ENVIRONMENTAL FACTORS ASSOCIATED WITH FREQUENCY OF COLLECTION AND SEMEN CHARACTERISTICS OF YORKSHIRE BOARS

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CHAPTER I

INTRODUCTION

Boars, as well as other mammalian males, are known to exhibit a decrease in semen quality during the hot summer months. This fact has been well documented by Cassady et al., (1953); Corteel, (1963); Cupps et al., (1960); Dutt and Hamm, (1957); Erb et al., (1942); Skinner and Louw, 1965; and others. Since multiple farrowing is becoming a more popular practice among swine breeders, the breeding season is continuous year around. The boar plays a major role in farrowing rates, so it is necessary to keep semen quality as high as possible the year around. Dutt and Bush (1955) report that lowering the environmental temperature for sheep during the warmer season of the year will keep them from exhibiting the normal lowered semen qualities.

The purpose of these two trials was to study the effect of four shelter modifications on semen qualities in boars, and to determine if controlling the environment would alleviate the depressed semen quality exhibited during the summer. Seasonal variation in swine semen characteristics were also observed and analyzed.

Also incorporated into the latter part of this study were several methods of estimating spermatozoa motility. The conventional method of estimating percent motile sperm subjectively has been criticized because it may be biased by sperm concentration, temperature, and other factors. Two other methods of estimating percent motile sperm that were utilized in this study were the hemocytometer method and the subjective method of estimating the rate of forward movement of spermatozoa.

CHAPTER II

REVIEW OF LITERATURE

Seasonal Variation

In recent years, researchers have become more concerned about the effect of temperature and other environmental conditions on the reproductive performance of farm animals. Most of the research in this area has been done with bulls and rams, but this review will present primarily the limited work that has been published con- concerning seasonal variation in boars. In all species, the results of these studies indicate that semen quality and quantity are affected by changes in temperature.

Okauchi and Hirakata (1962) found that the season of the year influences the composition of the ejaculate of the boar. They reported that semen volume was lowest during the seasons of maximum and minimum environmental temperature; however, the number of spermatozoa did not vary with the temperature. It was noted in this study that the ratio of live to total sperm fell during the hot season of the year.

Stevermer *et al.*, (1961) noted some seasonal variation in semen production in six boars. The semen volume increased in all but one boar in this trial during the cooler

season of the year. Both spermatozoa concentration and total number of spermatozoa per ejaculate were highest during the warm summer months.

At the French boar stud in Poitou, Cotreel (1963) noted that five times as many ejaculates were rejected for use for artificial insemination due to dead or a high percentage of abnormal spermatozoa from August to November then for any other time of the year.

Three shelter modifications (three-sided house, open shade, and air-conditioned house) were used in a study to determine the nature of seasonal changes in boar semen (Lawrence, 1967). The semen from eight Yorkshire boars were used in this experiment. The results in this study indicated that volume and total sperm per ejaculate were highest during cool weather, and percent abnormal sperm was lowest during the same period. The analysis for shelter effect suggested that, in general, the shelters which provided the most protection from the environment were the most conducive to good semen quality. However, the boars in the coolest shelter (air-conditioned house) did not produce the highest quality or quantity of semen. There was no apparent reason for this contradiction. Strained volume and percent motile sperm were highest in the group in the open shade. The percent abnormal sperm was lowest in the group in the three-sided house. Also, total sperm per ejaculate was highest in the three-sided house group.

Erb et al., (1942) studied the effects of seasonal changes in the semen characteristics of nine dairy bulls of four different breeds. These bulls were collected once a week for a year. The analyses of variance between months were found to be highly significant for initial motility, ejaculate volume, spermatozoa concentration per cubic millimeter, total sperm per ejaculate, and the number of abnormals per 1,000 spermatozoa. The pH of the semen was not significantly affected. The quality of the semen was significantly superior during the spring months and significantly inferior during the summer. Semen volume was found to be lowest in July, August, and September, and initial motility was also the lowest during the same three month period. The average number of abnormal sperm cells was twenty-five percent greater during these months than in the next poorest month. Sperm survival was least in August and September.

Mercier and Salisbury (1946) analyzed data from 328 ejaculates and 20,689 inseminations in dairy cattle located in New York. They found sperm production and fertility to be at their lowest level in June and July. The percent abnormal sperm was highest during March, April, and May.

Suffolk, Hampshire, and Rambouillet rams were used in an experiment by Cupps et al., (1960) to study seasonal changes in the semen of these rams. The rams were collected twice a week with either an electroejaculator

or an artificial vagina. They found a pronounced decrease in ejaculate volume, cell concentration, percent motile and percent live sperm in August and September. This coincided with the high average maximum daily temperatures for these months of 97.8° F. and 91.9° F., respectively.

Semen samples were collected from twelve fat-tailed rams once a week for a year by Hafez (1955). They were kept under natural conditions at Cairo, Egypt. The highest spermatogenic activity occurred at two different times during the year; March and April, and July and August. Semen quality was best in the spring and lowest in December. The total spermatozoa abnormalities were highest in the summer. Coiled tails was the main type of abnormality. Ejaculate volume was not correlated with any other semen characteristics.

Most researchers are in agreement that the male of all species of farm animals exhibits a decrease in semen quality during the late summer months, and that semen quality begins to improve in the fall. In general, research has indicated that spermatozoa motility decreases with an increase in environmental temperature and the percent of abnormal cells increase. Sperm concentration and volume are not adversely affected to as great a degree.

Controlled Environment

The use of artificial environments has been very helpful in studying many problems associated with the

effect of temperature on the physiology of reproduction. The use of controlled environments eliminates many of the variables that may influence fertility and semen production in males which cannot be controlled under normal conditions.

El-Azab (1966) studied the effect of local heat application to the scrotum on spermatogenesis, maturation of the spermatozoa in the epididymis, and semen quality in rabbits and bulls. In the first test, the testes of twenty rabbits were heated either by artificial cryptorchidism or by immersion of the scrotum in a water bath at 43° C. for twenty, thirty, forty, or sixty minutes. In the test involving bulls, the scrotum was heated for one, three, six, twelve, or eighteen hours by immersion in a water bath at 43° C. The results indicated that the first meiotic division and the spermatids were affected adversely by heat, and that this led to an increase in the number of primary abnormalities of the sperm. Sperm with coiled tails seemed to originate in the testis, while tailless sperm originated in the epididymis. The sperm in the cauda epididymis seemed to be more resistant to heat than those in the caput epididymis.

Heat stress and spermatogenesis in two species of cattle were studied by Skinner and Louw (1966). Several experiments were conducted to determine the critical duration of high ambient temperature (40° C.) required to adversely affect spermatogenesis in the bovine. Results indicated that spermatozoa were not as severely affected in

the Bos indicus as in the Bos taurus, but optimum spermatogenesis was impaired in both breeds. An exposure of as little as twelve hours proved to be critical and the site of virtually all damage was in the seminiferous tubules. A significant decrease in motility and percentage of live spermatozoa was recorded together with a significant increase in the percentage of morphologically abnormal spermatozoa. The authors concluded that even short-term exposures to heat stress can adversely affect spermatogenesis and fertility in the bovine.

Austin et al., (1961) conducted an experiment to evaluate effects of short periods of scrotal insulation on semen characteristics of bulls. Twelve Hereford bulls were divided in three groups; control, and scrotal insulation for twenty-four or seventy-two hours. The insulation raised the mean scrotal temperature 3^{o} F. In both treated groups, the number of live sperm decreased and abnormal sperm increased to sixty-five percent of controls in the second and third weeks after insulation. Sperm motility also decreased during this period. Sperm concentration decreased during the fourth through the seventh week in the seventy-two hour group. The authors also compared the effects of scrotal insulation and ionizing radiation. They demonstrated that the two types of injury produce similar defects, but the major effect of radiation is on the spermatogonia while insulation affects the more mature stages of sperm development.

Two heat chambers were utilized to study the effect of ambient temperature on four Guernsey bulls (Casady et al., 1953). The temperature in one chamber was progressively changed from 70° F. to 90° F. In the other chamber it was changed from 70° F. to 52° F., and then back to 86° in the same manner. Stress, as evidenced by restlessness, excessive salivation, weakness and tremors, was not apparent until temperatures approached 90° F. Spermatogenesis was impaired in two bulls by continuous exposure to 100° F., and in the other two bulls by continuous exposure to 86° F. for five weeks. Libido and volume were not affected by the high environmental temperatures, but initial motility, sperm concentration, and total sperm counts decreased very markedly during or following exposure.

Holstein, Brown Swiss, and Red Sindhi cross-bred bulls were used in a study of their physiological response to high temperatures and humidities (Johnson et al., 1963). The bulls were exposed eight hours per day for seven days to a maximum temperature of 104° F. and thirty millimeters of mercury vapor pressure. The minimum conditions were 82° F. and twenty-two millimeters of mercury pressure. The effects of this stress were lower initial motility, sperm concentration, and total numbers of sperm, and an increase in sperm abnormalities. These effects were most pronounced four to six weeks post treatment. Recovery of semen quality occurred by nine weeks after heat stress.

Simpson (1966) studied the response of semen production and fertility in rams following exposure to controlled ambient temperatures. One group of rams was exposed to 90° F. for one week, another group was placed in an environment of 100° F. for twenty-four hours, and the last group was exposed to 100° F. for six hours. The semen quality of rams exposed to the first two treatments was significantly poorer than controls. The semen quality of the group exposed to 100° F. for six hours, however, was not significantly poorer than controls. When rams were maintained in an air-conditioned room during the summer months, their semen was of better quality than that of controls kept under normal environmental conditions. Testicular biopsy was performed on the group in the treatment of 90° F. for one week. The biopsy revealed shrunken tubules and disorganization of the germinal epithelium. Fourteen days post treatment the tubules were found to be devoid of germinal elements other than spermatogonia.

Merino rams were used in a study to determine the effect of heat stress on morphological changes in spermatozoa (Rathore and Yeates, 1967). Treated rams were exposed in the climatic chamber for two daily exposures, each of eight hours at 105° F. and forty-five percent relative humidity. Unheated rams served as controls and all rams were collected twice a week. Semen collections were made one day before exposure and every fourth day up

to thirty-six post heating. The semen of the treated group contained a high proportion of spermatozoa with pyriform heads. They were first noticed eight days after the end of the heating period, and their occurance gradually increased up to fourteen to twenty-one days post heating. This condition returned to normal twenty-eight days post heating.

Simpson and Rice (1957), in a research study conducted at the Kentucky Agricultural Experiment Station, placed three rams in a room maintained at 90° F. for one week. The semen quality of these rams was compared to that of three control rams which were kept in an unheated barn during January. Collections were made once a week for an eight week period. The treatment had no effect on semen volume, but in the fourth week motility dropped to twenty-two percent and the number of abnormal cells rose to 51.1 percent. Total sperm count reached its lowest level in the fifth week. The treated group returned to normal semen production by the eighth week post treatment.

A second experiment with rams performed at the same station (Simpson and Dutt, 1958), utilized treatments of 100° F. for twenty-four hours, 90° F. for one week and a control group. During the six-week period following exposure to elevated temperatures, little difference was noted in semen qualities from the two treatment groups of rams; however, both produced semen of lower quality than did the control rams. The percent motile cells was lowest

four weeks after the treatments began when it was forty, thirty-three and seventy-nine percent for the three groups, respectively. The percent abnormal cells was also highest during the fourth week, averaging thirty-four percent for both treated groups and twenty-two percent for the controls. When rams were exposed to 100° F. for six hours, the only response observed was an increase in the percent abnormal spermatozoa.

The effect of locally heating the testis of rams on spermatogenesis was studied by Waites and Setchell (1964). They noted that locally heating the testis above 39° C. for two hours caused moderate or severe seminal degeneration. The most severely treated ram produced eighty-eight to one hundred percent dead sperm between days fifteen and thirty-nine. The number of sperm declined from day eleven. Recovery of spermatogenic function was rapid and appeared to be normal again on day fifty-three.

The effect of high environmental temperature on the semen production of sheared and unsheared rams was studied by Dutt and Hamm (1957). Six Southdown rams were used in the study. Two rams were subjected to each of two treatments and a control. The control animals were unsheared and maintained conventionally for winter conditions in Kentucky. Both treatment groups were subjected to an environment of 90° F. and sixty-five percent relative humidity for one week, the only difference being that one group was sheared and the other was not. Volume and libido

was unchanged by either treatment group, however, spermatozoa motility was adversely affected, especially in the unsheared groups. Motility fell to ten percent during the fifth week after treatment. Abnormal cells increased reaching a maximum five weeks after treatment. Sperm cell concentrations decreased more in the unsheared than in the sheared rams. Semen quality of all rams returned to normal after eight weeks.

The effect of low temperature on the fertility of rams was studied by using an air-conditioned chamber held within a range of 45° F. to 48° F. (Dutt and Bush, 1955). The reproductive performance of sheep maintained in this environment was compared to that of control animals in a similar chamber with a daily maximum temperature of 88.7° F. Semen from the rams kept in the air-conditioned room did not show the marked decrease in spermatozoa motility or the increase in percent abnormal that was noted in the control rams during the summer months.

The effect of daily immersion of the scrotum of rats and monkeys in a 44° C. water bath was studied by Vankalachalam and Ramanathan (1962). The scrotum of the rats was immersed for ten minutes per day and the scrotum of the monkeys for twenty minutes per day. This procedure was extended over an eight-week period. Both the rats and monkeys showed loss of testicular weight, atrophy of the tubules and degeneration of the germinal epithelium. Judged on the basis of reproductive performance, the rats

were found to be infertile. The epididymal smears of the monkeys showed no spermatozoa to be present. Partial regeneration of the germinal epithelium of the rat's testis was noted six weeks post treatment.

Steinberger and Dixon (1959) studied the effect of heat on the testicular germinal epithelium of rats. The results indicated that a fifteen minute exposure to a temperature of 45° C. produced a progressive destruction of the entire germinal epithelium. The earliest cytologic changes were observed in the spermatids. When the rats were exposed to temperatures below 43° C. for fifteen minutes, it produced inconsistent tubular damage. A fifteen minute exposure to 43° C. produced testicular damage, mainly of the spermatocytes.

Chowdhury and Steinberger (1964) conducted a similar experiment with rats. The results they obtained are in close agreement to Steinberger's earlier work. Testicular tissue was studied at intervals of two, four, six, eight, and twenty-six days after exposure to heat (43° C. for fifteen minutes). The frequency distribution of the various stages of spermatogenesis, the resting spermatocytes, spermatogonia, and Sertoli cells were not affected by exposure to the heat. However, the primary spermatocytes in the leptotene stage to, and including, the dividing spermatocytes were injured.

Rats were used by Bowler (1967) to determine the effects of repeated temperature applications to testis on

fertility in males. The rats were immersed up to penis level in a water bath at 43.5 ± 0.1° C. for twenty minutes. Eleven rats received a single heat application and recovered their fertility in 60.0 ± 1.58 days. Of the fourteen rats which received seven applications of the same heat treatment at six weekly intervals, twelve had a mean recovery time of 108.2 ± 7.2 days. The author suggests that the testis of the rat can withstand repeated heat applications and still recover its spermatogenic function, although the repeated treatments do cause a delay in recovery of fertility.

Male rabbits, all of which had at least sixty percent motile sperm at the start of the trial were subjected to a temperature of 110° F. for one hour in a laboratory experiment (El-Sheikh et al., 1955). The body temperature of the rabbits was raised 4.6° F., but in most cases sperm motility was not reduced. Treated and control males were then mated to normal females on days seven, twelve to fourteen and twenty-one to thirty-one post treatment, and the percent of fertilized ova was used to determine fertility. Fertility of the treated bucks was significantly lower than the controls. It was concluded that increased ambient temperature may affect male fertility without bringing about any apparent depression in sperm motility.

Burfening and Ulberg (1968) studied the effect of embryo survival in rabbits after being fertilized with

sperm that had been subjected to temperatures of 38° and 40° C. Split ejaculates of rabbit semen was incubated for three hours at 38° or 40° C. The semen was then examined and inseminated separately into the uterine horns of rabbits which were mated four hours earlier to vasectomized males. The eggs were recovered and examined for fertilization thirty hours after coitus. The eggs were then returned to the oviduct, and nine days later their survival was estimated by counting the implantation sites. Increased temperature had no effect on the fertilizing capacity of the semen, but embryonic survival rate was higher (75%) in the uterine horns inseminated with semen incubated at 38° C. than in those inseminated with semen incubated at 40° C. (53%).

Research that has been done with controlled environments indicates that high ambient temperatures are detrimental to spermatogenesis and semen quality. The more mature stages of spermatogenesis seems to be the most susceptible to heat damage, whereas, the spermatogonia and leydig cells remain unaffected. Unless heat exposure is very severe, spermatogenesis will again take place normally after a period of a few weeks.

Hemocytometer Method of Assessing Spermatozoan Motility

At the present time there is no quick and reliable method to determine the distribution of motility of individual sperm cells in a semen sample. Most methods

now in use for assessment of sperm motility are primarily subjective in nature, and the results are usually expressed in comparative rather than absolute terms. These subjective measurements are apt to be influenced by the concentration of cells unless care is taken to prevent this bias. There are several other methods which are commonly used by researchers that may possibly have more merit than estimating motility of sperm cells subjectively in undiluted semen. Some investigators and artificial insemination units depend on graded estimates of the vigor of swirls and wave formation in undiluted semen, as seen under the microscope, to assess motility. Many researchers just dilute the semen sample in order to view the individual cells easily at 200X or higher magnification. Rothschild (1948) devised a means of assaying sperm activity by using an a-c bridge and an oscilloscope to measure the electrical conductance of semen samples between two platinum electrodes. This type of method cannot be used with diluted semen, because less than 800,000 cells per cubic millimeter will not give an indication on the oscilloscope.

The only known thoroughly objective method to assess sperm motility is based on the use of the hemocytometer. This method was first described by Brady and Gildow (1939) who diluted semen 1:100 with physiological saline in one dilution pipette and with a one percent alcohol solution, which kills the spermatozoa in another. These separate dilutions were then placed in two chambers of the hemocy-

tometer. They determined the percent motility by counting the number of non-motile sperm cells in the sample diluted with physiological saline and subtracting this value from the total number of cells in the sixteen squares of the chamber which contained the semen diluted with alcohol. This method has been somewhat modified by Willet and Salisbury (1942) who used a one percent chlorazene solution to kill the spermatozoa; by Harvey (1945) who used a two percent solution of osmic acid in a glass capsule inverted over one section of the counting chamber; by Blom (1946), who used heat; and by Lasley (1951), who used freezing.

Bane (1952a) conducted a study involving the hemocytometer method in which he compared several extenders. Hemocytometer determinations were carried out on 372 ejaculates from eight normal bulls. The following average motility rate was recorded for the different extenders; Ringer's solution, 76%; Ringer's bicarbonate solution, 71.5%; glucose-phosphate diluter, 67.3%; and egg-yolk phosphate diluter, 58.9%. In a second comparative trial, Ringer's solution gave on an average 17.6% higher motility than the egg-yolk phosphate solution.

It was found that temperature has a great effect on motility. At 38° C. a 12.7% higher motility rate was obtained than when the same semen was tested at 18° C. The speed of motion of the cells was also reduced markedly at the lower temperature.

Lasley (1951) suggested that determining motility by hemocytometer may cause motility estimates to be too high, because many of the inactive sperm are moved about by the active ones, giving the false impression that nearly all the cells are motile. He found that the correlation between hemocytometer motility and conception rate was 0.314.

The hemocytometer method of estimating spermatozoa motility is time-consuming, but presently is probably the most accurate method. Salisbury and VanDemark (1961) deviates from the normal pattern of this method in that they do not use a known volume, but instead dilute the semen so that only a few spermatozoa are found in each microscope field and then quickly determine both the total number and the number of non-motile cells in each of several fields. The accuracy of this particular method depends on proper dilution, temperature control and the skill of the technician.

CHAPTER III

MATERIALS AND METHODS

Trial I

This trial began March 1, 1967, and terminated February 26, 1968. The study was conducted at the Oklahoma State University boar facilities and involved 12 purebred Yorkshire boars from the Oklahoma State University purebred swine herd. The identification numbers, initial and final weights and ages of each boar are given in Table I.

Four boars were randomly assigned to each of the three environmental lots. These were adjacent lots, each approximately 30 x 112 feet in size. The treatments imposed were determined by the type of shelter in each of the individual lots as follows:

Lot I. A 10 x 11 foot three-sided house that opened to the east. Bedding consisted of wet sand during the summer and straw during the winter.

Lot II. A six foot high, 14 x 18 foot open pole shade with a galvanized metal roof and a dirt floor. No bedding was provided for the boars in this lot.

Lot III. A fully insulated 9 x 10 foot house with a wooden floor and a 4½ foot ceiling. The door was designed so that it could be easily opened by the boars, but was

spring loaded so that it remained closed except when in use. A one horsepower air conditioner was used to hold the temperature inside this building as close to 75° F. as possible during the summer months. During the cooler months the air conditioner was turned off and no attempt was made to heat the building or to provide bedding in the winter.

TABLE I

DESCRIPTION OF THE BOARS USED IN TRIAL I

Boar No.	Lot No.[a]	Initial Age (Mo's.)	Final Age (Mo's.)	Initial Weight (lbs.)	Final Weight (lbs.)
69-10	I	16	28	475	475
63-5	I	16	28	490	550
62-15	I	16	28	420	490
69-7	I	16	28	435	480
70-3	II	16	28	450	480
69-8	II	16	28	400	480
70-5	II	16	28	485	510
71-10	II	16	28	505	530
66-9	III	16	28	470	520
69-14	III	16	28	410	485
62-16	III	16	28	475	530
74-7	III	16	28	415	505

[a] I = three sided shelter, II = open shade, III = air conditioned house

The 14 percent protein ration that was fed to the boars is shown in Table II. The boars were fed six pounds per day of the ration, except for the boars in Lot II which were fed at a higher level during the winter months in order to maintain a more constant weight. All boars were fed in individual feeding stalls. Water was provided ad libitum, by means of automatic waterers.

TABLE II

BOAR RATION

Ingredient	lbs/ton
Milo	1592
Soybean Meal	208
Tankage	50
Alfalfa Leaf Meal	100
Dicalcium Phosphate	25
Calcium Carbonate	10
Trace Mineral Mix	10
Premix	4.0

Semen collections were made three times weekly (Monday, Wednesday, and Friday) in an attempt to simulate production conditions, and reduce the variation between ejaculates that may be associated with less frequent collections. Each boar was collected by the same person whenever possible in an attempt to standardize conditions. The boars were trained to mount a padded, canvas covered, wooden breeding dummy for collection. Disposable plastic bags were placed over the end of the dummy to prevent contact of the boar's penis with the canvas so as to keep this area of contact sanitary. The ejaculation was stimulated by applying pressure with the hand upon the spiral portion of the penis (as described by Hancock and Hovell, 1959). This method, when properly carried out, is very effective and eliminates the use of the artificial vagina.

As each ejaculate was collected, it was strained through four layers of gauze into a 600 milliliter beaker to remove the gelatinous material which was discarded. Strained volume was the only data recorded for the ejaculates from the first collection day of each week. A 25 milliliter subsample was obtained from the ejaculates of six boars during the second collection day, and from the remaining six boars during the last collection day of each week. The test tubes containing the subsamples were placed in an insulated chest, and taken to a laboratory in the Veterinary Medicine Building as soon as possible after collection to be analyzed.

At the laboratory the subsamples were removed from the chest and placed in a water bath maintained at a constant temperature of 37° C. The following laboratory evaluations were carried out:

1. Motility estimates were made by subjectively estimating the percent of sperm cells that were motile in the undiluted semen. Two drops of the undiluted semen were placed on a warm microscope slide, swirled with a stirring rod, then covered with a coverslip, and immediately observed under 100 power magnification.

2. Spermatozoa concentration was estimated by means of a hemocytometer. The semen was diluted with a 3.2 percent sodium citrate solution in a red blood cell pipette at a ratio of one part semen to 100 parts of diluter. The cells were evenly dispersed by means of a mechanical

shaker. The first few drops from the pipette were discarded. then the two chambers on a hemocytometer were filled. The total number of spermatozoa in the five diagonal squares in each chamber were counted and avaraged. Sperm concentration was calculated as the average of the counts obtained and converted to a concentration per milliliter.

3. Total spermatozoa per ejaculate was calculated as the product of the concentration per milliliter and the strained volume of the semen in milliliters.

4. The percent of abnormal spermatozoa was estimated from the observation of 100 stained cells from slides prepared by the technique described by Wagelie et al., (1959), utilizing carbol fuchsin stain. Morphological forms were categorized on a multiple tally machine as normal, bent tails, coiled tails, broken necks, tailless heads, abnormal heads and cytoplasmic droplets. This semen criteria was not recorded until the first part of April, 1967.

The daily maximum and minimum temperatures were available from instruments located one mile from the boar pens.

Trial II

This trial began June 1, 1968, and terminated November 30, 1968. The same facilities were used in this trial that were used in Trial I. Nine purebred Yorkshire boars were used in this trial. Boar no. 69-10 died during

the third week of July. The identification numbers, lot number, initial and final age and weight of each boar are given in Table III.

TABLE III

DESCRIPTION OF THE BOARS USED IN TRIAL II

Boar No.	Lot No.[a]	Initial Age (Mo's)	Final Age (Mo's)	Initial Weight (lbs.)	Final Weight (lbs.)
62-15	I	31	37	490	485
63-5	I	31	37	570	600
69-10	I	31	37	485	---
71-10	II	31	37	520	510
69-8	II	31	37	485	490
70-5	II	31	37	515	520
69-14	III	31	37	490	500
74-7	III	31	37	505	505
66-9	III	31	37	540	575

[a] I = three-sided shelter, II = open shade with sprinkler, III = air-conditioned house

The boars in this trial were fed and managed in the same manner as were the boars in Trial I. The shelters in each lot were identical to those in Trial I except a sprinkler system was installed in the open shade in Lot II. The sprinklers were in use from June 21, 1968, to September 31, 1968. They were turned on by 9:00 o'clock each morning and were turned off by 7:00 o'clock every evening.

Daily temperatures were taken between two and four o'clock each afternoon by means of thermometers which were placed in similar shaded locations in each shelter. The daily maximum and minimum environmental temperatures were also recorded during this trial.

Collection procedures were identical to those carried out in Trial I with the exception that the boars were collected only on Monday and Friday of each week during this trial. This change was made because of the refusal of a number of boars to work consistently following several months of collecting three times a week.

The semen evaluations made were the same as in Trial I with the following exceptions:

1. Strained volume data was recorded twice each week, and the remaining data was recorded only once each week.

2. Subjective motility estimates were made at the collection barn as soon as the ejaculates were obtained. A drop of semen was placed on a microscope slide that had been heated to 37° C. by means of an electric slide warmer. Two drops of the extender described in Table IV were mixed with the semen on the slide. This was a modification of the Norman-Johnson (NJ-2) extender described by Johnson et al., (1968). It was added to allow the individual spermatozoa cells to be seen more easily. The number of motile spermatozoa was estimated and recorded as a percent of the total sperm present.

3. An estimate was also made of the rate of forward progress of the spermatozoa at the same time the motility estimate was made. A rating of one to five was given, as described by Wells, (1968), with one being no forward movement and five being very fast forward progress.

TABLE IV

COMPOSITION OF THE MODIFIED NJ-2 EXTENDER

Ingredient	Quantity per 100 ml. of extender
Sodium Citrate Dihydrate	2.16 gm
Calcium Chloride, Anhydrous	226 mg
Glucose	500 mg
Magnesium Chloride Hexahydrate	37 mg
Potassium Chloride	21 mg
Sodium Chloride	14 mg
Monosodium Phosphate Monohydrate	3 mg
Distilled water added to bring total to 100 ml.	

4. At the laboratory, motility estimates of each semen sample were made by the hemocytometer method as described by Brody and Gildow (1939). The semen was diluted 1:100 with the modified NJ-2 extender in one red blood cell pipette and with a 3.2 percent sodium citrate solution, which immobilized the spermatozoa in another pipette. Two separate hemocytometer counting chambers were then filled with each of these dilutions. The percent motility was determined by counting the number of non-motile sperm cells in the sample diluted with NJ-2 extender and subtracting this value from the total number of cells in the hemocytometer chamber containing the semen diluted with sodium citrate, in which all sperm cells had been immobilized. This value, which was an estimate of the

number of motile cells, was divided by the total number of cells and multiplied by one hundred to express the estimate of the percent motile cells. The sodium citrate solution was not effective in immobilizing the spermatozoa, so the procedure was slightly modified. The number of non-motile cells were counted in the pipettes containing the samples diluted with the NJ-2 extender, then the pipettes were placed in the freezer for five minutes to immobilize the spermatozoa. The total number of cells in the hemocytometer chamber were then counted and the procedure as explained above was followed to obtain an estimate of the percent motile cells. This method proved to be more satisfactory.

The monthly fluctuations of semen characteristics and changes in environmental temperature for each trial are graphically illustrated.

The analyses of variance for the effect of shelter modification on the various semen characteristics are somewhat complicated. The data was sorted so that only the days on which all boars in each respective treatment worked were considered. This type of analysis was used for evaluating the effects of shelter modification on all semen characteristics. Kramer's modification of Duncan's New Multiple Range Test was used in conjunction with the analyses of variance described above (Steele and Torrie, 1960). Simple correlations were used to determine the relationship between the various semen criteria.

CHAPTER IV

RESULTS AND DISCUSSION

Trial I

Collections

During the course of this 12 month study 1,752 individual collections were attempted, and 1,421 (81.0 percent) were successful. Successful collections per boar ranged from 59.3 percent to 95.0 percent of those attempted as presented in Table V. Ejaculates were obtained from 77.1, 76.1 and 89.2 percent of the collections attempted from the boars in Lots I, II, and III, respectively. An analysis of variance for percent successful collections showed that lot effect accounted for a major source of variation. Lot III (air-conditioned building) had a significantly ($P < .05$) higher percentage of successful collections than did either Lots I (three-sided shelter) or II (open shade). These results indicate that boars kept in a more sheltered environment tend to be more consistent in their response to collection (Figure 1). During the first part of this trial, all boars worked fairly consistently, but during the second half of the trial it became increasingly more difficult to obtain successful collections from all boars. The refusal of boars to mount the breeding dummy routinely during the

TABLE V

MEAN MEASUREMENTS FOR INDIVIDUAL BOARS IN TRIAL I

Boar No.	Lot No.	Percent motile sperm	Percent abnormal sperm	Volume (ml.)	Conc. per ml. x 10^6	Total sperm per ejac. x 10^9	Percent successful collections
62-15	I	82.3	10.3	193.6	247.0	47.44	59.3
69-7	I	76.9	11.1	169.9	187.3	33.00	68.4
63-5	I	68.3	10.3	298.4	177.9	54.35	88.6
69-10	I	65.0	14.9	272.3	185.5	50.49	92.2
69-8	II	62.3	10.3	126.3	321.6	39.52	71.8
70-3	II	57.0	10.6	126.7	296.3	38.40	59.3
70-5	II	44.2	10.6	178.5	188.5	34.03	85.7
71-10	II	55.4	9.1	204.2	255.4	50.36	87.4
69-14	III	45.6	12.7	166.3	249.3	44.56	92.1
74-7	III	69.7	19.9	203.8	231.0	46.52	95.0
62-16	III	70.5	9.7	225.6	196.1	41.67	81.0
66-9	III	79.7	9.5	217.4	220.2	44.73	88.8

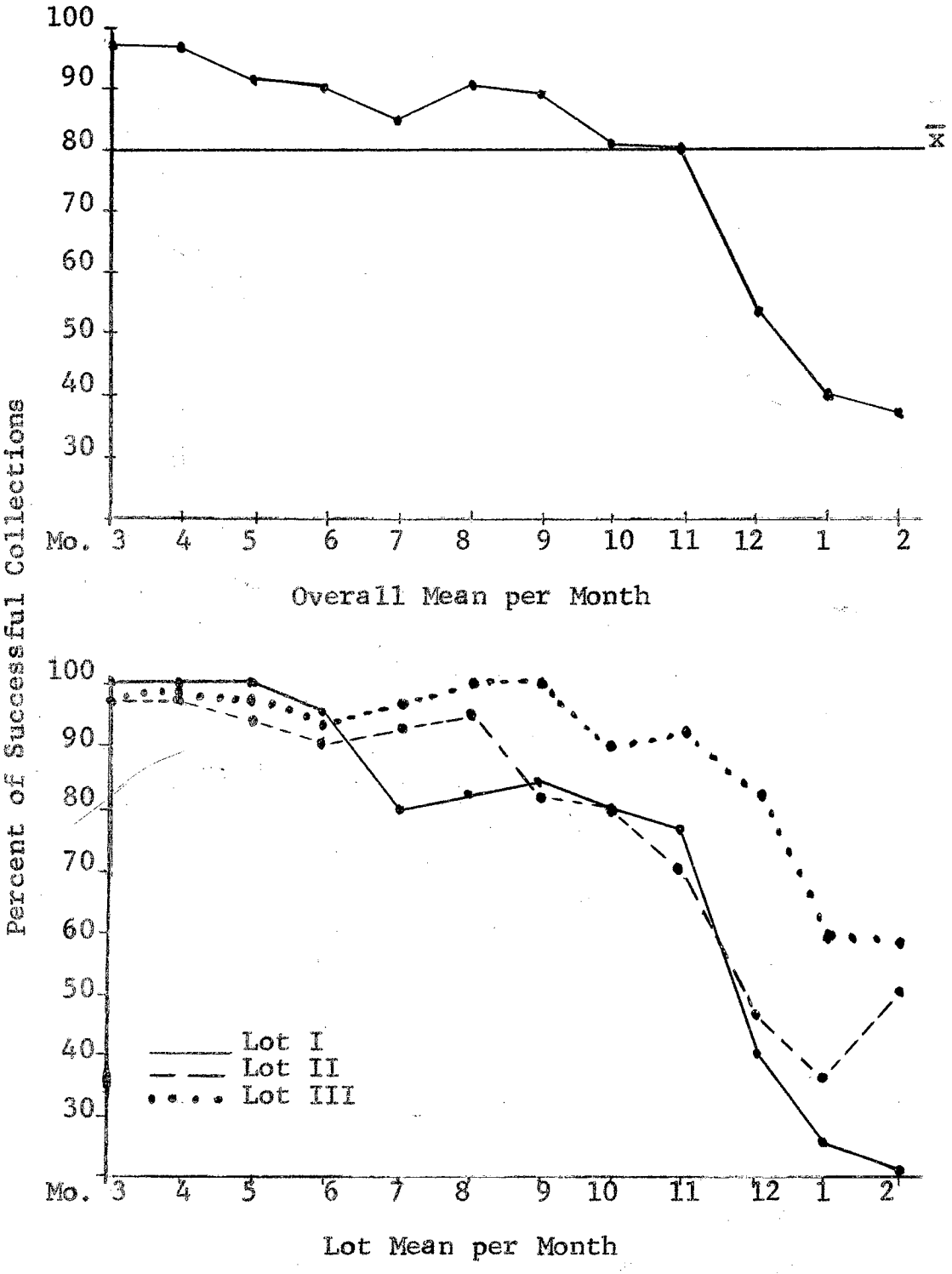

Figure 1. Pattern of Percent Successful Ejaculate Collections

second half of the experimental period has also been reported by Swierstra (1967). Collecting boars every other day may be too frequent if the boars are to be collected regularly for an extended period of time. The failure to obtain regular collections from trained boars have also been reported by Cox and Wilham (1961). The coldest environmental temperatures were during the latter part of this trial, and this also could have been a factor in the refusal of the boars to mount the breeding dummy during this period.

Semen Criteria

The number of observations, means, standard errors, and coefficients of variation for the semen characteristics used in this trial are given in Table VI. The means for the semen characteristics in this study are well within the ranges that have been established in the literature.

TABLE VI

POPULATION DESCRIPTION OF SEMEN (TRIAL I)

Semen Characteristics	N[1]	Mean ± S. E.	C.V. (%)
Strained volume (ml.)	1020	203.40 ± 2.06	32.4
Percent motile sperm	376	61.16 ± 1.44	45.7
Percent abnormal sperm	298	11.44 ± 0.50	74.5
Concentration/ml. x 10^6	376	220.35 ± 5.59	49.2
Total sperm/ejac. x 10^9	376	41.61 ± 1.00	46.4

[1]N = Number of observations used in Analysis of Variance

The average strained ejaculate volume of 203.4 ml. for the boars in this study is in close agreement with the 213 ml. value reported by Lawrence (1967) and the 205.5 ml. value reported by Gerrits et al., (1962). Turkheimer (1957) and Swierstra (1967) obtained somewhat lower values of 173 and 178 ml. respectively. Since they were using young Yorkshire boars their values are expected to be somewhat lower than the values obtained from more mature boars.

The average percent motile spermatozoa determined in this study was very close to the 58 percent reported by Turkheimer et al., (1958) and the 55 percent found by Lawrence (1967). Gerrits et al., (1962) however, obtained a higher value of 80.2 percent motile sperm.

Lawrence (1967) and Gerrits et al., (1962) reported 11.7 percent and 9.6 percent abnormal spermatozoa in boar semen, respectively, and this is comparable to the 11.4 percent value obtained in this trial.

The average spermatozoa concentration per milliliter found in this study was 220.4×10^6. This agrees very well with the value 213×10^6 obtained by Lawrence (1967) and 192×10^6 obtained by Gerrits et al., (1962). Borton et al. (1965) states that 150 to 250×10^6 spermatozoa per milliliter is the normal range for the average concentration of boar semen

Lawrence (1967) and Gerrits et al., (1962) found the value of total sperm per ejaculate to be 37×10^9 and 39.5×10^9, respectively, which is comparable to the 41.6×10^9

value found in this trial. Turkheimer (1958) found a somewhat higher value of 47 x 10^9 and Swierstra (1967) reported a lower value of 23 x 10^9.

Seasonal Effects

Semen quality values do not remain constant, but are found to fluctuate over a period of time. Environmental conditions, temperature, humidity, and type of shelter, are responsible for a large percentage of this variation in semen quality. The two part figures, Figure 2 through Figure 6 present the monthly patterns established for the semen criteria used in this study. Each point in the single lined graph of each pair of figures represents the average value for all 12 boars for each month irrespective of treatment. In the second figure of each pair, the average monthly values for the boars in each treatment group are plotted. The average value of the semen characteristics are presented in the first figure of each pair as a straight line on the X-axis. Figure 7 illustrates the average maximum and minimum daily temperature for each month during the trial.

Analyses of variance (Table VII) showed that the effect of time of year accounted for a major source of variation in each semen criteria studied. This was expected because other researchers have well established the fact that there is seasonal variation in the semen quality of boars. Table VIII shows which monthly averages

of all boars combined were signicantly different from other monthly averages as determined by Kramer's Modification of Duncan's New Multiple Range Test (Steel and Torrie, 1960). In the table all values not underlined by the same line are significantly differert from each other. The analyses of variance also indicated that there was a significant interaction between lots and time for all semen criteria. These interactions can be observed in Figure 2 through Figure 6.

In this study larger semen volumes were obtained during the cooler months of the year (see Figure 2). Semen volume was lower during the summer and early fall months (July-October) than during any other time during the year. These results agree very well with Erb et al., (1942) who found that in bulls the average semen volume was least in July, August, and September. Hafez et al., (1955) also found that the average semen volume of sheep was lowest during the summer months and highest during the spring and winter months. Okauchi and Kirakata (1962) reported that semen volume in boars is lowest during the seasons of maximum and minimum environmental temperatures.

Figure 3 shows that spermatozoa motility was highest during the early winter months and lowest during the late spring and summer months. Generally most researchers agree that initial spermatozoa motility is lowest during the warmer months and highest during the cooler months. Erb et al., (1942) reports that average initial motility in

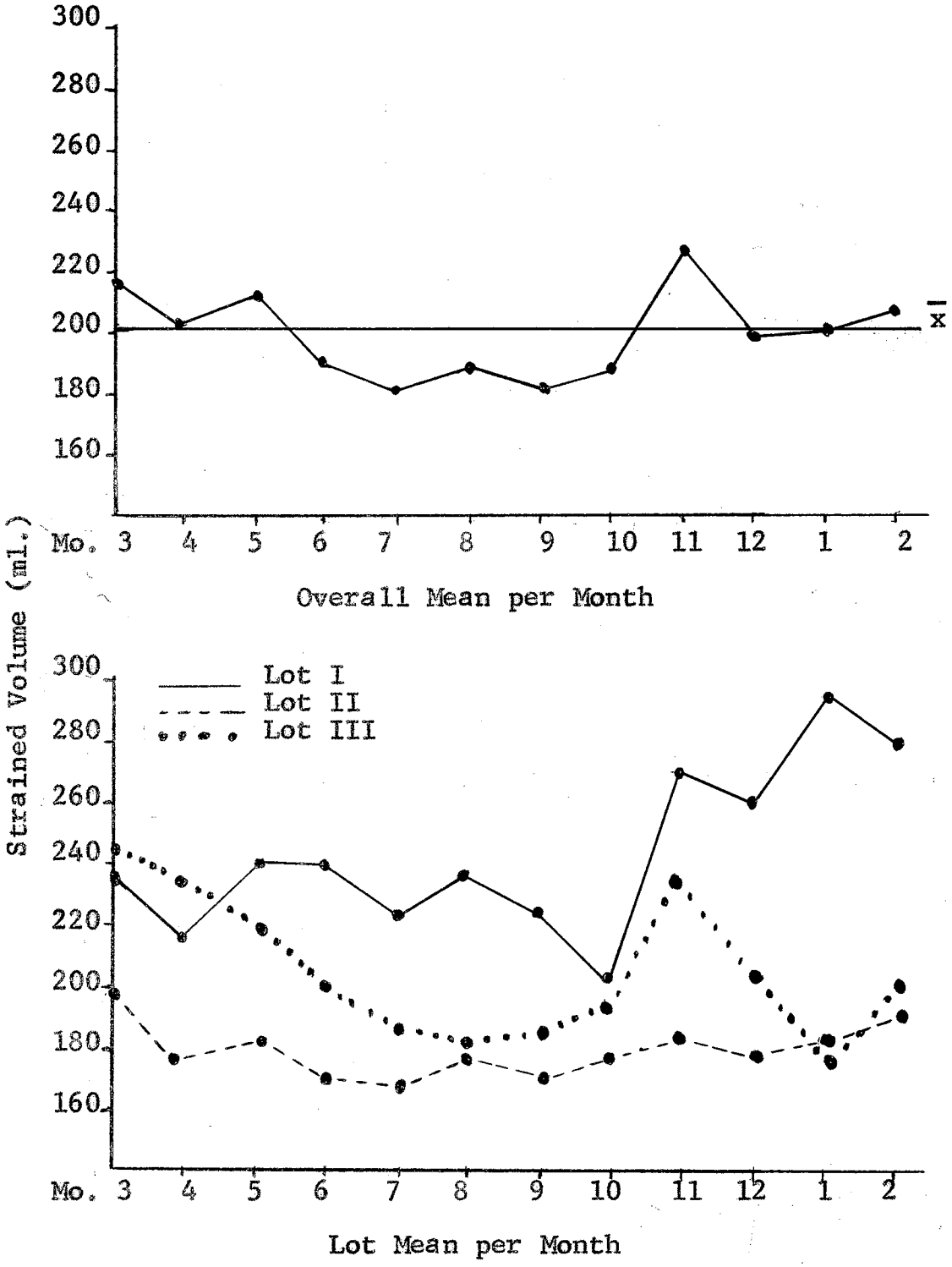

Figure 2. Pattern of Ejaculate Strained Volume

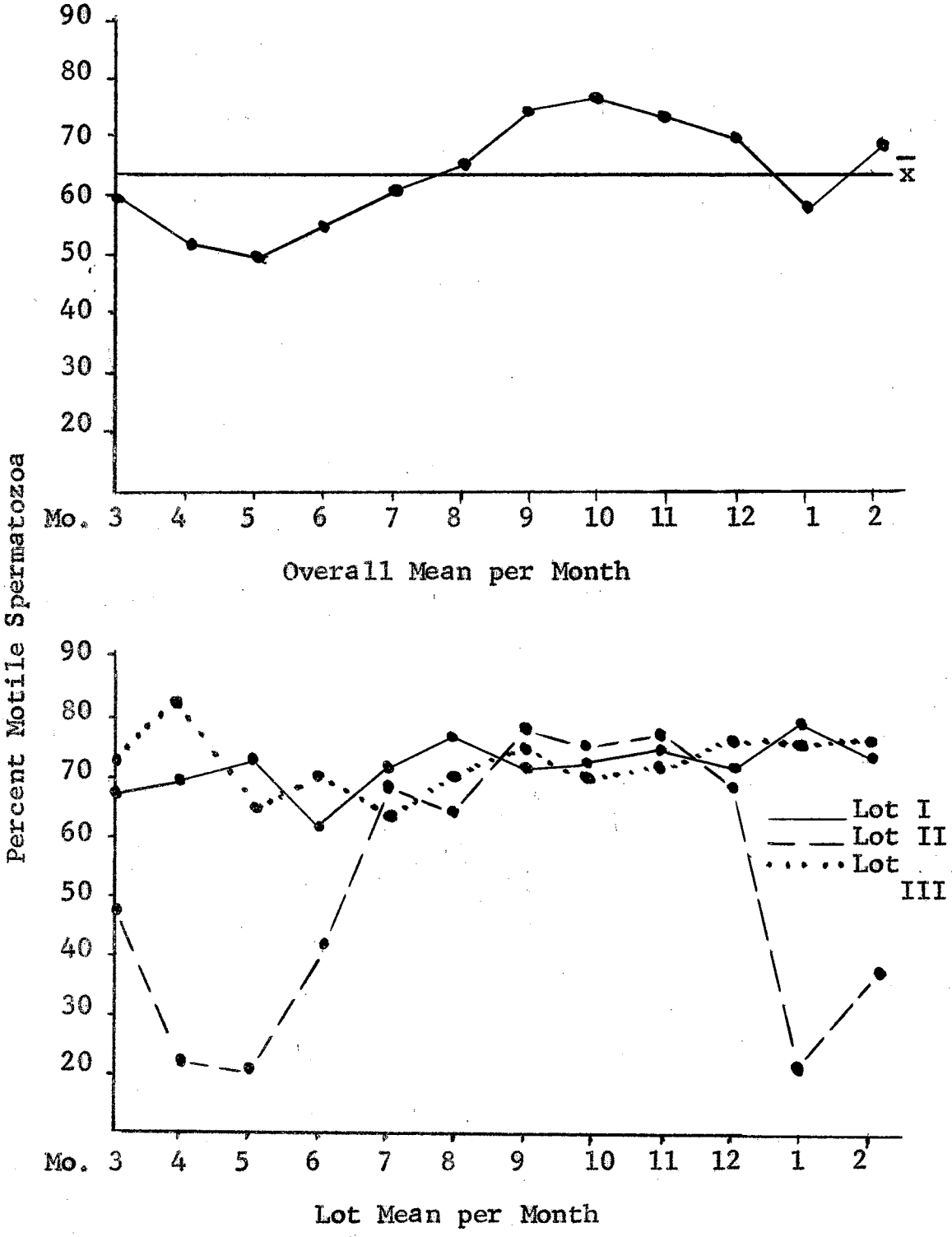

Figure 3. Pattern of Percent Motile Sperm

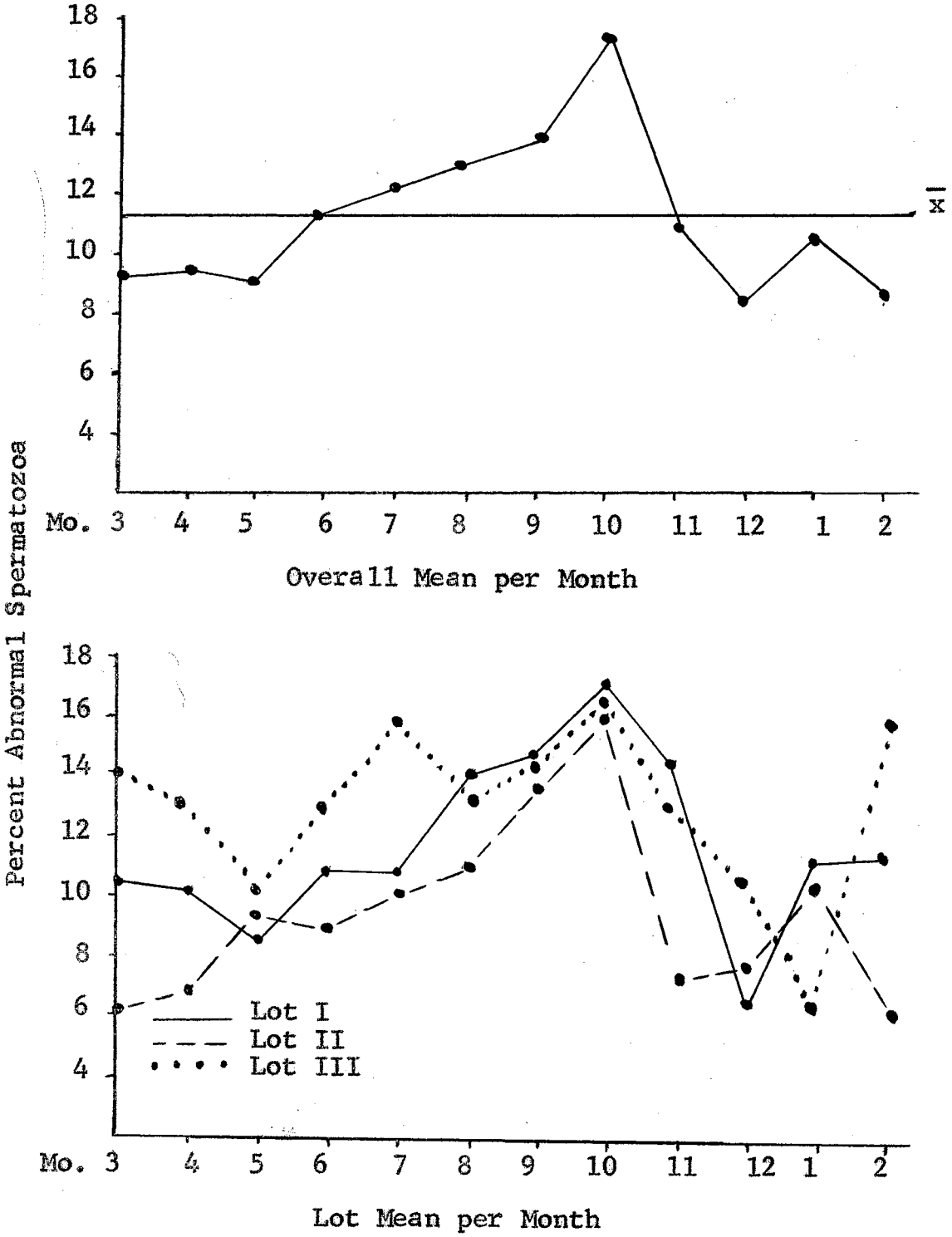

Figure 4. Pattern of Percent Abnormal Sperm

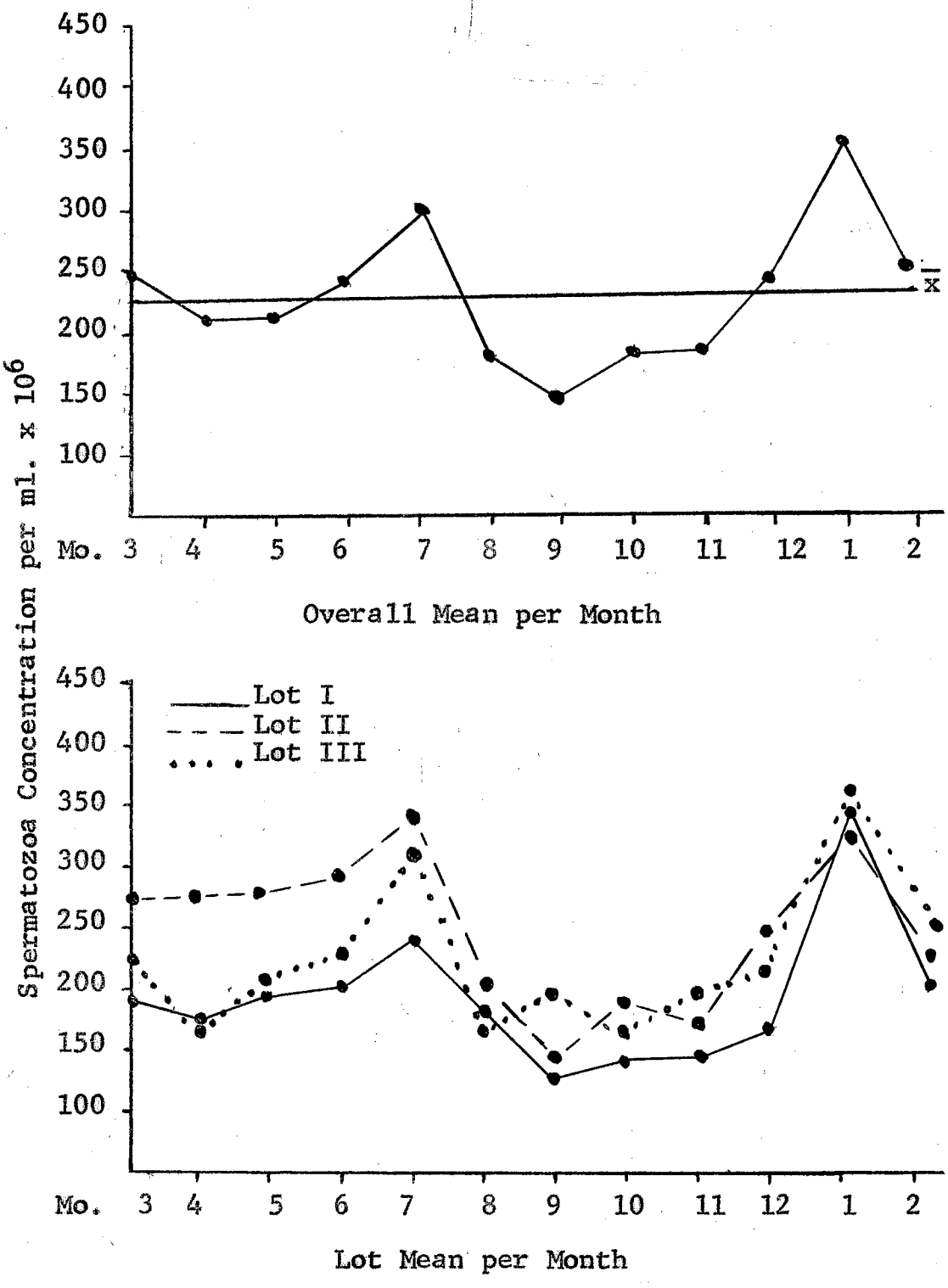

Figure 5. Pattern of Sperm Concentration Per ml. x 10^6

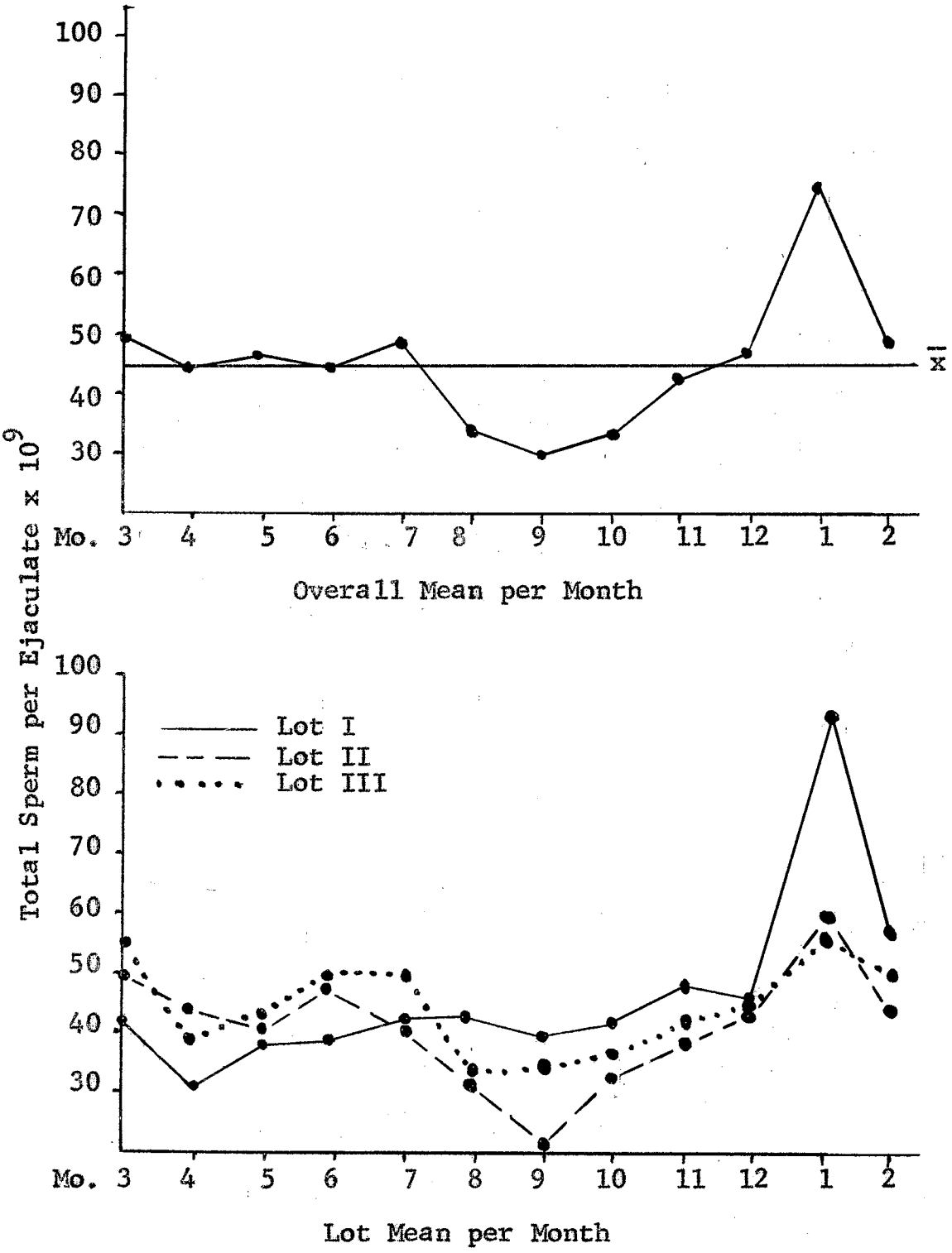

Figure 6. Pattern of Total Sperm per Ejaculate x 10^9

bulls was least in July, August, and September. The same trend is also shown in sheep by Cupps et al., (1960). The results of this trial, omitting the data from Lot II, are in agreement with most researchers. Lot II (open shade), however, exhibited the lowest initial motility during the early spring months. The boars in Lot II had the least protection from the environment, and the cold weather during the winter months may have been more detrimental to spermatozoa motility than the higher temperature during the summer. The motility values for all three lots were very similar during the summer months.

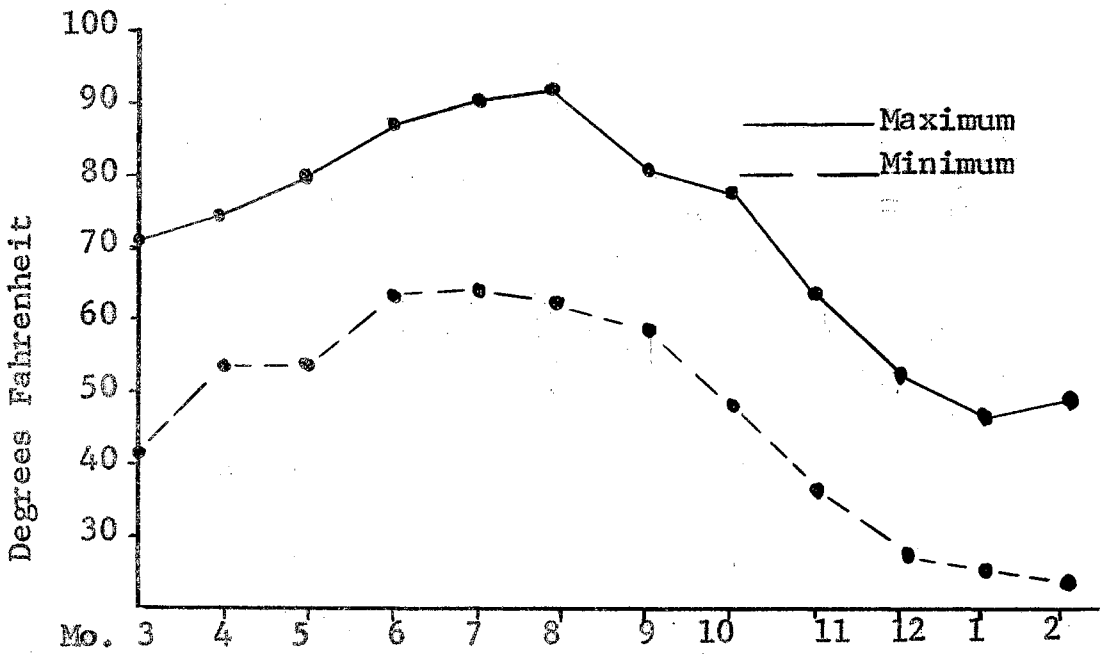

Figure 7. Average Maximum and Minimum Daily Temperatures per Month

TABLE VII

TABULATED ANALYSES OF VARIANCE OF TIME EFFECT ON SEMEN CHARACTERISTICS (TRIAL I)

Source	D.F.	Mean Square	F Value
Volume (ml.)			
Day in treatment	252	4327.0	2.03***
Error	756	2069.3	
Percent motile sperm			
Day in treatment	182	910.9	2.99***
Error	182	303.8	
Percent abnormal sperm			
Day in treatment	143	71.91	1.28***
Error	143	56.33	
Concentration/ml.			
Day in treatment	182	14548.34	3.04***
Error	182	4788.86	
Total sperm/ejac.			
Day in treatment	182	496.14	2.22***
Error	182	223.94	

*** Level of significance = $P < .001$

In this study, the average maximum ambient temperature was highest in August (Figure 7) and the percent abnormal spermatozoa was the highest in October (Figure 4), approximately eight weeks later. These results are comparable to those found in rams by Cupps *et al.*, (1960) and in bulls by Erb *et al.*, (1942). However, Lawrence (1967) at this same station found the percent abnormal spermatozoa to be the highest during December. Heat and other forms of stress, such as exposure to irradiation, seems to have a latent effect on the semen qualities of most male mammals. Skinner and Louw (1966) have shown that when bulls

TABLE VIII

DUNCAN'S NEW MULTIPLE RANGE TESTS[1] OF MONTHLY MEANS FOR SEMEN CRITERIA (TRIAL I)

Strained Semen Volume

Month:	July	Sept	Oct	Aug	June	Dec	Jan	Apr	Feb	Mar	Nov	May
Value:	184	184	189	190	197	203	204	206	213	221	229	248

Percent Motile Sperm

Month:	May	Apr	June	Jan	Mar	July	Aug	Feb	Dec	Oct	Sept	Nov
Value:	50	56	57	58	60	64	65	69	70	72	74	75

Percent Abnormal Sperm

Month:	Dec	Feb	May	Apr	Jan	Nov	June	July	Aug	Sept	Oct
Value:	8.1	9.4	9.4	9.6	10.8	11.1	11.3	12.4	12.7	14.0	16.8

TABLE VIII (CONTINUED)

Concentration/ml.[2]

Month:	Sept	Nov	Oct	Aug	Apr	May	Feb	Mar	Dec	June	July	Jan
Value:	153	171	176	184	213	214	232	234	237	238	307	363

Total Sperm/ejaculate[3]

Month:	Sept	Oct	Aug	Apr	May	Nov	June	Dec	July	Feb	Mar	Jan
Value:	30.8	33.9	35.4	40.0	40.9	43.5	45.6	46.1	47.1	48.6	49.6	72.6

[1] Kramer's modification for unequal numbers (Steel and Torrie, 1960).

[2] $\times 10^6$

[3] $\times 10^9$

were exposed to heat stress of 40° C., it was approximately four weeks later before the maximum symptoms were observed. Ladd and Murphee (1964) and Willham and Cox (1961) conducted irradiation experiments with boars, and they report this latent period to be eight or nine weeks. The percent abnormal spermatozoa seems to be more sensitive to temperature than are the other semen characteristics. This has also been reported in sheep by Simpson and Dutt (1958).

The values for sperm concentration per milliliter and total sperm per ejaculate observed in this study are in agreement with most researchers. Two peaks of semen production were noticed; one during June and July and one during December and January (Figures 5 and 6). Since there is a negative relationship between sperm concentration and volume, the values obtained during these two periods were to be expected. Stevermer (1961) found the greatest sperm production in boars to be during June, July, and August. Lawrence (1967), however, found the highest peak to be in December and January. If his study had encompassed a complete year, he might also have found another peak sperm production during the summer months.

Lot Effect

The lot effect on each semen criteria was evaluated by analyses of variance for a completely randomized experiment, however, the data was sorted so that only complete data for each lot was used in the analysis. The results of

these analyses are tabulated in Table IX. Duncan's New Multiple Range Test was used to locate the differences between lots. The results of the multiple range tests are presented in Table X.

The shelters in each lot were designed to be applicable to production conditions and therefore temperature, humidity, and light were not strictly controlled. The air-conditioned house in Lot III provided the most protection from environmental extremes, while the open shade in Lot II provided the least protection. It was hypothesized that the boars in Lot III would be under less environmental stress than the boars in the other two lots and would therefore exhibit the highest semen quality. The semen criteria averages for each lot are presented in Table X. The analyses of variance indicate that of all the semen characteristics involved, only percent motile cells and sperm concentration per milliliter were significantly affected by the lot treatments. Analyses of variance also showed that there was a significant amount of variation between boars in the same treatment.

The boars in Lot III were kept cooler in the summer and warmer in the winter than were the boars in the other lots, however, the overall semen quality was higher for the boars housed in the three-sided shelter. These results are in agreement with those reported by Lawrence (1967). During the hot summer months, the temperature was maintained at cooler temperatures in the lot with the air-

TABLE IX

TABULATED ANALYSES OF VARIANCE OF LOT EFFECT ON SEMEN CHARACTERISTICS (TRIAL I)

Source	D.F.	Mean Square	F Value
Volume (ml.)			
Between lots	2	258978.35	1.87
Error	9	138293.41	
Percent motile sperm			
Between lots	5	11117.98	4.05*
Error	6	2743.43	
Percent abnormal sperm			
Between lots	5	367.15	1.58
Error	6	232.57	
Concentration/ml.			
Between lots	5	156797.77	8.51**
Error	6	18434.14	
Total sperm/ejac.			
Between lots	5	346.97	0.31
Error	6	1133.92	

* Level of Significance = $P < .10$
** Level of Significance = $P < .05$

TABLE X

DUNCAN'S NEW MULTIPLE RANGE TESTS OF LOT MEANS FOR SEMEN MEASUREMENTS (TRIAL I)

Semen Criteria	Lot I	Lot II	Lot III
Volume (ml.)	233.6^{a1}	158.9^{a}	203.2^{a}
Percent motile sperm	73.13^{a}	54.72^{b}	66.39^{ab}
Percent abnormal sperm	11.63^{a}	10.15^{a}	12.95^{a}
Concentration/ml x 10^6	199.40^{a}	265.50^{b}	224.20^{ab}
Total sperm/ejac. x 10^9	46.32^{a}	40.58^{a}	44.35^{a}

[1]Lot means of same measurement with the same superscript do not differ significantly ($P < .05$).

conditioned building but the boars in Lot I had much more ventilation and air movement. This could possibly be a factor which caused the boars in Lot I to exhibit the highest overall semen quality. Dutt and Bush (1955) reported that semen from rams kept in air-conditioned buildings at 45° F. did not show the marked decrease in motility of cells or an increase in percent abnormal cells which was found in the control rams during the summer months. The air-conditioned building that they used, however, was kept much cooler in the summer than was the building used in this trial. If the air-conditioned building had been maintained at a cooler temperature, the boars may not have exhibited the depressed semen qualities during the summer months. Simpson and Dutt (1958) and Dutt and Hamm (1957) observed that when rams were exposed to increased temperatures, semen qualities declined.

Interrelationships of Semen Criteria

Table XI shows the simple correlations between the semen criteria studied in this trial. These correlations were pooled for the complete trial and the variance due to the time of the year was removed.

Sperm concentration and total sperm per ejaculate were found to be closely associated with one another ($r = 0.698$). Semen volume was also significantly associated with these two semen criteria. These relationships are to be expected since total sperm per ejaculate is calculated as the product

TABLE XI

CORRELATION COEFFICIENTS BETWEEN SEMEN CHARACTERISTIC MEASUREMENTS (TRIAL I)

	Percent motile sperm	Percent abnormal sperm	Concentration	Total Sperm
Volume	.12	.03	-.35**	.33**
Percent motile sperm		-.22**	.02	.08
Percent abnormal sperm			.05	.04
Concentration per ml.				.70**

**Level of significance = $P < .01$

of semen volume and sperm concentration per milliliter. The significant negative correlation between percent motile sperm and percent abnormal sperm was also expected, because sperm motility is associated with high semen quality, and abnormal sperm with low semen quality. The correlations obtained in this study are in excellent agreement with Lawrence (1967). However, since these semen criteria are not very highly correlated with each other, it would appear necessary that each measurement must be individually determined in order to get the best estimate of semen quality.

Trial II

Collections

During the course of this six month trial, 383 individual collections were attempted and 309 (80.7 percent)

were successful. The successful collections per boar ranged from 41.0 to 95.3 percent of those attempted as presented in Table XIII. Ejaculates were obtained from 93.8, 78.2 and 72.3 percent of the collections attempted from the boars in Lots I, II, and III, respectively. An analysis of variance for percent successful collections showed that lot effect accounted for a major part of the variation. Lot I had a significantly higher ($P<.01$) percentage of boars that were successfully collected than either of the other lots. Lot III included one boar which failed to work consistently, thus lowering the pen average greatly. This boar was crippled for several weeks during the trial, which may account for his refusal to mount the breeding dummy. Lot III had the highest percentage of boars that were successfully collected in Trial I, however. It was noted in this trial that all boars worked more consistently during the first half of the trial than they did during the second half. This was also observed in Trial I. The variation between months was not as drastic in this trial as it was in Trial I. (Figures 1 and 8). This may be due to the fact that Trial II was not conducted during the extremely cold months. In Trial I the boars mounted the breeding dummy less consistently during the colder months. The boars in Trial II were only collected twice a week as compared to the three times weekly collection in Trial I. It may very well be that boars collected less frequently may be collected more consistently over a

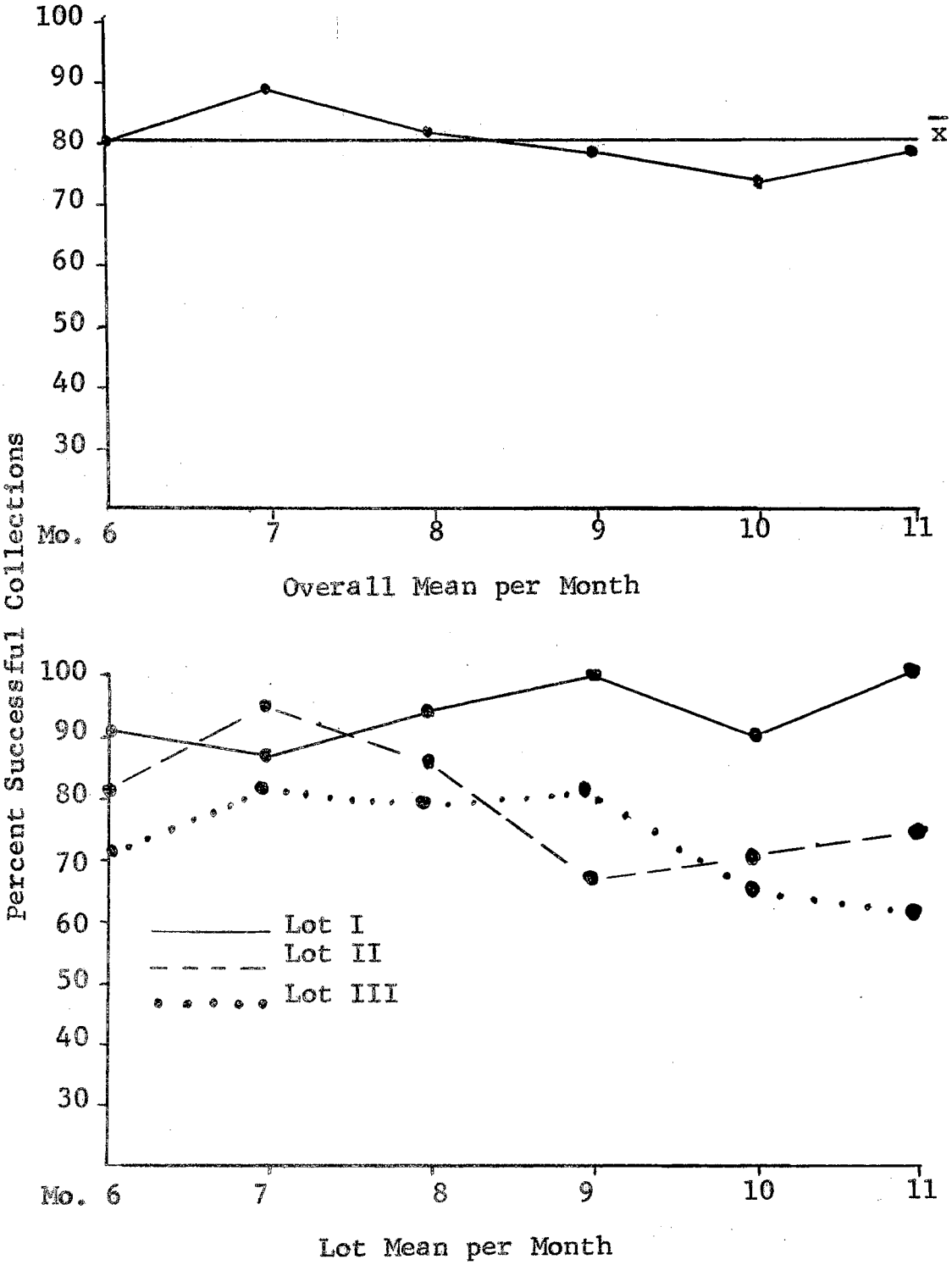

Figure 8. Pattern of Percent Successful Ejaculate Collections

period of time than boars that are collected every other day.

Semen Criteria

The number of observations, means, standard errors, and coefficients of variation for the semen characteristics used in this trial are given in Table XII. All the semen criteria evaluated in this trial are somewhat higher than those values obtained in Trial I, however, these values are still well within the normal ranges.

TABLE XII

POPULATION DESCRIPTION OF SEMEN (TRIAL II)

Semen Characteristics	N[1]	Mean ± S. E.	C.V. (%)
Volume	189	232.99 ± 7.74	45.6
Percent Motile Sperm (S)[2]	99	71.01 ± 1.46	20.5
Percent Motile Sperm (H)[3]	92	77.55 ± 1.42	17.6
Rate of Forward Movement	93	3.19 ± 0.09	8.9
Percent Abnormal Sperm	98	13.75 ± 1.13	80.9
Concentration/ml. x 10^6	99	265.05 ± 12.99	48.9
Total Sperm/Ejac. x 10^9	99	55.21 ± 2.19	39.3

[1]N = No. of observations used analysis of variance.

[2]S is subjective method

[3]H is hemocytometer method

Two other methods of assessing spermatozoa motility were utilized in this trial besides the conventional sub-

TABLE XIII

MEAN MEASUREMENTS FOR INDIVIDUAL BOARS IN TRIAL II

Semen Characteristics	Boar No.							
	62-15	63-5	69-8	71-10	10-5	69-14	74-7	66-9
Lot No.	I	I	II	II	II	III	III	III
Percent motile sperm (S)[1]	79.8	75.5	78.3	60.2	71.5	59.5	72.8	67.5
Percent motile sperm (H)[2]	79.7	77.2	79.0	66.0	72.5	63.6	79.4	82.4
Rate of forward progress	3.75	3.22	3.80	2.62	3.13	2.55	3.47	3.12
Percent abnormal sperm	7.7	13.8	7.6	19.1	12.3	25.4	12.4	8.5
Volume (ml.)	205	368	135	218	208	169	212	216
Concentration per milliliter x 10^6	266	182	421	248	220	386	303	265
Total sperm per ejaculate x 10^9	49.7	65.5	55.0	53.5	45.9	60.9	68.8	53.9
Percent successful collections	92.2	95.3	54.8	88.0	91.7	89.7	85.8	41.0

[1] S = Subjective

[2] H = Hemocytometer

jective estimate method. These two methods were the hemocytometer method and subjective method of estimating the rate of forward movement of sperm in undiluted semen. These methods are explained in detail in the materials and methods section of this thesis.

Seasonal Effects

The two part figures, Figure 9 through 15, present the monthly patterns established for the semen criteria used in this study. Each point in the single lined graph of each pair of figures represents the average value for all eight boars for each month, irrespective of treatment. The average value of each semen characteristic is presented in the first figure of each pair as a straight line on the X-axis. In the second figure of each pair, the average monthly values for the boars in each treatment group are plotted. Figure 14 shows the average maximum and minimum daily temperature for each month during this trial.

The time of the year was found to be a major source of variation in semen characteristics in this trial also. Table XIV shows the combined analyses of variance for time effect and Table XV shows which monthly averages were significantly different from each other as determined by Kramer's Modification of Duncan's New Multiple Range Tests, (Steel and Torrie, 1960). All values not underlined by the same line are significantly different from each other.

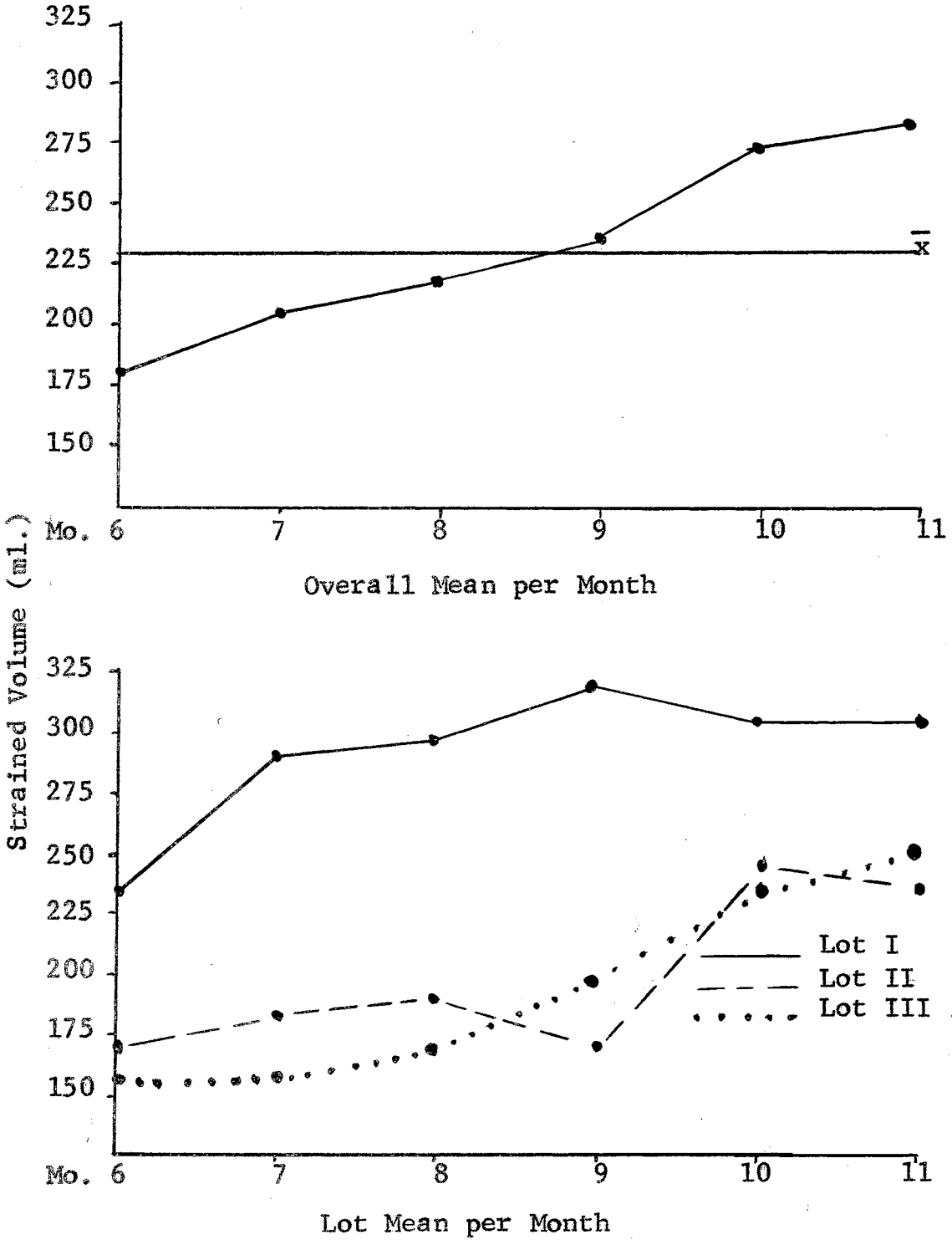

Figure 9. Pattern of Ejaculate Strained Volume

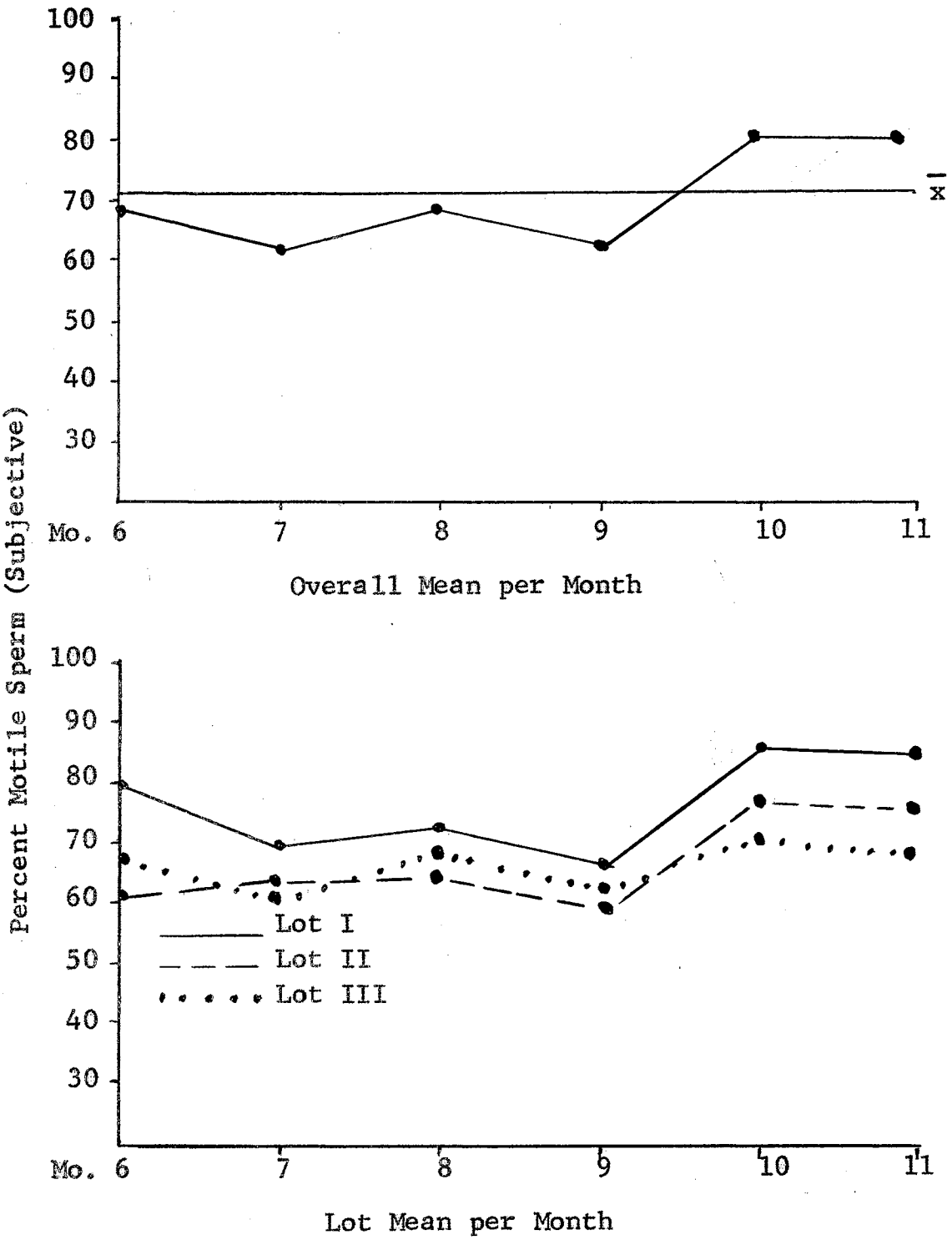

Figure 10. Pattern of Percent Motile Sperm (Subjective)

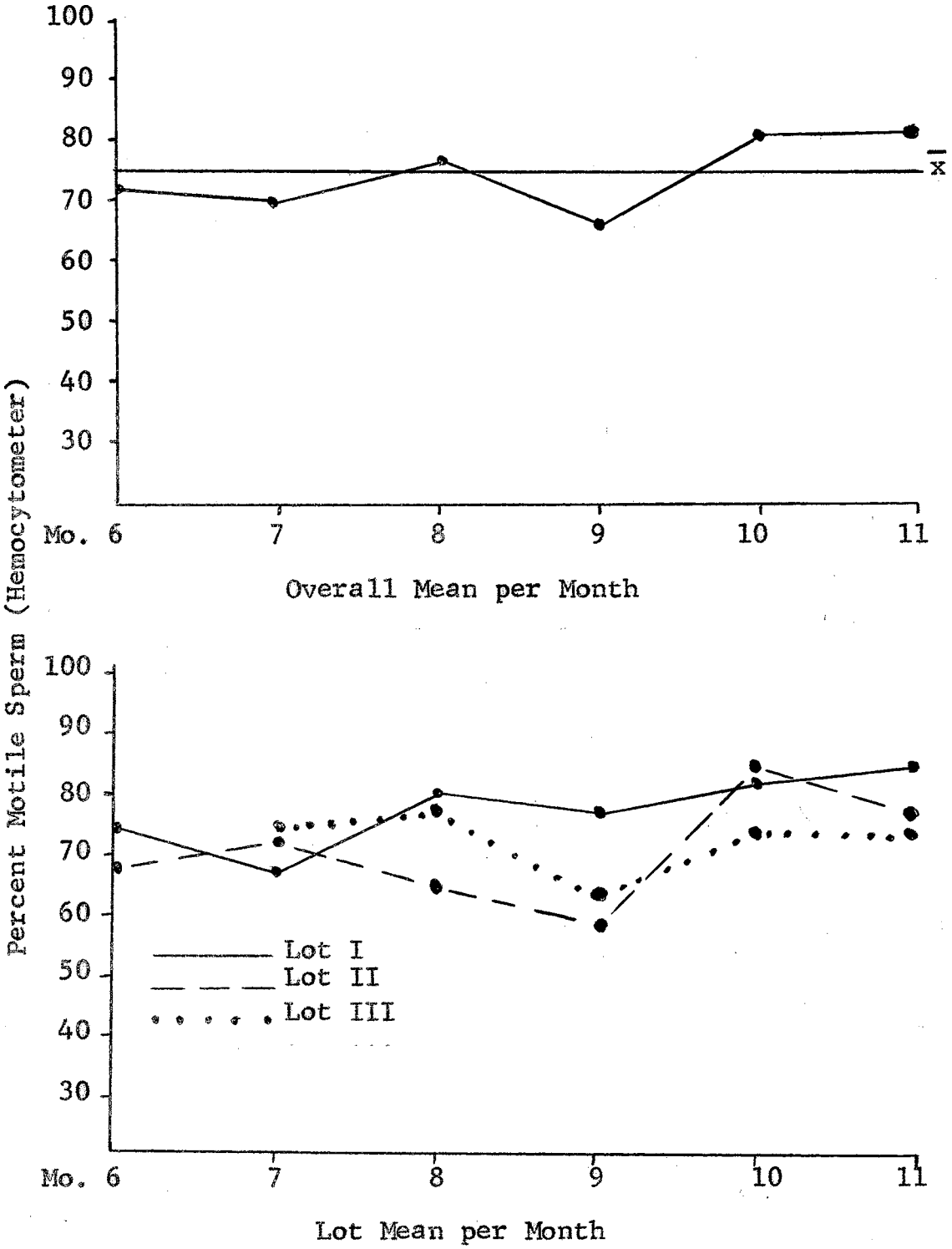

Figure 11. Pattern of Percent Motile Sperm (Hemocytometer)

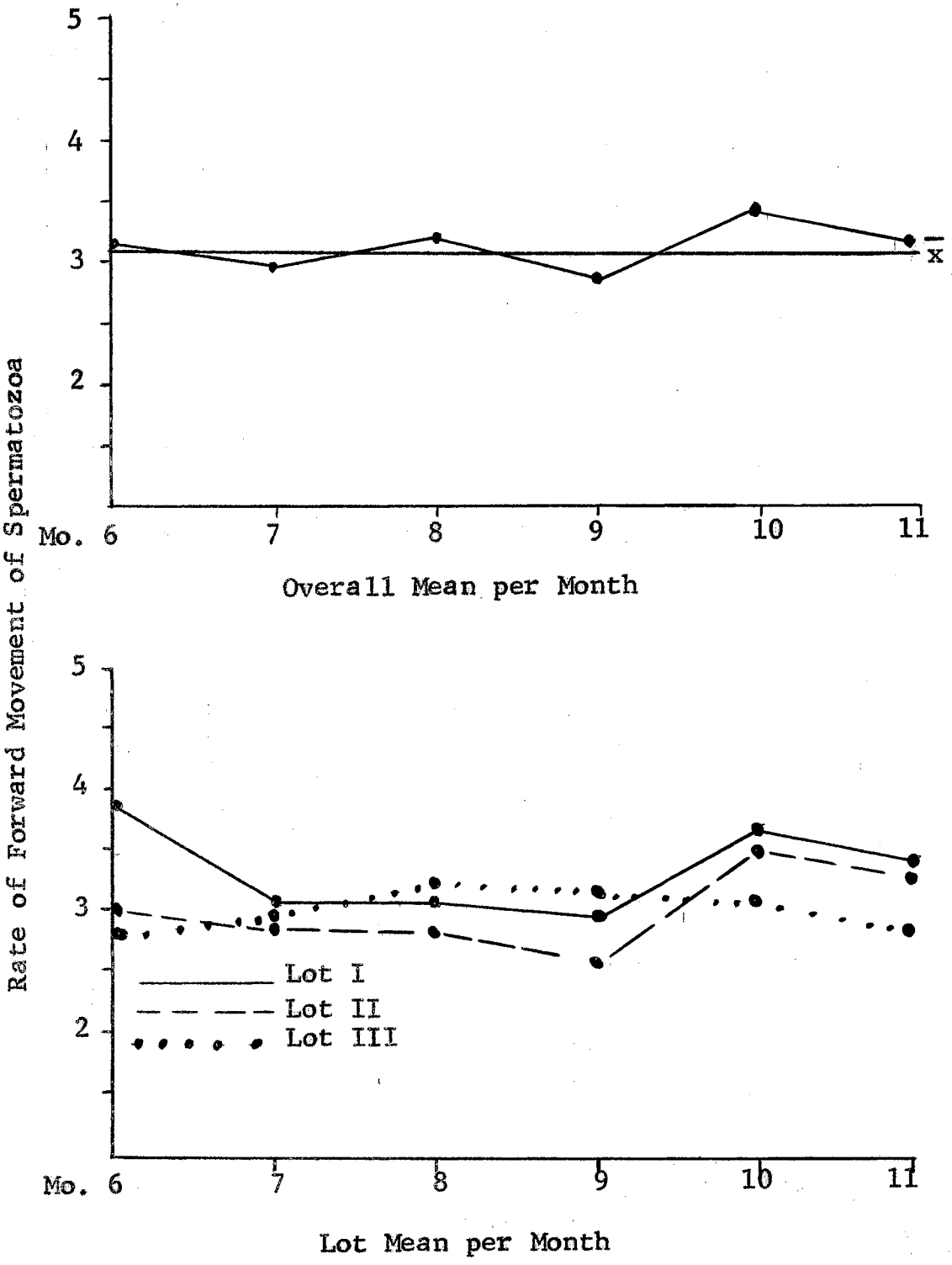

Figure 12. Pattern of Rate of Forward Movement of Spermatozoa

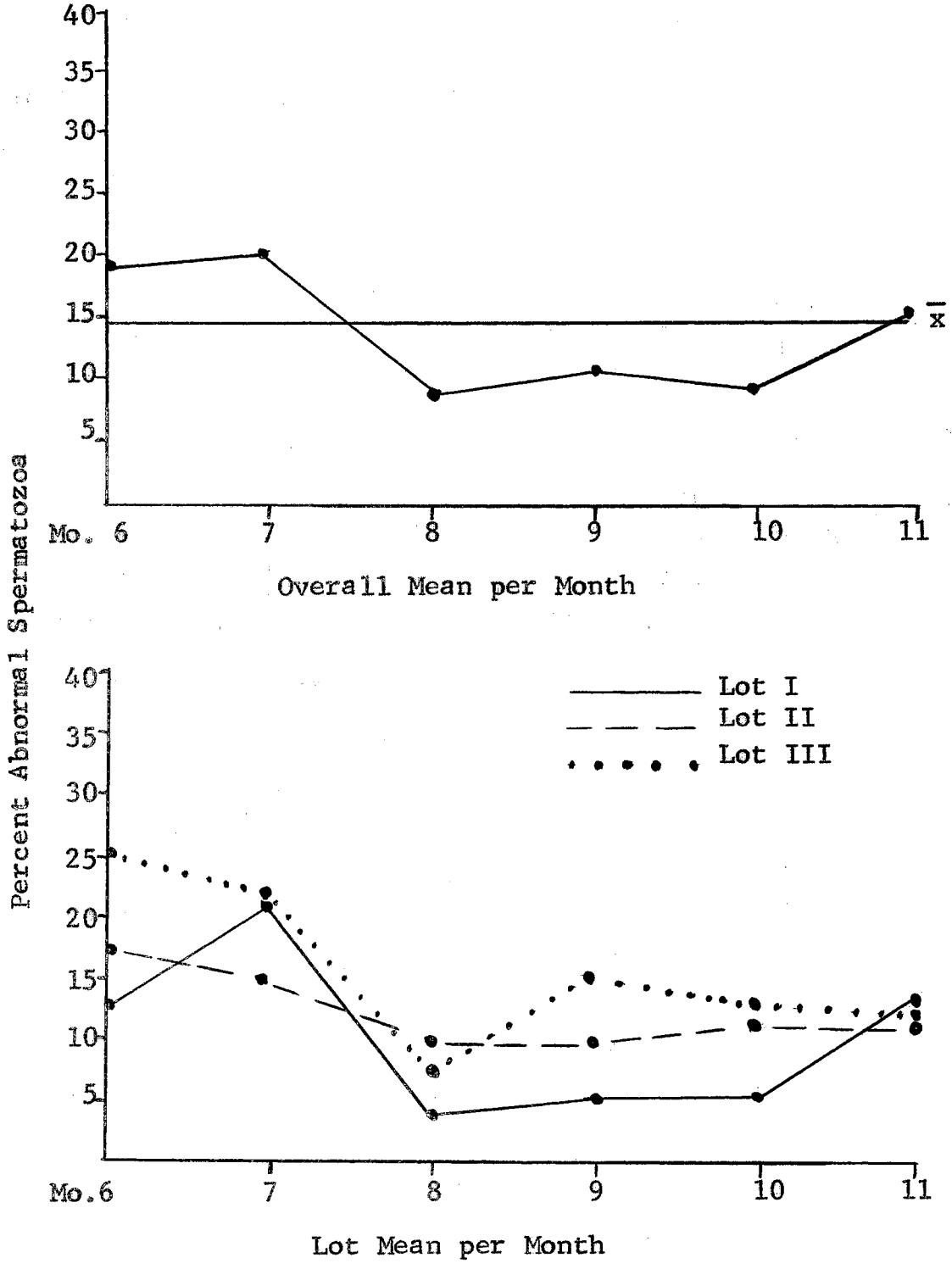

Figure 13. Pattern of Percent Abnormal Sperm

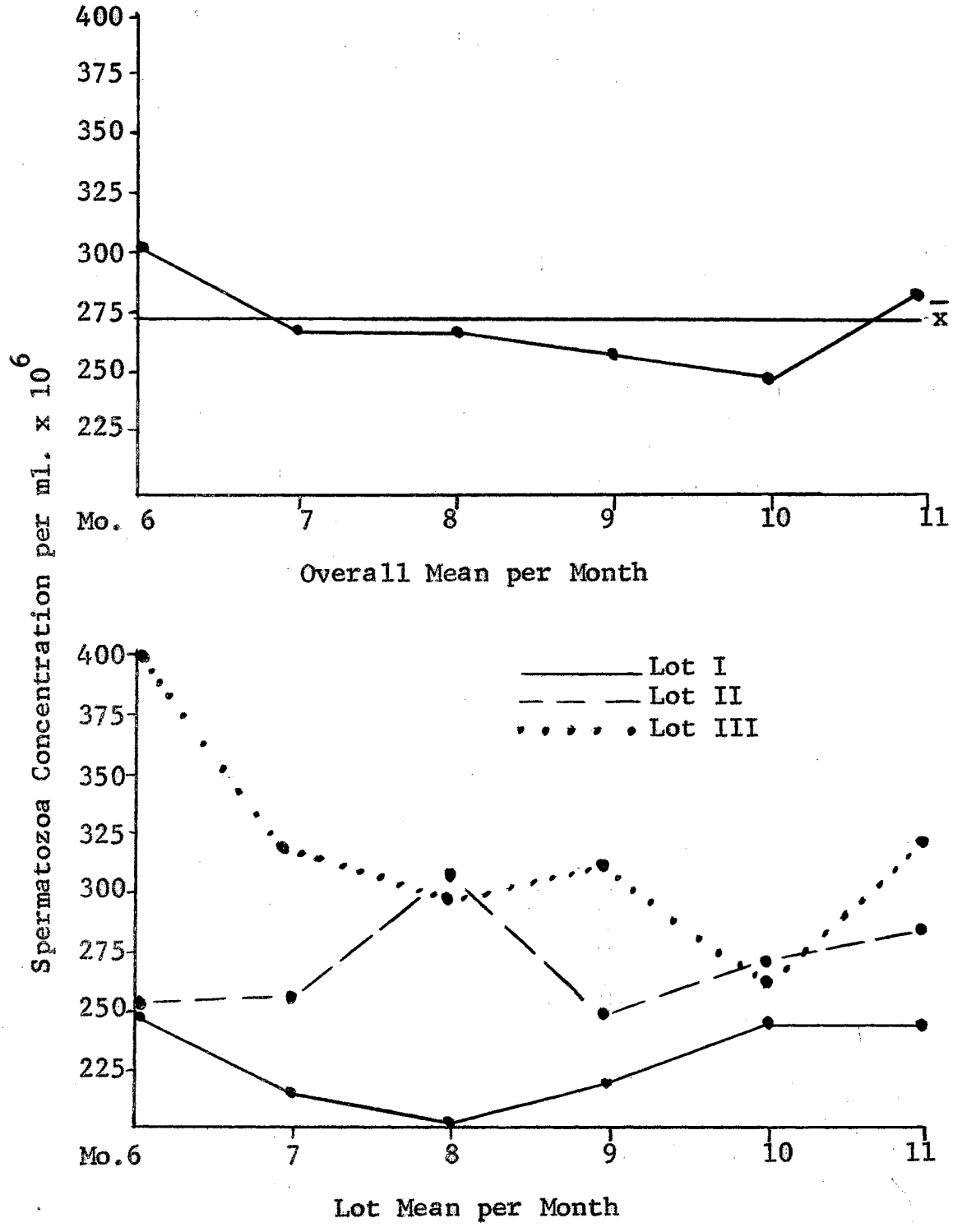

Figure 14. Pattern of Sperm Concentration per ml. x 10^6

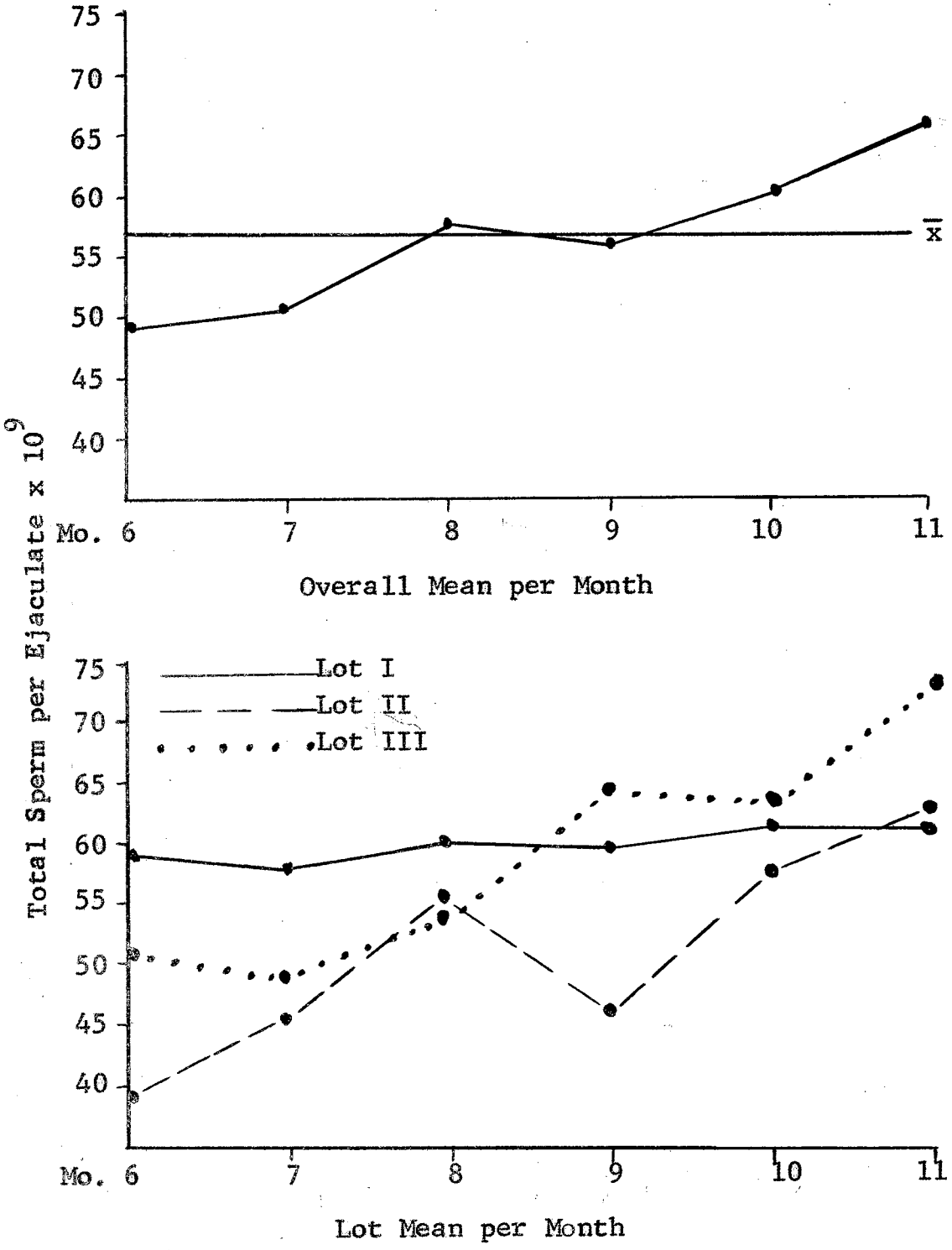

Figure 15. Pattern of Total Sperm per Ejaculate x 10^9

This trial agrees very well with Trial I concerning the effect of time on semen criteria fluctuations. Generally, the months that exhibited the highest values in Trial I were the months that showed the highest values in Trial II.

In this trial, significantly larger semen volumes were obtained during October and November than any other time during the trial (Table XV). This is in agreement with the results obtained in Trial I and also with other workers.

Figures 10 and 11 show that the percent motile sperm was lowest during the summer months and higher during the fall months. These results are the same as those found in

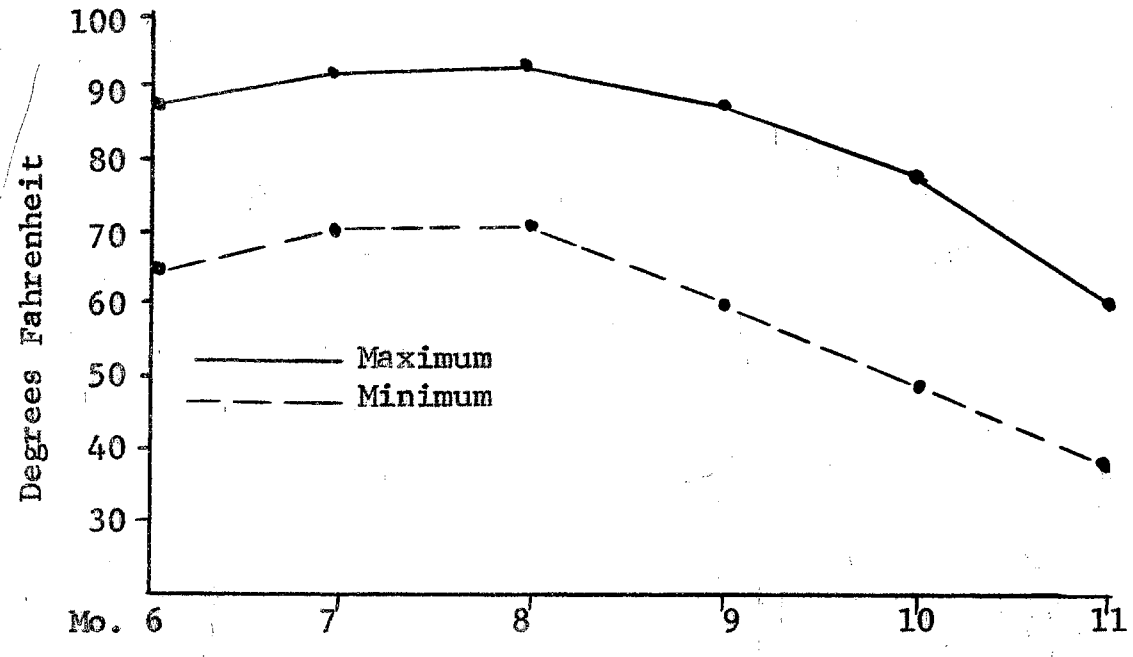

Figure 16. Average Maximum and Minimum Daily Temperatures per Month

TABLE XIV

TABULATED ANALYSES OF VARIANCE OF TIME EFFECT ON SEMEN CHARACTERISTICS (TRIAL II)

Source	D.F.	Mean Square	F Value
Volume (ml.)			
Day in treatment	74	6115.53	1.28***
Error	107	4768.35	
Percent motile sperm (S)[1]			
Day in treatment	36	257.92	2.45***
Error	55	105.50	
Percent motile sperm (H)[2]			
Day in treatment	34	216.30	1.46*
Error	50	147.70	
Rate of forward progress			
Day in treatment	34	0.80	1.51*
Error	51	0.53	
Percent abnormal sperm			
Day in treatment	36	111.38	1.67**
Error	54	66.83	
Concentration/ml.			
Day in treatment	36	15364.21	1.90**
Error	55	8107.62	
Total sperm/ejac.			
Day in treatment	36	635.43	1.69**
Error	55	375.29	

[1] S = Subjective method

[2] H = Hemocytometer method

* Level of Significance = $P < .10$

** Level of Significance = $P < .05$

*** Level of Significance = $P < .01$

TABLE XV

DUNCAN'S NEW MULTIPLE RANGE TESTS[1]
OF MONTHLY MEANS FOR SEMEN
CRITERIA (TRIAL II)

Strained Semen Volume

Month:	June	July	Aug	Sept	Oct	Nov
Value:	183	211	217	232	259	266

Percent Motile Sperm (Subjective)

Month:	Sept	July	Aug	June	Nov	Oct
Value:	62.3	64.7	69.3	69.7	78.4	80.0

Percent Motile Sperm (Hemocytometer)

Month:	Sept	July	June	Aug	Oct	Nov
Value:	66.0	71.7	72.0	75.3	80.5	81.2

Rate of Forward Movement

Month:	Sept	July	Nov	June	Aug	Oct
Value:	2.87	2.92	3.17	3.19	3.20	3.53

Percent Abnormal Sperm

Month:	Aug	Oct	Sept	Nov	June	July
Value:	7.73	10.11	11.56	15.07	18.43	18.93

TABLE XV (CONTINUED)

	Concentration/ml. x 10^6					
Month:	Oct	Sept	July	Aug	Nov	June
Value:	257	260	270	270	282	301

	Total Sperm/ejaculate x 10^9					
Month:	June	July	Sept	Aug	Oct	Nov
Value:	49.5	50.8	56.4	57.4	61.0	66.6

[1]Kramer's modification for unequal numbers (Steel and Torrie, 1960).

Trial I. The two measurements of percent motility utilized in this trial tended to vary together over the months. The rate of forward movement of sperm also fluctuated in the same pattern. The hemocytometer method had a higher average value than did the subjective method, but both estimates exhibited the same fluctuation. Most researchers agree that the hemocytometer method estimates the percentage higher than does the subjective method. Lasley (1951) states that the motility estimates are higher when using the hemocytometer method because many of the inactive sperm are moved about by the active ones, giving a false impression that more cells are motile than really are.

The only semen criteria that did not exhibit the same pattern was percent abnormal sperm. In Trial I the months with the highest percentage of abnormals were July

through October. In this trial, however, the percent abnormal sperm was highest during June and July. The average daily maximum temperatures were very similar during both trials. The treatment imposed on the boars in Lot II was modified in this trial. A sprinkler system was installed in the open shade structure and this seemed to have some merit. The second part of Figure 13 shows that the boars in this lot did not exhibit the increase in abnormal sperm as they did in Trial I (Figure 4). The boars in Lot III had a high percentage of abnormal sperm during July and September. The peak in percent abnormal sperm in Lot III during September is probably due to the fact that the air-conditioner was not functioning during one week in July and the boars had no cooling system while it was being repaired.

The values for sperm concentration per milliliter and total sperm per ejaculate agree very well with results obtained in Trial I (Figures 14 and 15). Both trials indicate a low sperm production during the summer months. These results have also been obtained by other workers as discussed in the previous trial.

Lot Effect

The lot effect on each semen criteria was evaluated by analyses of variance for a completely randomized experiment, however, the data was sorted so that only complete data for each lot was used in the analysis. The results of

these analyses are tabulated in Table XVI. No statistically significant differences were found due to the small number of animals used in this trial. The graphs in Figures 9 through 15 indicate that some differences exist, but these differences were not large enough to be detected

TABLE XVI

TABULATED ANALYSES OF VARIANCE OF LOT EFFECT ON SEMEN CHARACTERISTICS (TRIAL II)

Source	D.F.	Mean Square	F Value
Volume (ml.)			
Between lots	2	248581.10	1.86
Error	5	133443.17	
Percent motile sperm (S)[1]			
Between lots	2	785.01	0.97
Error	5	807.87	
Percent motile sperm (H)[2]			
Between lots	2	27.59	0.06
Error	5	427.52	
Rate of forward movement			
Between lots	2	2.10	1.03
Error	5	2.03	
Percent abnormal sperm			
Between lots	2	1338.85	3.89
Error	5	344.02	
Concentration/ml.			
Between lots	2	104917.19	1.23
Error	5	85579.48	
Total sperm/ejac.			
Between lots	2	370.72	1.08
Error	5	344.85	

[1]Subjective

[2]Hemocytometer

by the small number of degrees of freedom associated with the error term which had to be used. Table XVII presents the semen criteria averages for each lot and Table XVIII presents the maximum bimonthly ambient temperatures and the corresponding afternoon temperatures in each shelter.

The boars in Lot III were maintained at the coolest temperature but during the week the air-conditioner was not in use, they were stressed rather severely. This had a detrimental effect on the boars as is evidenced by their depressed semen quality. The boars in Lot I exhibited the highest overall semen quality. This is in agreement with the results obtained in Trial I and also with the results reported by Lawrence (1967). Although the difference between lots were not significant, the trend indicates that boars kept in a three sided shelter have higher semen quality than boars that were maintained in the other two shelters. The addition of the sprinkler system to the lot with the open shade structure (Lot II) improved the semen quality of the boars as compared to the boars in this same lot a year earlier without the sprinkler. The sprinkler system seemed to have the greatest merit in preventing the increase in the percent of abnormal sperm during the summer months. (Figure 4 and 13) Whatley et al., (1957) conducted a trial utilizing sprinklers in open shade structures for pregnant gilts during the summer months. He observed that the sprinklers reduced the rectal temperatures of gilts as compared to control gilts which were

maintained in open shades without access to sprinklers. Although no rectal temperatures were taken for the boars used in this trial, it is possible that the body temperature, as well as the scrotal temperature, of the boars in the lot with the sprinklers could have been lowered enough to prevent the increase in percent abnormal sperm which is usually observed during the summer months. This type of cooling device is very inexpensive and would be very practical for use by most swine breeders.

TABLE XVII

SEMEN CRITERIA AVERAGES FOR EACH LOT (TRIAL II)

Semen Criteria	Lot I	Lot II	Lot III
Volume (ml.)	286.5	187.3	199.2
Percent motile sperm (S)[1]	77.67	70.00	66.61
Percent motile sperm (H)[2]	78.42	72.5	75.13
Rate of forward movement	3.49	3.18	3.05
Percent abnormal sperm	10.76	12.97	15.44
Concentration/ml. x 10^6	223.95	295.97	318.13
Total sperm/ejac. x 10^9	57.56	51.46	61.20

[1]Subjective

[2]Hemocytometer

The results of this study as well as the study conducted by Lawrence (1967) indicate that the type of air-conditioned shelter used in these trials does not improve semen quality in boars. In future studies of this nature,

it would be profitable to maintain the boars in a larger air-conditioned building at a cooler temperature than that used in this study. The boars should also be kept in the air-conditioned building during the entire day instead of being allowed to go in and out at will. Observations made during this study revealed that the boars spent the major part of each day outside the shelter and were therefore not utilizing it to the greatest degree. This suggests that the building was too small to accomodate the boars comfortably and that it was not cooling them adequately.

TABLE XVIII

CORRESPONDING MAXIMUM DAILY ENVIRONMENTAL AND AFTERNOON SHELTER TEMPERATURES

	Environmental Bimonthly Maximum	Type of Shelter		
		Three sided Shelter	Open Shade	Air Conditioned Building
June 17-30	87.8	83.8	83.0	81.3
July 1-15	88.5	85.3	85.7	83.9
July 16-31	95.0	89.5	89.0	83.5
Aug 1-17	95.5	90.6	89.1	81.1
Aug 18-31	91.0	86.7	85.5	81.4
Sept 1-15	85.5	79.7	78.9	77.9
Sept 16-30	86.1	81.5	80.3	78.9

Interrelationships of Semen Criteria

The simple correlations between the semen criteria studied in this trial are shown in Table XIX. These

correlations were pooled over the complete trial and the variance due to time of the year was removed. These correlations are very similar to those obtained in Trial I. Volume and total sperm per ejaculate showed a significant correlation. A high negative correlation existed between volume and concentration per milliliter. The three estimates of motility utilized in this trial were all significantly correlated with one another. The highest correlation was between the subjective estimate of sperm motility and the rate of forward movement. This correlation was probably high because both are subjective estimates and they both may be biased to a certain degree by the sperm concentration at the time the estimates were made. All three estimates of motility showed a significant negative correlation with percent abnormal sperm. Sperm concentration was found to be highly correlated with total sperm per ejaculate.

None of the correlations were high enough to be able to predict one semen characteristic by measuring another. A much higher correlation between the subjective estimate of motility and the hemocytometer method was expected. Several factors may have influenced erroneous values for either or both methods. The sperm concentration at the time of making subjective estimates may not have been constant for all observations, thus biasing the value. A lower motility value is usually given to the semen with the least concentration, even though its motility may be

TABLE XIX

CORRELATION COEFFICIENTS BETWEEN SEMEN CHARACTERISTIC MEASUREMENTS (TRIAL II)

	Percent motile sperm (S)[1]	Percent motile sperm (H)[2]	Rate of forward movement	Conc. per ml.	Total sperm/ ejac.	Percent abnormal sperm
Volume (ml.)	-.01	.20*	-.11	-.61**	.22*	-.05
Percent motile sperm (S)		.42**	.57**	.01	-.05	-.41**
Percent motile sperm (H)			.22*	-.12	.05	-.48**
Rate of forward progress				.02	-.10	-.36**
Conc./ml.					.55**	.24*
Total sperm per ejac.						.24*

[1]S = Subjective

[2]H = Hemocytometer

* Level of Significance = P< .05

** Level of Significance = P< .01

similar to a semen sample which is more concentrated. The advantages of the hemocytometer method is that the individual cells can be observed and the concentration of sperm is more uniform, however, it is a very time consuming procedure. The extender used to dilute the semen for use in the hemocytometer method may have been too basic or acidic for the spermatozoa. The initial pH of the extender was determined and it was acceptable, but as the extender set over a period of time it could possibly have changed. Except for boar 66-9 (Table XIII) the averages for both estimates of motility for each boar were very similar. The monthly fluctuation patterns for both types of estimates were also very similar. From this study, the subjective estimate of motility seems to be the most feasible.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study was divided into two trials. Trial I extended over one complete year and utilized 12 Yorkshire boars that were collected three times per week. Trial II was six months in length and included eight Yorkshire boars that were collected twice weekly. Three shelter modifications were used in each trial. Semen evaluations were made to determine the effects of shelter and season fluctuations in semen characteristics over the period of each trial. In Trial II three different ways of estimating spermatozoa motility were evaluated, and their relationship to each other was studied.

The results of this study indicated that semen volume and total sperm per ejaculate were highest during the cooler months of the year. In general, sperm motility was highest during the cooler weather and lowest during the warmer season of the year, but the boars in the open shade structure, which provided the least protection from the environment, exhibited the lowest motility during the coolest period of the year. The percent abnormal spermatozoa was highest during the late summer and early fall months.

The analyses for shelter effects suggests that the three sided shelter was the most conducive to high semen quality in boars. The results of this study indicate that boars housed in shelters with maximum ventilation have higher semen quality during the summer than those housed in cooler shelters that have inadequate ventilation. During the winter months, however, semen quality of boars was lowest in the lot with the least protection from the environment. The cooling of boars by means of the sprinkler system utilized in Trial II apparently was effective in lowering the percent abnormal spermatozoa during the summer months as compared to the other lots.

In both trials, the boars mounted the breeding dummy and could be collected more consistently during the first half of each trial than during the latter half. Results also indicate that boars provided with the best shelter during the winter months tended to be more consistent in their response to collection. Collecting boars three times a week for one year seems to be too often. During the first six months of the trial, 90% of the collections attempted were successful, but by the end of the trial, the boars were only successfully collected about 40% of the times attempted. After terminating Trial I, the boars were allowed to rest sexually for two months before Trial II began. They were then collected twice a week for six months. They worked more consistently than when they were collected every other day, but during the latter half of

the trial they became less consistent in collection. Whether this is a carry over effect from Trial I or an indication that continued biweekly collection is too frequent could not be determined.

The estimates of spermatozoa motility by each of the three methods studied in Trial II all followed the same general fluctuation pattern over a period of six months. In general, the boars with the highest subjective estimate of motility exhibited the highest estimate by the other two methods. Only one boar did not follow this pattern and this was responsible for the correlations among the three estimates of motility being low. Since all methods of estimating sperm motility followed the same pattern, the subjective method is recommended for use, because the hemocytometer method is very time consuming.

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