

RELATIONSHIP OF SEMEN PRODUCTION IN DAIRY BULLS  
TO SELECTED AND CONTROLLED AMBIENT CONDITIONS

by

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TABLE OF CONTENTS

	Page
INTRODUCTION _____	1
REVIEW OF LITERATURE _____	2
METHODS AND PROCEDURES _____	7
Aspects Common to Experiments I-II _____	7
Experiment I _____	9
Experiment II _____	10
RESULTS _____	13
Experiment I _____	13
Experiment II _____	16
DISCUSSION _____	22
Experiment I _____	22
Experiment II _____	25
SUMMARY AND CONCLUSION _____	26
ACKNOWLEDGMENTS _____	28
REFERENCES _____	29

## INTRODUCTION

The artificial breeding of dairy cattle in the United States has increased from 23.2% of the national dairy herd in 1955 to 43.7% in 1965 when a total of 7,264,835 dairy cows were artificially bred (5, 6). This increase can be attributed to improved methods of sire evaluation, more effective semen processing procedures, efficient transportation methods and greater use of superior progeny tested sires. Present sire evaluation methods are at least twice as effective as previous methods (29).

With the apparent demand for quality semen, a more complete knowledge of the factors that influence the quality and quantity of semen is needed. This encompasses further investigation of spermatogenesis in the bull with reference to the ambient environmental factors which influence increased semen production.

Extensive investigations have been made of spermatogenesis in small laboratory animals but bull studies have been limited with few exceptions to factors such as physical condition of the bull, frequency of collection and methods of processing. A more complete understanding of spermatogenesis in the bull could result in improved management practices, increased use of seasonal schedules for collection and relocation of bull studs to a climate more suitable for maximum yields of semen (2, 24).

This study was undertaken as a pilot trial for the eventual histological investigation of spermatogenesis in the bovine. The object was to determine the possibilities of altering previously

observed seasonal trends (24) and subsequently, were this possible, to determine if, at a predicted time, a bull could be rendered in a predictable spermatogenic state as indicated by semen quality. If this could be done, testicular biopsies taken in various known spermatogenic states could advance the study of spermatogenesis.

#### REVIEW OF LITERATURE

Because of the lack of data on the effects of ambient climatic conditions on semen quality, it is necessary to compare non-return rates to seasonal climatic conditions. Erb et al. in Indiana, Fryer et al. in Kansas, Johnston and Branton in Louisiana, Stott in Arizona, Weeth and Herman in Missouri, found significantly lower conception rates from late July through September (19, 24, 32, 58, 63). The results were attributed to high environmental temperatures. Mercier and Salisbury in eastern Canada (39), and New York (40, 41) and Sweetman in Alaska (60) also found the lowest conception rates in the late summer, but attributed the cause to the greater length of day. Anderson in Kenya (2) and Erb and Waldo in Washington (20) found the lowest non-return rates in the coldest season. Swanson and Herman in Missouri (59) did not find monthly variations for volume, concentration or total abnormal sperm. Phillips et al. in Maryland (48) found seasonal differences in semen with respect to percent motility, concentration, total sperm produced and total abnormal sperm, but failed to find differences in volume. Johnston and Branton in Louisiana (31) noted increases in morphologically abnormal sperm and decreases in motility with increasing ambient temperatures. Patrick et al. in Louisiana (47) maintained bulls

in a chamber at a temperature of 80F and 73% relative humidity from June until August. The quality of the semen produced by these bulls during July and August was better than that of bulls exposed to the hotter uncontrollable temperatures. Naelupaa et al. (45) produced cyclic climatic conditions with daily changes ranging from 104F with 54% relative humidity to 82F with 79% relative humidity. Dairy bulls were exposed to these conditions for one week and then returned to the cooler natural environment. Semen was collected twice each week. Marked reductions in percent motility, concentration and morphologically abnormal sperm were not found until 1 to 2 weeks after the treatment. Casady et al. (15) in 1953 maintained bulls in two chambers with temperatures from 70 to 90F for varying periods ranging from 2.5 to 6 weeks. Light was limited to 12 hours daily. Volume of semen was not affected by increasing temperatures in one chamber but volume increased as temperatures decreased in the other. It was also found that percent motility, concentration and total sperm count decreased in all semen samples either during or after exposure to high ambient temperatures. Dutt and Simpson in Kentucky (18) studied the effects of controlled temperature and humidity on semen quality in rams. The experimental animals were kept in a chamber at temperatures of 45 to 48F with 70 to 80% relative humidity; and the control group had normal environmental conditions. Natural light was provided for both groups. Semen from the experimental group was of significantly higher motility, greater concentration and had less abnormal sperm than did semen from the control group.

Among the environmental factors that affect semen production

are light, solar radiation, humidity, ambient temperature, air movement, altitude and nutrition (36). When these factors are above or below the capacity of the bull to acclimate, climatic stress may result (36). Respiratory rate and rectal temperatures are reliable measures of climatic stress and have been used by a number of workers (22, 23, 25, 30, 32, 34, 37, 38, 53, 61). Casady et al. (16) and McDowell (38) found an increase in respiratory rate preceded any increase in rectal temperature. In 1945 Anderson in Kenya stated that body temperature was affected by humidity and ambient temperature (2). Rectal temperature (8) and respiratory rate (32) were found to be positively correlated with humidity. Humidity has little affect on climatic stress at temperatures below 75F, but at higher temperatures an increasing correlation between respiratory rate and rectal temperature becomes evident as humidity increases (22, 34, 38, 51, 52, 61). Two separate groups of investigators found that under field conditions, humidity had little affect on body temperature (49, 50, 55). In European cattle it was found that thermo--regulatory mechanisms became active at 60F as indicated by increased respiratory rates (12). Casady et al. (15) observed an abrupt increase in respiratory rates at 70F. Findlay (22) found increased respiratory rates most pronounced when ambient temperatures were above 80F. At 80F increased respiratory rates were no longer an effective thermo-regulatory mechanism and rectal temperatures rose rapidly (12, 13, 15, 22, 34). Casady et al. (16) observed no increase in rectal temperatures or respiratory rates in older bulls until chamber temperatures exceeded 70F. They concluded the effect

was due to thermo-regulation being more efficient in the adult. Many workers found that the degree of climatic stress produced by heat, to which an animal could acclimate was an indirect function of the stage of maturity (16, 22, 52). Measurements other than increased respiratory rates and rectal temperatures can appear when cattle are subjected to increasing ambient temperatures. Some of these are: panting (9, 22), tachypnea (15), restlessness (15, 22), protrusion of the tongue (22), profuse salivation (22), decrease in body weight (12, 33), decrease in feed intake (10, 12, 13, 15, 33, 36), and relaxed posture (36).

Mercier and Salisbury (41) found a statistically significant correlation between length of daylight hours and fertility with bulls 6 to 10 years of age but not with bulls less than 6 years of age. Brody (11) in 1945 observed that domestication in some species involves release from the photoperiodic influence. Roussel et al. (53) in 1963 observed bulls exposed to artificial light (incandescent) produced increased percent motility and fewer morphologically abnormal sperm, but no significant difference in concentration or volume. Roussel et al. (54) in 1964 noted the absence of a complete explanation as to how light influences the reproductive performances of bulls.

In summary then, increased humidity, temperature and age have been shown to influence the physiological thermo-regulatory mechanisms of the bull. Also, length of day had measurable affect on semen quantity and quality.

The spermatogenic studies in the bull which have been reported

were patterned after other species more adaptable to laboratory conditions. The results of such studies indicate that semen production is directly related to the functional state of the seminiferous epithelium. Estimates for the time required for spermatogenesis have been made by two methods. One involved halting the spermatogenic process by destruction of the seminiferous epithelium; the other was radioactive labeling of young spermatocytes. The investigators with the most consistent results in estimating the time required for spermatogenesis have utilized  $^{32}\text{P}$  to label germ cells. Radioactive phosphorus becomes incorporated in deoxyribonucleic acid (DNA) of the young spermatocytes at the preleptotene stage, after which no further phosphorus exchange occurs. After  $^{32}\text{P}$  treatment, semen was collected twice each week until labeled sperm appeared. The frequency of collection did not affect the time for the appearance of labeled sperm. The time in days from injection of  $^{32}\text{P}$  until appearance of labeled sperm was considered to be the time required for spermatogenesis and sperm transport. Radioactive sperm were found in the lumen of the seminiferous tubules 40 days after  $^{32}\text{P}$  injection in the bull. The time from injection until labeled sperm were ejaculated ranged from 48 to 55 days (17, 27, 35, 46). Moore (42, 44) surgically produced cytorchidism in the guinea pig and rabbit and observed degeneration of the seminiferous epithelium. He hypothesized that the higher temperature of the abdominal cavity was unfavorable to spermatogenesis. Young (62) investigated the effects of heat on semen production by treatment of intact guinea pig testes with hot water



(46C for 30 minutes and 47C for 15 minutes). He concluded that such treatment caused immediate degeneration of the seminiferous epithelium. Using a similar treatment, Moore (43) found different degrees of injury in the same testis, varying from severe degeneration of seminiferous epithelium to normal tubules. MacLeod and Hotchkiss (37) in 1941 raised the rectal temperature of eight men to  $40.5 \pm 0.5C$  by heating the entire body. Total sperm counts were made and the lowest counts occurred 25 to 55 days after treatment. A low sperm concentration was sustained for periods of 14 to 50 days; then sperm counts returned to normal. The authors concluded that mature sperm in the epididymis were resistant to heat and therefore injury to the germinal epithelium was not manifested immediately.

The wide biological and environmental variation between studies, species and subjects made it difficult to draw conclusions regarding spermatogenesis in the bull with any degree of accuracy. The estimated duration of time from sperm formation to ejaculation ranges between 48 and 55 days in the bull (17, 27, 35, 46).

#### METHODS AND PROCEDURES

##### Aspects Common to Experiments I and II

The facilities used for these studies included a climatic chamber and an adjacent bull barn at Kansas Artificial Breeding Service Unit (KABSU), Manhattan, Kansas.

The chamber measured 11.3 x 15.5 x 7.2 feet, and had individual stalls. Twelve hours of light was provided daily by four 100 watt bulbs. Regulated air flow provided uniform distribution of temperature and relative humidity throughout the chamber. Drinking water

was maintained at 85F. The adjacent barn contained individual tie stalls and ventilators. There, climatic conditions dictated the ambient temperatures, relative humidity, hours of light and temperature of the drinking water.

Two sets of Holstein twin bulls were paired so that two siblings did not compose a group. The groups were designated experimental if kept in the climatic chamber and control if kept in the bull barn. The feed and feeding procedures were the same for both sets of twins. The bulls were fed a 14% protein concentrate made to the specifications of KABSU (6) twice daily. A daily supplement of 5000 I.U. of vitamin A and 37,000 I.U. of vitamin D were added. Roughage consisted of alfalfa and prairie hay fed alternately and water was provided ad libitum. Body weight was recorded weekly. Bulls were preconditioned to the climatic chamber for four months prior to the experiment. During this time, siblings were alternated weekly between the barn and the chamber.

In order to keep the chamber and bulls in a reasonably sanitary state, it was necessary to wash the chamber floor and the flanks and underline of the bulls daily. The bulls within the barn were bedded with wood shavings and did not require washing.

The bulls were collected in an area within the bull barn and adjacent to the laboratory by means of an artificial vagina. Two ejaculates were taken at each collection as suggested by Anderson (1). Each bull was allowed a false mount before the first ejaculation and the second ejaculate was taken as soon as possible thereafter. Immediately after semen collections the bulls were returned to their

respective quarters. The same technician did all the collecting and semen evaluation to insure uniformity. Semen volume was measured in milliliters. Motility was estimated as a percent motile by placing a drop of fresh semen on a preheated slide and examining it microscopically at a magnification of 100X. Motility estimates were derived from the movement patterns. Concentration was estimated in sperm per milliliter by placing "raw semen" diluted 1 to 50 with 2.9% sodium citrate in a Cenco-B<sub>2</sub> Photometer calibrated for semen concentration estimates. Total motile sperm per ejaculate was calculated as a product of volume, motility and concentration. "Weighted" averages of the two ejaculates per bull were calculated for motility, concentration and total motile sperm.

#### Experiment I

This experiment was an attempt to reverse seasonal effects on semen production. The experimental group was maintained in an "air conditioned" climatic chamber at  $64 \pm 4$ F with the relative humidity at  $82 \pm 12$ %. The control bulls were exposed to the existing summer climate. The experiment was conducted during a seven week period from June to mid-August, 1965. The monthly mean temperatures in Manhattan, Kansas during June, July and August, 1965 were 73.6, 78.6 and 77.1F respectively, an average of 2.2 degrees below normal. The mean maximum monthly temperatures for June, July and August were 84.7, 89.7 and 89.8F, an average of 2.1 degrees below normal (3). Semen quantity and quality were compared to determine possible reversal of semen production trends. If time and operating funds had permitted, the duration of this experiment would have been increased, probably

starting a month earlier and continuing for a minimum of 12 weeks.

The experimental group involved bulls 79 and 90 and the control group bulls 78 and 89. At the beginning of Experiment I, bulls 78 and 79, age 19 months, weighed 1404 and 1384 pounds and bulls 89 and 90, age 17 months, weighed 1314 and 1293 pounds, respectively. Bulls 78 and 79 received 16 pounds of concentrate and 14 pounds of hay daily and bulls 89 and 90 received 15 pounds of concentrate and 13 pounds of hay.

Semen was collected on the third and sixth day of each week. The semen quality and quantity of the two groups were compared by calculating a common regression line for each group according to Snedecor (56). The regression lines were then compared by the Student's "t" test as outlined by Steel and Torrie (57).

#### Experiment II

This experiment was designed to determine if a bull could be rendered in a predictable spermatogenic state indicated by semen quality and quantity at a predicted interval. The experimental procedure was patterned after the triple reversal trial of Cannon et al. (14). There were four experimental periods, each five weeks in length. The experimental group in the first period was designated the control group in the second period and vice versa. "Group reversing" was continued as shown in Table I throughout the experiment.

The experiment was conducted for 20 weeks extending from late October, 1965 to March, 1966. Ideally the periods should have been extended to nine weeks in order to overcome any individual biological variations.

Table 1

## EXPERIMENT II DESIGN - TRIPLE REVERSAL TRIAL

Bulls	Period 1	Period 2	Period 3	Period 4
79	Experimental	Control	Experimental	Control
89	Experimental	Control	Experimental	Control
78	Control	Experimental	Control	Experimental
90	Control	Experimental	Control	Experimental

At the beginning of Experiment II, bulls 78 and 79, age 23 months, weighed 1628 and 1696 pounds and bulls 89 and 90, age 21 months, weighed 1620 and 1648 pounds, respectively. Bulls 78 and 79 received 17 pounds of concentrates and 14 pounds of hay each day and bulls 89 and 90 received 16 pounds of concentrates and 13 pounds of hay. Bull 78 was recovering from azoospermia due to an infection and was placed in the first control group. Bull 90 was chosen to complete the first control group leaving bulls 79 and 89 as the experimental group.

The experimental animals were kept in a chamber maintained at  $83 \pm 2.5^{\circ}\text{F}$  and  $75.0 \pm 8\%$  relative humidity. Control bulls were kept in an unheated barn. The mean monthly outdoor temperatures during the experiment were, respectively, 47.9, 40.3, 26.9, 32.9, and 48.4 $^{\circ}\text{F}$  for the months from October through February. Each day before feeding, the respiratory rate was taken by counting flank movements for one minute. Rectal temperature was also taken by means of a 4 inch clinical thermometer.

Semen was routinely collected once each week during this experiment. The percent of morphologically abnormal sperm for each bull per collection period was obtained by counts of sperm smears stained with Methylene Blue. The data were subjected to statistical analysis to determine differences in semen quality and quantity by a triple reversal analysis of variance as outlined by Cannon et al. (14). This method compares periods by the formula  $(-a + 3b - 3c + d)$ . The analysis of variance was calculated by periods containing all 5 weeks and then only weeks one and two and only weeks four and five of each period. The analysis of selected weeks was used to determine possible

"carryover" effects. Simple correlations were also made to test "carryover" effects. Correlations were calculated for all criteria with various combinations of selected weeks within all periods. These combinations of weeks included: (1) all 5 weeks, (2) weeks 1 and 2, (3) weeks 4 and 5 and (4) weeks 1, 2, and 3. Correlations were made between sets of twins and groups for the entire 20 weeks and between twins for 15 weeks after a five week shift of data to align treatment groups. The 15 week correlations excluded the control data for Period 1 and Period 4 as illustrated in Table 1. If higher correlations between twins of the same set should occur, this would indicate that the effects of the treatment were insufficient to override genetic influence on semen production.

## RESULTS

### Experiment I

To compare the control group with the experimental group for any one of several semen quality measurements, the regression coefficients of each group were used to calculate "t" values. The experimental group's line ( $r = .09$ ) did not vary significantly from the control group's line ( $r = .06$ ) when plotted for volume (Fig. 1). This was substantiated by a "t" test that indicated no statistical significance between groups (Table 2).

Sperm concentration for the experimental group ( $r = -2.91$ ) remained relatively unchanged for the duration of the experiment. Sperm concentration for the control group ( $r = -40.71$ ) decreased continually throughout the experiment (Fig. 1). This difference in slope was not statistically significant at the 90% confidence level

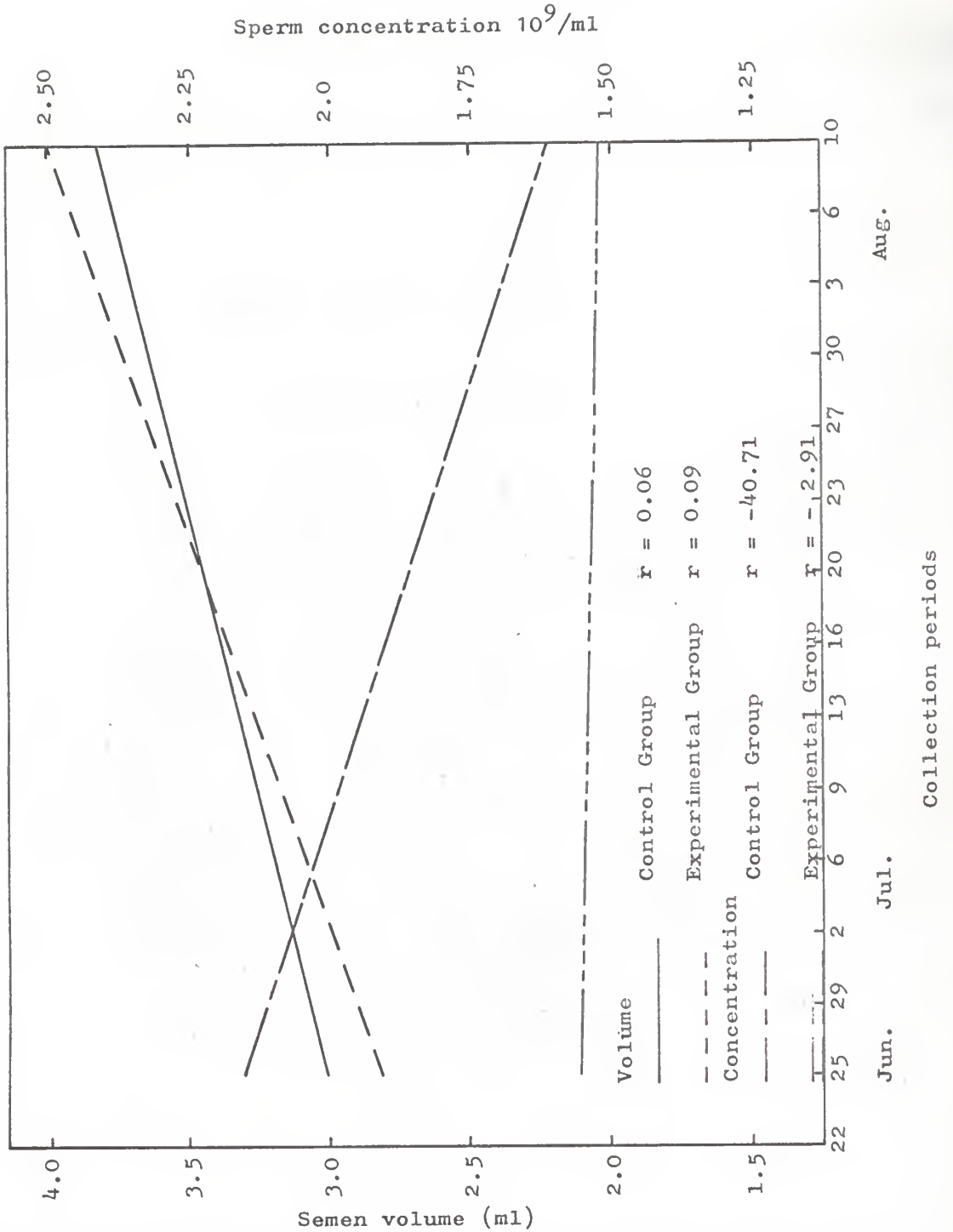


Fig. 1. Regression lines for semen volume and sperm concentration for ejaculate of the experimental and control group.



Table 2

COMPARED DIFFERENCES IN REGRESSION LINES BETWEEN  
EXPERIMENTAL AND CONTROL GROUPS

Measurements	Calculated <sup>a</sup> "t" values	Statistical Significance
Volume	0.527	N.S.
% Motility	11.484	***
Sperm Concentration/ml	1.061	N.S.
Total motile Sperm/ejaculate	1.672	N.S.

<sup>a</sup> All calculated "t" values contain  
24 degrees of freedom

\*\*\* Significant at 99% level

N.S. Not significant

(Table 2).

Percent motility decreased in the control group ( $r = -1.35$ ) and increased in the experimental group ( $r = 1.15$ ) (Fig. 2). As of July 27 and thereafter there was a marked drop in percent motility in the control group. The differences in motility percentages were significantly different between groups at the 99% level (Table 2).

Mean total motile sperm for the experimental group per ejaculate ( $r = 127.73$ ) rose sharply (Fig. 2), but decreased continually for the control group ( $r = -76.53$ ) (Fig. 2). Although the regression lines were opposite in slope these values were not statistically significant at the 90% level between groups (Table 2).

Semen collections were as scheduled for all bulls. However, on three occasions (July 16, 20 and 23), bull 89 made a poor false mount and would not mount to be collected. The artificial vagina was applied over his slightly extended penis as he stood "flat footed" behind the teaser animal, whereupon he thrust with vigor. Semen of quality comparable to that of the preceding week was obtained.

The bulls were weighed on the last day of the experiment and all gained more than 2 pounds per day with no significant difference between groups.

#### Experiment II

In this study the bulls lost an average of 0.85 pounds per day while in the heated chamber (experimental) and gained an average of 2.2 pounds per day when in the barn (control). The calculated Chi Square Value with one degree of freedom was 6.491 which was statistically significant at the 99% confidence level between groups for

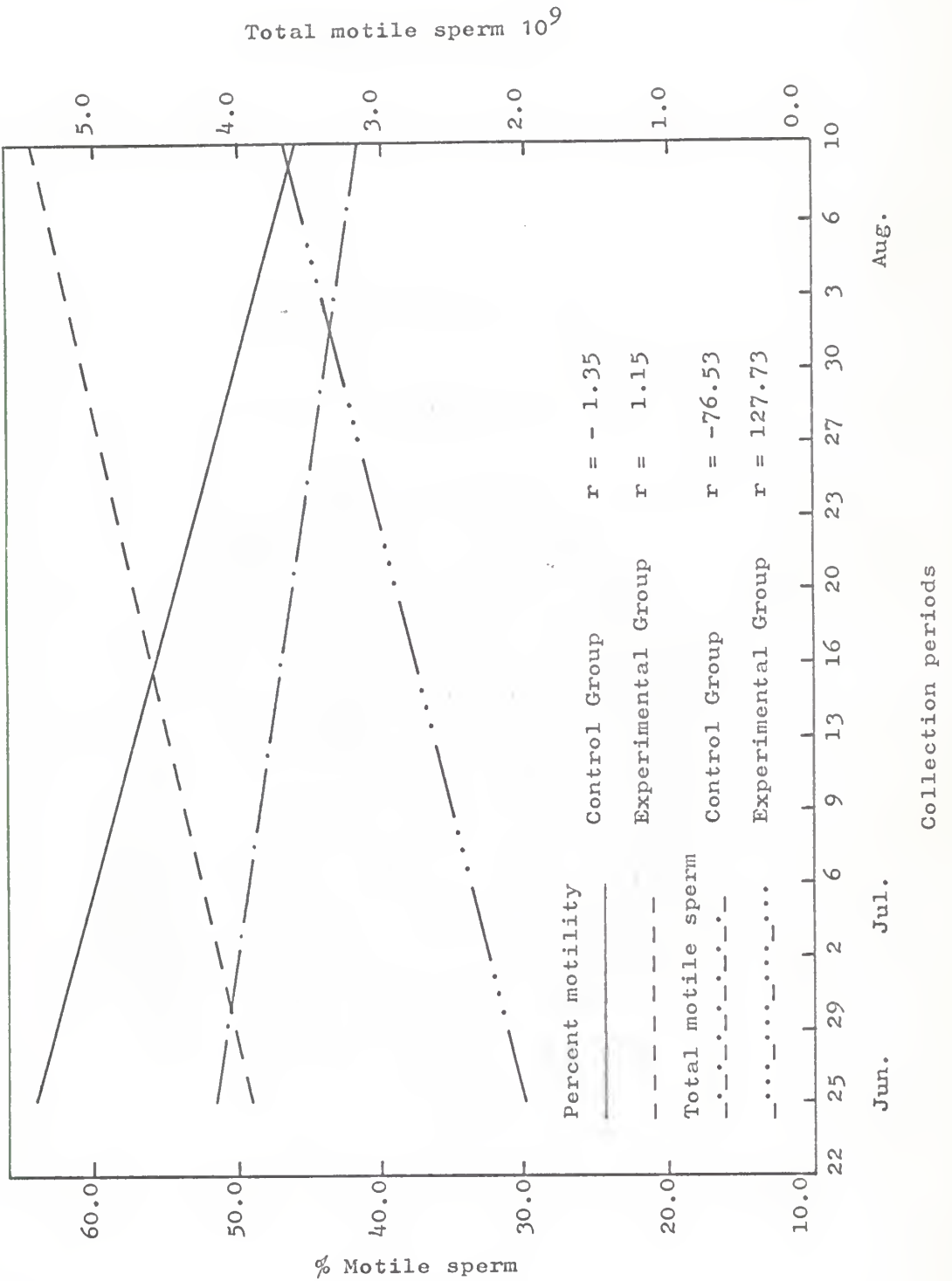


Fig. 2. Regression lines for percentage of motile sperm and total motile sperm of the experimental and control group.

weight change. The bulls in the experimental group ate slowly and often did not clean up their feed. Before the bulls acclimated to the heated chamber, tachypnea with rates exceeding 100 per minute, and increased salivation were observed. Acclimation usually occurred in 5 to 7 days after introduction into the heated chamber. When respiratory rates, rectal temperatures and salivation rates returned to pre experimental levels, acclimation was considered to be complete. Restlessness, indicated by increased tail switching, was observed in all bulls while in the heated chamber. This was accompanied by relaxation of posture: the bulls stood wing shouldered with the head lowered and the rear legs extended posteriorly.

Visual inspection of semen quality data in a graphic form indicated unexpected differences within groups may exist. There were definite variations in semen quality without detectable pattern. At this point statistical analysis was used in an effort to expose an otherwise undetectable pattern, such as common variables. Correlations were obtained for volume, motility, concentration per milliliter, body temperature and respiration rate, for each bull for the 20 weeks of Experiment II without regard to placement in trial. There were positive correlations between rectal temperature and respiratory rates and between percent motility and concentration of sperm per milliliter of all four bulls. These correlations were statistically significant at the 99% confidence level. Whenever respiratory rates increased, rectal temperatures increased and vice versa. No other consistent correlations at the 90% or higher confidence levels were observed in all four bulls (Table 3).

Table 3

CORRELATIONS BETWEEN MEASUREMENTS AND THEIR  
STATISTICAL SIGNIFICANT LEVELS

	Volume	Motility	Concentration per ml	Temperature (Body)	Respiration
78					
Volume	1.000	- .025	- .079	.126	.155
Motility		1.000	*** .672	- .055	.020
Concentration/ml			1.000	* .407	** .517
Temperature(Body)				1.000	*** .793
Respiration					1.000
79					
Volume	1.000	.095	.321	.402	.247
Motility		1.000	*** .744	.475	.473
Concentration/ml			1.000	* .428	** .515
Temperature(Body)				1.000	*** .828
Respiration					1.000
89					
Volume	1.000	.563	.294	.033	.240
Motility		1.000	*** .650	- .029	.201
Concentration/ml			1.000	.028	.262
Temperature(Body)				1.000	*** .772
Respiration					1.000
90					
Volume	1.000	.091	-.046	-.442	-.378
Motility		1.000	*** .723	-.459	-.338
Concentration/ml			1.000	-.115	.180
Temperature(Body)				1.000	*** .685
Respiration					1.000

19 d.f. with all correlation values

\* = 90%

\*\* = 95%

\*\*\* = 99%

Negative correlations for the oldest twins (78 and 79) were significant at the 90% level between concentration per milliliter and rectal temperatures, and at the 95% level between concentration per milliliter and respiratory rates. The same correlations for bulls 89 and 90 were not statistically significant.

Semen quality data included measurements of volume, percent motility, concentration per milliliter, total motile sperm, and percent abnormal sperm. Statistical analysis included data from all five weeks, weeks one and two, and weeks four and five of each period. The degrees of freedom were one and two because of the small number of animals, and the calculated "F" values were small. There were no significant differences between the experimental and control groups for any of the semen measurements. Table 4 contains the comparison values  $(-a + 3b - 3c + d)$  calculated from individual weighted averages for total motile sperm per ejaculate. The large variance indicated variable degrees of response to treatment by individual animals. Several correlations were made to determine degrees of interaction. Correlations for all semen quality measurements, whether by period or group or treatment were not statistically different at the 90% level. Percent motility and total motile sperm correlations by twins and paired groups for 20 weeks were calculated (Table 5). Correlations within twins were computed regardless of the experimental phase. The correlations for percent motility and total motile sperm were greater within twins than between paired groups in all comparisons.

The following correlations were calculated to detect possible

Table 4

COMPARISON VALUES FROM  $(-a + 3b - 3c + d)$  FOR AVERAGE  
TOTAL MOTILE SPERM FOR 4 PERIODS (5 WEEKS EACH)

	1st and 3rd periods experimental for bulls:		2nd and 4th periods experimental for bulls:	
	79	89	78	90
Weeks 1	-17600	- 4078	-1024	-6808
Weeks 2	-12050	-16625	-6258	-8004
Weeks 3	6717	- 8466	8494	10881
Weeks 4	- 5769	4690	9269	-7157
Weeks 5	- 1389	-25965	7146	-15141

differences between "within twin" values regardless of treatment, as compared to effect of treatment. For analysis purposes data were shifted five weeks for one group to align the experimental and control groups. The control group's data were excluded from period 1 and period 4 leaving 2 x 5 week treatment periods and 1 x 5 week control period for 5 week correlations (Table 1). Correlations for percent motility and total motile sperm for 15 weeks were calculated within twins and the 15 week correlations were compared to the 20 week correlation within twins (Table 5). None of the 15 week correlations were greater than the 20 week values.

#### DISCUSSION

##### Experiment I

Late in July, bull 89, when moved from the air conditioned chamber to the collection area refused to mount the teaser animal. However, semen was collected "flat footed". The semen produced at that time was comparable in quality to the preceding week. Loss in libido with no change in semen production was attributed to the effect of radical change in ambient air temperature.

Bulls of both groups gained more than two pounds per day with no significant differences between groups. Semen volume was comparatively unchanged between the experimental and control groups; the differences were statistically non-significant ( $P > .10$ ). These results paralleled those of Dutt and Simpson (18) working with rams housed in air conditioned facilities and compared to those exposed to natural summer conditions. These results did not substantiate those of Casady et al. (15) who observed that semen volume increased



Table 5

CORRELATIONS FOR 20 WEEKS BY TWINS AND  
PAIRED GROUPS AND FOR 15 WEEKS BY TWINS

Measurements	Bulls Paired Groups	20 Weeks Paired Groups	Bulls Twins	20 Weeks Twins	15 Weeks Twins
Percent Motility	79, 89	-.224	78, 79	-.376*	-.336
	78, 90	.138	89, 90	.341	.020
Total Motile Sperm	79, 89	-.085	78, 79	.122	-.084
	78, 90	.141	89, 90	.477*	-.304

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\*Significance at 90% level

as temperature decreased.

Percent motility increased in Experiment I in the (air conditioned) experimental group and decreased in the (untreated) control group. Percent motility trends varied sufficiently to indicate statistical significance at the 90% confidence level. The reversal of seasons was in agreement with Dutt and Simpson (18) and Casady et al. (15). Sperm concentration per milliliter for the experimental group remained the same throughout Experiment I while the concentration decreased in the control group (Fig. 1). This difference between groups was not statistically significant at the 90% confidence level, however, a decrease in sperm concentration was apparent. This was in agreement with Casady et al. (15) who concluded that sperm concentration decreased as temperature increased. Experiment I did not substantiate the findings of Dutt and Simpson (18) that sperm concentration increased as temperature decreased. This may be due to the different relative starting temperatures. The alteration of the mean total motile sperm per ejaculate trend was indicated in Fig. 2 by the opposite slopes produced from the regression lines. This was not statistically significant at the 90% confidence level but closely approached this level.

In spite of the fact that average ambient air temperatures were not as high for those housed in the barn as expected because the mean monthly temperatures averaged 2.2 degrees below normal for summer, semen quality trends were significantly altered. The trends indicated in Fig. 1 and 2 continued to the end of the experiment indicating that increased affects could be expected if the experiment had been of longer duration. This would further substantiate that seasonal

semen trends could be reversed as they have been in rams.

#### Experiment II

There was a high correlation between rectal temperature and respiratory rate which was statistically significant at the 99% confidence level. The bulls in the experimental group lost weight and those in the control group gained. Average gains per group differ significantly at this level. The physiological changes observed in the experimental group were: decrease in appetite, restlessness, relaxation of posture and increased tail switching. These observations paralleled those of many other investigators for climatic stress (10, 12, 13, 15, 22, 33, 36).

Analysis of variance was calculated for all semen measurements by periods containing various combinations of weeks per periods. The degrees of freedom were one and two because of the small number of animals. The calculated "F" values were small but this was attributed to the larger variance. These factors resulted in a lack of significant differences in all semen measurements between the experimental and control group. This held true regardless of combination of weeks per period when testing for "carryover" effects. Experiment II paralleled the findings of Experiment I in that volume of ejaculates is least affected by changing environmental conditions.

Correlations by siblings and correlations by paired groups, for the 20 week study, were calculated for percent motility and total motile sperm. This was investigated because of the lack of significances in correlations by period. The correlations for both measurements were greater between twins than between paired groups

in all comparisons. This indicates the genetic similarity between twins had more effect on semen production than did the treatment. This was confirmed when data were translocated five weeks for one group and the same correlations calculated for 15 weeks by twins. The 15 weeks were compared to the 20 week correlation values by twin sets, none of the 15 week correlation values were greater than the 20 week values. The raw data indicated definite decreases in semen quality. These decreases occurred at different times for each bull and for varying lengths of time. The computed positive correlations between rectal temperature and respiratory rates and general physiological observations when bulls were introduced into the heated chamber indicated that climatic stress occurred. The degree of injury to the seminiferous epithelium may differ between bulls because of biological variations. There were indications that longer periods may have eliminated "carryover" effects. This also would have simplified interpretation of the data.

#### SUMMARY AND CONCLUSIONS

Reversal of seasonal semen production trends, and the possibility of producing predictable spermatogenic states were investigated in the bull. Semen was estimated by the quality and quantity per ejaculate.

The results of Experiment I indicated that semen volume was not altered by ambient temperatures. Sperm concentration per milliliter decreased as temperature increased, but did not increase as temperature was decreased. Percent motility and total motile sperm varied inversely as the ambient temperatures.

The results of Experiment II indicated that volume was not affected by ambient temperatures. There were positive correlations between rectal temperatures and respiratory rates and between sperm concentration and percent motility. Climatic stress in animals subjected to raised environmental temperatures was indicated by an increase in rectal temperatures, respiratory rates, restlessness, salivation, poor posture and by a decrease in appetite and body weight. Although semen quality deteriorated under raised environmental temperatures, consistent correlations were not evident.

The following conclusions were drawn from the results of these experiments.

1. Volume of semen is not altered by lowering ambient temperatures to 65F.
2. Air conditioned facilities can prevent a decline in semen concentration, percent motile and total motile sperm during the summer in Kansas.
3. Further studies on the effects of environmental temperatures on semen quality are necessary to establish a predictable state of spermatogenesis in dairy bulls.
4. Testicular biopsy studies will not be feasible until it is possible to put a dairy bull in a predicted state of spermatogenesis

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RELATIONSHIP OF SEMEN PRODUCTION IN DAIRY BULLS  
TO SELECTED AND CONTROLLED AMBIENT CONDITIONS

by

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AN ABSTRACT OF A MASTER'S THESIS

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A more complete knowledge of the factors that influence the quality and quantity of bovine semen is needed because of the continually increasing demand for quality semen. The purpose of this study was to determine the possibilities of altering seasonal effects on semen, and if a bull could be induced into a predicted spermatogenic state as would be indicated by semen quality.

The facilities used for these studies included a climatic chamber and an adjacent bull barn at Kansas Artificial Breeding Service Unit, Manhattan, Kansas. The chamber was regulated to provide uniform temperatures, relative humidity and duration of light. The existing climate provided the environment which prevailed in the adjacent barn.

Two sets of Holstein bull twins were paired so that siblings did not compose a group (control or experimental). Two ejaculates were taken at each collection with the use of an artificial vagina. Semen was evaluated for volume, percent motility, sperm concentration, total motile and abnormal sperm per ejaculate.

Experiment I was conducted during a seven week period from June to mid-August, 1965, to determine reversibility of seasonal effects on semen.

The chamber was maintained at  $64 \pm 4F$  and relative humidity at  $82 \pm 12\%$ . The mean maximum monthly temperatures for June, July and August 1965 were 84.7, 89.7 and 89.8F. This was an average of 2.1 degrees below normal. Results of twice weekly collection indicated the volume of semen was not affected. Sperm concentration for the group in the chamber remained relatively unchanged but decreased in

the control group. Percent motility and total motile sperm trends indicated that both measurements were inversely proportional to the ambient temperatures. However, regression coefficients between groups showed no statistically significant difference for total motile sperm.

Experiment II was conducted to determine the feasibility of producing a predicted spermatogenic state indicated by semen quality. This experiment was conducted for a 20 week period from late October to March, 1966. The chamber was maintained at  $83.5 \pm 2.5^{\circ}\text{F}$  and  $75 \pm 8\%$  relative humidity. The barn was unheated and the mean outdoor temperature was  $39.5^{\circ}\text{F}$  during the experiment. A triple reversal trial was performed with four experimental periods of five weeks each and the two groups alternated in the chamber. Rectal temperatures, respiratory rates and body weight was recorded in addition to semen quality evaluations.

When the bulls were placed in the warm chamber there was tachypnea and increased salivation and rectal temperatures until acclimation occurred. A decrease in appetite and weight, relaxation in posture and increased tail switching occurred throughout the entire period in the chamber, indicating climatic stress on the bulls. Physiological conditions returned to normal within a day after the bulls were returned to the barn. Raw data indicated definite variations in semen quality at different times and for varying durations in each bull, making analysis difficult. The data were statistically analysed but the variance was so large that significant differences were not detectable. Variance was due to the

differences between bulls.

The results from Experiment I indicate that the decreases in bull semen quality during inclement weather can be minimized if not eliminated with climate controlled facilities. The results from Experiment II were inconclusive. It is suggested that this experiment should be repeated with more bulls, longer periods to minimize "carry over" effects, and temperatures controlled a few degrees higher.