenal weights, and luteal progesterin concentrations.

Sub Group	Corpus Luteum Weight in gms	Cow Weight in Pounds	Adrenal Weight in gms	Luteal Tissue Progesterone		Luteal Tissue 20-B-ol		Luteal Tissue Progestins	
				mcg	mcg/gm	mcg	mcg/gm	mcg	mcg/gm
C-14-D <sub>NER</sub>	5.74	812	16.60	220.9	39.9	80.5	14.4	283.4	51.1
C-14-D <sub>ER</sub>	5.35	702	24.42	206.9	39.6	57.3	10.7	264.1	50.3
H.S.-14-D <sub>NER</sub>	6.33	1216	20.20	192.8	33.9	74.9	13.7	247.3	43.8
C-32-D <sub>ER</sub>	6.07	768	16.29	278.0	46.6	112.3	19.7	376.3 <sup>a*</sup>	62.9 <sup>a**</sup>
C-32-D <sub>NER</sub>	5.67	709	22.70	445.7	76.9	153.1	28.8	598.8 <sup>a, b</sup>	105.4 <sup>a, b</sup>
C-32-D <sub>ED</sub>	5.54	640	17.06	305.1	57.8	170.0	33.7	475.1	91.5
H.S.-32-D <sub>NER</sub>	6.59	1240	27.86	232.6	37.1	N.D.	N.D.	232.6 <sup>b**</sup>	37.1 <sup>b**</sup>
H.S.-32-D <sub>ER</sub>	5.95	756	14.40	225.1	37.5	114.5	18.8	323.2	53.7

<sup>a, b</sup> Identical letters indicate a significant difference.

\*P < 0.05.

\*\*P < 0.01.

N. D. - Not detectable.

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