A STUDY OF FACTORS ASSOCIATED WITH CHANGES IN BOAR SEMEN AND A PHOTOELECTRIC METHOD FOR THE DETERMINATION OF SPERMATOZOA CONCENTRATION

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INTRODUCTION

Multiple farrowing has become more prevalent with the advent of mechanized, confinement swine operations, and requires an almost continuous year round breeding schedule. The boar's contribution to the farrowing rate has been demonstrated by First et al. (1963) and Borton et al. (1965), and numerous studies have shown that reproductive performance in the bull and ram varies, with climatological conditions, over time. Very little research has been performed to establish this pattern in boars.

It was the purpose of this study to determine the extent of seasonal variation in swine semen, and when the lowest semen quality values are evidenced in the period of this study. Also incorporated into this study were three shelter modifications to which the boars had access during the course of the study. The effect of these shelters on alleviating semen quality degradation was examined to determine if increasing environmental control decreased seminal changes. The use of improved shelters for housing boars would be justified if the boars reproductive performance could be improved or maintained at a constant level.

Estimating spermatozoa concentration by direct count is a very time consuming process, and the literature

reports that faster and more reproducible estimates can be obtained by photoelectric methods. Toward this end a regression equation was developed, with the semen from this study, for the estimation of spermatozoa concentration from an optical density measurement. The developing of this method provides estimates which should facilitate rapid semen evaluation in future research.

REVIEW OF LITERATURE

Seasonal Variation

Only a limited amount of published research has been concerned with the seasonal variation in the reproductive function of male farm animals. The results that have been reported indicated definite differences in semen quality and/or fertility. The major factors involved in this seasonal variation have been reported to be environmental temperature, amount and quality of light, relative humidity, and nutritive value of available feedstuffs.

Seasonal changes in the semen characteristics of nine dairy bulls of four different breeds were studied by Erb, et al. (1942). Collecting the bulls once a week for a year, they obtained highly significant differences between months in initial motility, ejaculate volume, spermatozoa concentration per cubic millimeter, total sperm per ejaculate and the number of abnormals per 1,000 spermatozoa. The only criteria observed that was not significantly affected was the pH of the semen. In this study, conducted at Purdue University, initial motility and volume were lowest during July, August, and September. The number of abnormal cells was twenty-five percent greater during these months than in the next poorest month.

Spermatozoa concentration was maximum during April, May and June.

Mercier and Salisbury (1946) analyzed data from 328 ejaculates and 20,689 inseminations in dairy cattle located in New York. Sperm production was at the lowest level, and fertility was below average in June and July. Fertility continued to drop in August before increasing during the subsequent fall months. The percent abnormal sperm was highest during March, April and May.

A six year study conducted by Erb and Waldo (1951), in conjunction with a Washington artificial insemination co-op, analyzed the seasonal variations in fertility in dairy bulls. The 60-90 non-return rate from 93,113 first and second services was lowest during the first four months of the year and gradually increased to its highest levels in September, October and November. The authors state that monthly correlations between fertility and spermatozoa concentration, motility and other semen characteristics were greatly variable from one period to another.

Fourteen dairy bulls from the Louisiana State University Dairy Improvement Center were employed in a fifty-seven week study by Johnston and Branton (1953). The bulls were maintained on pasture with a fourteen percent protein concentrate fed when necessary to maintain their weight. The non-return rate started to decline during late July and continued until it reached its lowest point in early September. Significant ($P<0.01$) correlations between six

weeks moving averages of fertility and maximum temperature, minimum temperature and vapor pressure of -0.46; -0.45; and -0.55 respectively, were obtained. The similarity of these correlations can be largely attributed to the high relationships between the environmental criteria.

Anderson (1945) investigated the seasonal variations in the reproductive capacity of beef and dairy bulls in Kenya, where the extreme average daily temperatures are very mild. The highest maximum average daily temperature during the twenty-seven months involved in this study was less than 90° F. The optimum semen values were associated with the warmer periods. The effect of hours of daily sunshine, relative humidity, and the amount of rainfall were also considered, but none of these were isolated as being responsible for the highly significant ($P<0.01$) changes in semen quality.

Nutrient intake and available vitamin A are two factors that are influenced by seasonal changes, and the latter is known to play an important role in maintaining the histological integrity of the testis. Sapford (1951) took this into consideration in designing an eighteen month experiment with twenty-two Merino rams. One-half of the rams were fed on a low plane of nutrition and the other half on a high plane of nutrition both with a ration which was deficient in vitamin A. Crystalline carotene was supplied to four animals within each of these groups. The results of this work showed an overall summer depression

of semen characteristics regardless of the nutritional regime, but the effect was more severe in the group on a low plane of nutrition and in subgroups with low plasma vitamin A levels. This trial was performed under the mild summer temperatures in Sydney, Australia, and Sapford speculated that some climatological factor other than temperature might be responsible for the summer depression.

The Suffolk, Hampshire and Rambouillet breeds were represented in a California study by Cupps, et al. (1960) in which they had from three to five rams on trial from April, 1956 to September of 1958. Data obtained from biweekly collections indicated a pronounced decrease in the ejaculate volume, cell concentration, percent motile and percent live spermatozoa occurred in August and September. This coincided with the high average maximum daily temperatures for those months of 97.8° F. and 91.9° F., respectively.

Fat-tailed sheep used by Hafez et al. (1954) have no distinct breeding season in Egypt where they conducted their work. The data which evolved, from this twelve month experiment with twelve rams, indicated two peaks of high semen quality at about the time of the spring and autumn eqiunoxes. Semen quality was lowest in July and August when the days were hottest and longest, and in December and January when the temperatures were lowest and the days the shortest. Seasonal fluctuations were significant for volume ($P<0.05$), motility ($P<0.01$), and

concentration ($P<0.01$), with the highest values in the spring (March, April and May) and the lowest in the winter (December, January and February).

Stevermer, et al. (1961) were concerned with the effect of feed intake on the reproductive performance of boars, but noted some seasonal variation in semen production as the fifteen and one-half month long Wisconsin trial progressed. Volume per ejaculate increased, in five of the six boars involved in this study, during the cool fall months. Both spermatozoa concentration and total number of spermatozoa per ejaculate were highest during the warm months of June through August. This information was reported as a general observation and was not statistically analyzed.

From the research concerned with sheep and cattle, it would appear that semen quality begins to deteriorate with the occurrence of the environmental conditions typical of summer and rebounds to a peak in the fall. Inherent to field trials, of the nature described above, are numerous variables over which the researcher has no control. The most prominent variable in studies concerned with male fertility, is the fertility level of the females on which he is used. Research concerned only with semen quality relies on measurements which may not always be indicative of the fertility potential at that time.

Controlled Environments

The creation of artificial environments has been used extensively to study the effect of specific environmental components on various physiological processes.

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, in varying intervals from seventeen to forty-two days, from 70° to 90° F. In the other chamber it was changed from 70° to 52° F. and then back to 86° in the same manner. Stress, as evidenced by restlessness, excessive salivation, weakness and tremors, became apparent at 90° F. Spermatogenesis was impaired in two bulls by continuous exposure to 100° F., and in two other bulls by continuous exposure to 86° for five weeks. A marked decrease occurred in initial motility, concentration and in total spermatozoa per ejaculate during or following exposure to the high environmental temperatures. A young bull (nineteen and twenty-four months of age) and an older one (twenty-eight and thirty-four months of age) were put on each treatment. The authors report that spermatogenic function was restored in both of the older bulls, but not in the younger males which were raised under different management. The fact that libido and volume were described as unaffected, suggests that there was no alteration of interstitial cell function.

Simpson and Rice (1957), in a research study conducted at the Kentucky Agricultural Experimental Station, placed three rams in a room maintained at 90° F. for one week. The semen production of these rams was compared to that of three control rams which were kept in an unheated barn during January. Eight weekly collections were made starting with the week of treatment. Semen volume remained unchanged by the treatment, 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 semen characteristics of the control group remained normal throughout the trial, and the rams in the treated group had recuperated by the eighth week as evidenced by the return of their semen picture to its pre-treatment values.

A second experiment with rams, performed at the same station, utilized treatments of 100° F. for twenty-four hours, 90° F. for one week and a control group (Simpson and Dutt, 1958). 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 and averaged thirty-four percent for both treated groups, and twenty-two percent for the controls. When rams were exposed to 100° F. for only six hours, an increase in the percent abnormal spermatozoa was the only response observed during the subsequent

six weeks.

Male rabbits, all of which had at least sixty percent motile sperm at the start of the trial, were incubated for one hour at 110° F. in a laboratory experiment by El-Sheikh et al. (1955). The body temperature of the bucks was raised 4.6° F. by the treatment, but motility of the spermatozoa was not affected. 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 on day seven (96.2 vs. 73.5 percent) and for days twelve to fourteen (92.2 vs. 53.1 percent) but there was no apparent difference in the fertility of the two groups on days twenty-one to thirty.

The results obtained in this experiment with rabbits agrees with the conclusions drawn by Ulberg (1958) in a review of the influence of high temperature on reproduction. Ulberg postulated that increases in ambient temperature above a certain level, cause an increase in body temperature which adversely affects fertility before semen quality declines.

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 but subjected to environmental temperatures with an average daily maximum of 88.7° F. Twenty Hampshire ewes and three Southdown rams were randomly assigned to each of the two chambers which were designed to utilize natural lighting. Semen evaluations revealed significantly ($P<0.01$) less deterioration, as measured by the percent motile and abnormal spermatozoa, for the treated rams during the trial period. Two services were required per conception when treated rams were mated to ten control ewes, and 1.9 when mated to treated ewes. Control rams mated to treated ewes required 5.3 services per conception, and the difference between the two groups of rams was highly significant.

Dutt and Simpson (1957) repeated the ram treatments described in the previous paragraph, but bred sixty untreated ewes to each group of three rams. The average maximum daily temperature during this June through September trial ranged from 78.8° F. to 89.0° F. One-half of the ewes in each group were slaughtered shortly after breeding and twenty-six percent of the ova from thirty ewes bred to the control rams were fertilized, compared to sixty-four percent for the treated rams ($P<0.01$). When thirty ewes bred to each group of rams were allowed to lamb, the treated rams again had a significantly ($P<0.01$) higher fertilization rate (50.0 vs. 13.3 percent). In this study the treated rams had significantly ($P<0.05$) less deterioration in semen quality as measured by

concentration, percent motile sperm and percent abnormal cells.

Goldsveig and Smith (1956) examined the reproductive function in male rats in which they had induced extreme hypothermia. Through a combination of hypoxia, hypercapnia and cold, the body temperature was forced to between +15° C. and +20° C. in one group, and between 0° C. and +1.5° C. in a second group. Respiration and circulation were arrested for one hour in the rats in the latter group. Sex drive and fertility were reduced for one to two weeks after treatment in the first group and for eight weeks after reanimation in the second. This work demonstrated that severe hypothermia damages developing spermatozoa and spermatids, but not the spermatocytes or spermatogonia.

Several inferences can be drawn from the research which has been done with controlled environments. The species studied appear to be more adaptable to temperatures below optimum than to temperatures above it. The deleterious effects of extreme temperatures on spermatogenesis lag behind exposure, and in most cases are of a temporary nature. Evidence indicates that interstitial cell function remains unaffected by a wide range of temperatures, if libido and volume are accurate indices of androgenic secretion as suggested by Mann (1948) and Anderson (1951).

Delayed Response

Recent irradiation research has been instrumental in adding to our understanding of the delay that exists between a male's exposure to thermal stress and ejaculate symptoms of seminal degradation. Irradiation has an immediate affect on the testicular constituents, therefore, the time from application until the effects are apparent in the ejaculate can be measured rather precisely.

Spermatogenesis is a continuous process occurring in waves along the seminiferous tubules. The pattern of irradiation induced desquamation, of the various germ cell forms, led Wilham and Cox (1961) to speculate that a reduction of the cells to a specific class of type "A" spermatogonia, may be responsible for the characteristic synchronization of the spermatic cycle. Glover (1959) explains that thermal sterility and testicular degeneration take the course of passing from the most highly differentiated cells to the simpler forms. That is, desquamation of the spermatids occurs before that of the secondary and primary spermatocytes, and the response would be expected to be more gradual than that induced by irradiation.

Weekly collections were made on fifteen ten month old boars that had been divided into groups receiving either 200 or 400 r (roentgen units) of gamma radiation or serving as controls (Pace et al., 1959). The semen evaluations revealed that the percent of abnormal cells increased

during the fourth week and rose to the highest level during the seventh to ninth week after treatment. The percent live spermatozoa and oxygen consumption reached their lowest levels by the sixth week. Sperm numbers in the 400 r group went from a normal value of 50.0×10^9 to a low of 1.0×10^9 which was recorded during the ninth week. In the thirteenth week, the total spermatozoa per ejaculate value had approached its preirradiation level.

The pattern of spermatogenic response in irradiated boars has been shown to be influenced by the collection frequency (Cox and Wilham, 1961). Collections in this experiment were made three times a week, weekly or twice a month on six month old boars. The two trials in this experiment involved the exposure of groups of boars to a single dose of 0, 300, 450, 600 or 900 r of irradiation directed locally to the testicles. Total spermatozoa per ejaculate was the only criterion used and almost aspermic ejaculates were obtained forty-seven, fifty-nine and seventy days after irradiation for the decreasing collection frequencies, respectively.

The time lapse between treatment and recovery was not hastened by more frequent collections, which suggests that the production of sperm cells was not affected. Post irradiation recovery back to 2.0×10^9 cells (50.0×10^9 was the control average) was found to be at seventy-two to seventy-four days for all groups, but a second decline was evident at 140 days to 160 days in groups that received the

higher doses.

Ladd and Murphree (1964) irradiated one group of mature boars with 50 r at seven day intervals, and another group with 100 r at fourteen day intervals until a total of 600 r had been administered to both groups. A third group of unexposed boars served as a control. Sperm output started to decline at the eighth week, and ejaculates were aspermic by the seventh week after initial exposures. It was not until the forty-eighth week after the first treatment that the spermatozoa output, from the group exposed to the 50 r doses, had increased to fifteen percent of that maintained by the controls. All of the boars were castrated, and the left testicle weighed, forty-nine weeks after the start of the study. The average testis weight was 319 grams for the controls, compared to about 216 grams for both treatments.

The large epididymis of the boar affords a large spermatozoan reservoir which may account for the time lapse, after treatment, before minimum counts are obtained. Singh (1962_a) used radioactive phosphorous (P^{32}) to estimate the time required for spermatozoa to pass along the epididymis of mature boars. The data indicated that fourteen days were required for the labeled spermatozoa to pass through the epididymis when collections were made two or three times a week.

In a second phase of this study, (Singh, 1962_b), the epididymides of eight boars from one to two years of age

were homogenized for one or three minutes after having been divided into three parts. Volumetric measurements and concentration analysis of the homogenates were used to estimate spermatozoan reserves. Estimates ranged from 68.0×10^9 to 143.9×10^9 spermatozoa per epididymis, or a mean per pair of 198.8×10^9. On a percentage basis the largest reserve was in the caudal portion (54.4 percent) with smaller fractions being stored in the corpus (26.9 percent) and caput (18.7 percent) areas.

The estimated spermatozoan reserve estimated by Singh (198.8×10^9) and a later estimate of 193.0×10^9 by Kennelly and Foote (1964) are remarkably close. The estimated daily spermatozoa production from the two studies were 27.2×10^9 and 31.3×10^9, respectively.

Exposure to radiation provides a means by which almost instantaneous effects can be produced on the testis. With this as the base point it is possible to determine, at any assigned frequency of collections, the time lapse involved until the maximum effects are apparent in the ejaculate. Research results are consistent in establishing this interval at seven to nine weeks when collections are made two or three times a week.

Differences in the physiological processes involved and in testicular and epididymal size would be expected to introduce some variation in the time lapse. It should generally be valid to relate spermatozoa production, as evaluated from the ejaculate, to influences which were

exerted on the testis seven to nine weeks earlier.

Optimum Temperature for Swine

No literature was found that was directly concerned with the seasonal variation of, or the effect of altered ambient temperature on semen production in the boar. Jensen (1964) described swine as non-perspiring homeotherms with a relatively inefficient thermoregulatory system. Body temperature regulation is primarily dependent upon heat dissipated by evaporation from the lungs, and to a lesser extent by conduction, convection and radiation.

Research has proven that environmental temperature influences the feed intake and heat production of an animal, and has a role in governing the availability of energy in ingested food, net energy available for production purposes and the composition of gain. Temperatures above the "comfort zone" lower an animals energy requirement because of the bodies need to decrease heat production. Conversely temperatures below the optimum are responsible for an increase in feed consumption as the heat required to warm and humidify the inspired air increases (Winchester, 1964). Therefore, it might be justified to assume that the optimum environment for productive purposes and gross body physiology would also be most favorable for spermatogenesis.

Heitman and Hughes (1949), and Heitman et al. (1954) employed a psychrometic chamber to investigate the effect

of ambient temperature on weight gains in swine. They found that maximum average daily gains for pigs under 125 pounds were attained at 75° F., and 61° F. was best for pigs from 150 to 350 pounds. More recent work by this group substantiates those results (Heitman et al., 1958). Temperatures from 40° F. to 110° F. were examined, and the data indicated 61° F. and 73° F. were best for the production of 350 and 100 pound hogs, respectively.

The relative merit of six different environmental modifications was examined under summer conditions in California (Heitman et al. 1956; Heitman et al. 1959). Briefly, the modifications consisted of a shade only, a wallow only, a shaded wallow, a fanned wallow, access to a small air-conditioned house, and a small pen within a larger hog barn. During the trial the mean temperature for the four outside groups was 75.0° F., 71.5° F. in the air-conditioned house, and 77.6° F. inside the hog house. The performance of forty-eight growing-finishing pigs was observed during a seventy day period when the diurnal temperature range was from 58.5° F. to 94.4° F. The average daily gain of pigs with access to a shade only was lower than any of the other groups, but it was not significant. The amount of feed consumed per pound of gain was least in the air-conditioned group, but this difference was also nonsignificant. The shade was used 90% of the time when the temperature was above 69° F., while pigs with access to the air-conditioned house used it only 60% of the time.

Physiological Responses

Some physiological responses of rams to increased environmental temperatures were reported by Dutt and Hamm (1957). Two Southdown rams were subjected to each of two treatments and a control. The control animals were unsheared and maintained conventionally for winter conditions in Kentucky. Treatment one and two involved incubation at a constant 90° F. at 60% humidity for one week, and differed only in treatment one animals being sheared while those in treatment two were not. Rectal temperatures, pulse rate per minute and respiration rate per minute were recorded before and during the treatment. Significant ($P<0.01$) increases during treatment were calculated for rectal temperature and respiration rate in both treatments, and for pulse rate in treatment one.

In two trials cited earlier (Simpson and Rice, 1957; and Simpson and Dutt, 1958) the response of rectal and testicular temperature to increased ambient temperature were measured by making a tissue probe with an electronic thermometer. Both trials utilized the same facilities and were under the direction of the same person, so the results will be combined. Control animals had body, scrotal and testicular temperatures of 101.8° F., 90.7° F. and 93.4° F., respectively. When rams were maintained at 90° F. for one week, the same temperatures increased to 104.5° F., 96.1° F. and 98.0° F., respectively. Rectal and testicular

temperatures increased 3.7° F. and 7.1° F. above that of the control animals when the treatment was incubation at 100° F. for twenty-four hours, and 1.5° F. and 4.4° F. when the 100° F. period was only six hours.

Testicular and body temperature of five sheared and five unsheared rams were measured in a study by Foote et al. (1957) comparing three ambient temperatures. At the end of a forty-eight hour exposure to 70° F., 82° F. and 95° F. environments the testis temperature averaged 95.46° F., 95.36° F. and 98.75° F., respectively. This effect of ambient temperature on testicular temperature was statistically significant ($P<0.01$). The effect on rectal temperature was also significant at the same level, but the increase of ambient temperature from 70° F. to 95° F. caused a greater change in testicular temperature than in rectal temperature (3.31° F. vs. 2.14° F.)

Body temperature and respiration rate in gilts and barrows were determined after fifteen or thirty minute exposure to a summer environment which had a maximum average of approximately 88° F. (Tidwell and Fletcher, 1951). The 116 to 183 pound pigs were normally maintained under cool, shaded conditions and were subjected to the environmental temperatures only on days when the temperature was above 80° F. at 2 p.m. Body temperature was raised an average of 0.94° F. after a fifteen minute exposure and 1.97° F. after a thirty minute period. Respiration rate was increased 49.8 and 80.8 respirations

per minute for the fifteen and thirty minute exposures, respectively.

Ingram (1964) studied the effect of ambient temperature on the body temperature, respiratory frequency and pulse rate in three month old Landrace pigs. All seven of the thirty-nine to sixty-two pound pigs were exposed to -5°, 10°, 25° 30°, and 35° C. for six hours in a random sequence. Rectal temperatures were found to be lowest at 25° C. (77° F.) and increased in both warmer and cooler environments. Skin temperature increased 0.6° C. for every 1° C. rise in body temperature when the ambient temperature was 35° C. The respiratory frequency was 12-18 respirations per minute at 30° C., but rose to 181 per minute when the temperature was raised to 35° C. Each increase in ambient temperature initiated an increase in pulse rate which was greatest at 35° C.

No significant increase was found in the rectal temperature of seven month old Berkshire gilts maintained for twenty-eight days at 70° F. and then for twenty-eight days at 85° F. (Sanders et al. 1964). The study utilized a psychrometric chamber with a constant 50% humidity. Analysis indicated a significant ($P<0.01$) difference between the body temperatures of the gilts irrespective of the ambient temperatures.

The effect of varying ambient temperatures is manifested in the animal by increases in respiratory frequency, pulse rate and by body and testicular temperature.

Temperatures which are most conducive to normal values for these criteria are also found to contribute to maximum production performance.

Modes of Action

The mechanism by which ambient temperature exerts its effect on spermatogenesis is not completely known. It may act directly on the testis, or as a secondary response to an altered general metabolism of the animal (Turman, 1950).

Chang and Fernandez-Cano (1959) suggested that reproductive failure in female rats may be the result of a physiological shift of hormone production from the anterior pituitary. When put under stress the organism produces ACTH for its own welfare, but at the expense of gonadotropin production. A mock adrenalectomy was used on normal and previously adrenalectomized rats to create stress (Barrett and Hodges, 1956). They found increased ACTH levels in both groups after the operation.

Aldosterone, a mineralocorticoid which is responsible for salt retention in the body, was found to increase significantly in the urine of males under heat stress (Hellmann et al. 1956). Men were subjected to alternate periods of work and rest for four hours at 100° F. The urinary output of the five other adrenocortical steroids studied, including cortisone, was not significantly altered.

Thyroid function has been associated with both changing ambient temperatures and spermatogenic function. Bogart and Mayer (1946) found that the spermatogenic decline, associated with "summer sterility" in the ram, could be alleviated by the administration of thyroxine or thyro-active substances. Their work also indicated that symptoms of this temporary reduction in fertility could be induced by administration of thirouracil which is a goitrogenic substance. Neither treatment affected volume or motility which they considered to be under the control of the interstitial components of the testis.

Using I^{131} release from the thyroid gland and the conversion ratio of inorganic to organic iodine, Ingram and Slebodzinski (1965) estimated the secretory activity of the thyroid in young pigs housed at 25° C. and 35° C. for six weeks. Secretory activity declined with time, but it was always greater in pigs housed at the cooler temperature. Oxygen consumption rates of the pigs were also higher in the cooler environments.

In all probability it is not a single factor, but a combination of many which decrease spermatogenic processes during heat stress. The sensitive balance of the hypophyseal-gonadal axis, and the delicate nature of spermatozoa could render spermatogenesis suceptable to any number of intrinsic and extrinsic variables.

Photometric Concentration Determinations

Determining the concentration of sperm cells by a direct count from a hemocytometer is a long and tedious operation, and subject to several errors. Several more rapid methods have been used to make these determinations. One of these, first used in 1939, employs a photometer to measure optical density of the semen sample (Salisbury and Vandemark, 1961). It is based on the principle that the specimen becomes proportionately more opaque as the number of cells per unit volume increases.

The accuracy of the photometer (or nephelometer) depends upon the accuracy of its calibration against semen of known concentration. The concentration for this calibration must be established by direct count. Freund and Carol (1964) studied the factors affecting the accuracy of hemocytometer counts. In this study three technicians, with one to three years experience in the use of the hemocytometer, examined forty-six semen specimens from seven human donors. Duplicate pipettings were made of each specimen, and two hemocytometer chambers were filled from each pipette.

An analysis of variance was calculated on the technique and it described 57.4% of the variance as being among the technicians. The remainder (42.6%) of the variance was among duplicate determinations by the same technician. Components of this 42.6% were 1.3% between pipettings,

21.3% between chambers within pipettings and 20.0% between counts within chambers. A 95% confidence interval for counts among technicians, on a single specimen, was equal to $\pm$52% of the mean of this study.

The large variance associated with the direct count should be considered when calibrating a photometer, for it is the basis on which estimates will be made. The number of samples counted, and the refinement of the technique will determine the reliability of the calibration.

Comstock et al. (1943) developed a regression equation for the prediction of spermatozoa concentration in ram semen. Duplicate hemocytometer counts were made on twenty-four different semen samples, and the count per 0.0001 cubic millimeter averaged 337 with a standard error of 26.8. The regression of the count on 2-$\log_{10}$ of the galvanometer reading accounted for 95.6 percent of the variance ($r = +0.978$).

Photometer scales are least sensitive at both extremes, where the amount of light that is transmitted through the specimen approaches 0 and 100 percent. Willett and Buckner (1951) used a bull semen to diluter ratio of 1:40 to keep the majority of readings in the more sensitive area of the scale (twenty to seventy percent light transmittance). Duplicate determinations on twenty-five ejaculates from twenty bulls were made for both the direct count and the instrument reading. A -.97 correlation was found between the optical density and the

logarithm of the count, but the meter gave the more reproducible results.

The study also involved the comparison of nine photometers with five diluted samples. The maximum deviation from the average reading of the nine machines was four percent light transmittance. This indicated that it was not essential that each instrument be calibrated, but individual calibration would improve accuracy.

Three colorimeters and one spectrophotometer, from different manufacturers, were compared using dog semen diluted at ratios of 1:4 and 1:9 (Foote and Boucher, 1964). Correlations of about +0.99 were obtained for readings between all of the photoelectric instruments that were tested. Even with two dilution ratios and four instruments involved, the correlations were +0.92 and higher between the hemocytometer count and the density reading.

The main source of error in photoelectric determinations is the inherent property of the seminal plasma, which is independent of the cellular concentration. No appreciable increase in accuracy was realized when the semen is centrifuged and only the precipitate diluted for photometric analysis (Willett and Buckner, 1951).

Glover (1955) stated that the presence of opaque matter in the accessory sex secretions would be detrimental to the accuracy of the photoelectric method of estimating cell concentration in boar semen. The proportion of the ejaculate that is contributed by the secretion of the accessory

sex organs is far greater in the boar than in either the ram or bull.

Recent work with optical density determinations with boar semen suggests that Glover's reservations were not entirely justified. Young et al. (1960) obtained correlations of 0.95 and higher between hemocytometer counts and photometer readings, expressed as two minus the logarithm of the 0 to 100 reading, with boar semen. The twenty-five ejaculates that were used for this study were diluted at ratios of 1:4, 1:9, 1:19 and 1:39, with the two intermediate dilutions giving readings in the most sensitive range. With a 1:9 dilution, the estimated concentration was equal to 862.1 times two minus the logarithm of the reading, minus 72.4.

Once a photometer has been calibrated for sperm cell concentration determination, it provides faster and more repeatable results than are attained by a direct count. The photoelectric method also involves fewer technique errors. Opaque matter, that is inherent to the seminal plasma, has been a measurable source of error in some studies, and is of particular concern in boar semen.

Testis Characteristics

Testicular and epididymal weights and histological characteristics have been examined in several studies concerned with male reproductive processes. Attempts to relate these measurements to sexual maturation phenomena

have been very successful. Less success is obtained when these some measurments are studied in relation to seminal characteristics of mature animals.

Philips and Andrews (1936) reported the first appearance of spermatozoa in the boar testis at 147 days when the average seminiferous tubule diameter was 165.7 micra. Data obtained by Turman (1950) indicated the presence of fully formed spermatozoa at 116 days when the tubule diameter averaged 104 micra. This work also showed a close relationship to exist between testicular weight and tubule diameter in young, maturing boars.

The curvilinear relationship between body weight and seminiferous tubule diameter was presented graphically by Green and Winters (1944). At a boar weight of 350 pounds the tubule diameter was about 175 micra and still increasing, but at a decreasing rate.

A high correlation has been shown to exist between body weight and testicular development, and between seminiferous tubule diameter and the number of spermatogenic cell types found in the tubule (Green and Winters, 1944). The analysis of measurements associated with the development of the ram testis disclosed that seminiferous tubule diameter increased in a quadratic manner with age, and with the weight of the body and testis (Carmon and Green, 1952).

McKenzie et al. (1938), in a classic works on the reproductive process in the boar, presented data on the

size of the testis and epididymis. Nine of the boars used had a live weight of between 315 and 435 pounds, and the total testes weight in these animals ranged from 539 to 804 grams. The average testes to live weight ratio was 1:250 as compared to 1:800 for the epididymides which ranged from 168 to 219 grams. Like the testis, the left epididymis was generally the heaviest.

Hauser et al. (1952) found the left testicle to be significantly heavier than the right in a group of boars from 125 to 175 days old. Holst (1952) took testicular weights on thirty sterile and fifteen fertile boars. The average testicular weights for the normal boars were 594 grams for the right and 632 grams for the left, with comparable average of 432 and 440 grams in the sterile boars. The significance of these measurements and their relationship to fertility is dubious.

Mean total testicle and epididymis weights of 718.4 and 169.4 grams, respectively, were calculated in a comprehensive study on the semen and sexual behavior of boars (Hancock, 1959). After analyzing the data from this work, the author noted that normal semen characteristics are apparently compatible with seemingly abnormal testis characteristics and vice versa.

Four areas of the boar testis were compared to determine their relative merit in studying spermatogenesis (Kennelly and Foote, 1961; Kennelly and Foote, 1964). Three of the areas were adjacent to the surface of the

testis on the side opposite the epididymis, and were equally representative of the testicular parenchyma. The histological make up of the fourth area was significantly ($P<0.01$) different than the other three and was located deep in the testis, adjacent to the mediastinum.

No significant difference was found between the histological structures of the right and left testis. The parenchyma was estimated to consist of an average of 29.6 percent non-tubular material and 70.4 percent tubule components. These averages varied significantly between the six mature boars that were examined.

The gross and histological anatomy of the testis is often studied as an adjunctive part of various experiments. Several real relationships have been established between testis measurements and semen characteristics with young, maturing boars. Variation in testis measurements still exist among mature animals, but the relationships between these measurements and semen production or body size are generally lost.

MATERIALS AND METHODS

The data obtained for this study were collected in the seven month interval between June 25, 1965, and January 28, 1966, at the Oklahoma State University boar facilities at Stillwater. This was the first time that these facilities had been used for a study of this nature. Consequently, it was necessary to make some revisions in both procedure and facilities during the course of the trial. The revisions which might have had a bearing on either the results or the interpretation of the data will be described.

Boars and Facilities

Nine purebred Yorkshire boars from the Oklahoma State University Purebred swine herd were used in this study. The litter and individuals numbers, lot number, initial age and weight and the final weight of each boar are given in Table I.

Three boars were randomly assigned to each of the three environments provided, but the refusal of boar 61-7 to serve under the artificial conditions resulted in data being obtained on only two boars in lot II. Each lot was approximately 30 by 112 feet and the three lots were adjacent to one another.

TABLE I

DESCRIPTION OF THE YORKSHIRE BOARS

Litter No.	Individual No.	Lot No.	Initial Age (mo's.)	Initial Weight (lbs.)	Final Weight (lbs.)
55	7	I[a]	18	465	480
61	8	I	18	510	560
39	5	I	19	510	510
61	7	II	18	435	---
61	6	II	18	440	450
54	13	II	18	560	580
73	1	III	21	685	680
54	12	III	18	415	460
53	11	III	18	460	460

[a] I = Shade, II = 3 Sided shelter,
III = air-conditioned house

Within the lots the boars had free access to the following shelters:

Lot I. A six foot high, 14 by 18 foot pole shade with a galvanized metal roof was provided for the three boars in this lot. No bedding was provided, and the boars had no alternative but to lie on the bare ground.

Lot II. A 10 by 11 foot three sided shelter, open to the east, with a six foot ceiling was located in this lot. The two boars in this lot were provided with wet sand during the hot season and straw bedding during cold weather.

Lot III. A fully insulated 9 by 10 foot air-conditioned house with a wooden floor and a 4½ foot ceiling was provided for the boars in this lot. The door was designed so that it was easily opened by the boars, but was spring loaded so that it normally remained closed. A

one horsepower air conditioner was used to cool this building. On October 28 the air conditioner was turned off and no attempt was made to heat the building or provide bedding in the winter.

Daily temperatures were taken between three and five o'clock each afternoon by means of thermometers placed in similar, shaded locations in each of the shelters. The daily maximum and minimum temperatures were available from instruments located less than a mile from the boar pens which recorded climatological data for the Environmental Science Services Administration.

Each boar received the same amount of a fourteen percent protein, milo and soybeam meal ration. Originally the ration was fed at five pounds per head per day in a single feeding, but in an attempt to maintain constant boar weights the daily allotment was increased to six pounds on October 28. Group feeding, within lots, was practiced until August 8 when individual feeding stalls were constructed.

Water was available <u>ad libitum</u>, except for the period between August 18 until November 6 when boars in lots II and III were hand watered twice daily. Lot one was watered by an automatic waterer, but lots II and III were originally watered by means of fifty-five gallon drums with a valved cup at the base. The valves on the drums often stuck open and created wallows, and in order to eliminate the wallows the drums were removed from these lots and hand

watering was practiced until automatic waterers were installed.

Collection Procedure

Collections were made three times a week in an attempt to simulate production conditions, and reduce the variation between ejaculates that is associated with less frequent collections. Data obtained by Gerrits, et al. (1962) indicated that this collection frequency, over an extended period of time, resulted in maximum volume and sperm production.

Collections were made while the boar was mounted on a padded, canvas covered, wooden breeding dummy. Disposable plastic bags were placed over the end of the dummy to prevent contact of the boars penis with the abrasive canvas, and to keep this area of contact sanitary.

The ejaculation was obtained by applying pressure with the hand upon the spiral portion of the protruded penis (as described by Hancock and Hovell, 1959). This method proved very effective, and contamination from the contents of the preputial pouch, which has been reported in studies using artificial vaginas (Paredis, 1962), was avoided.

Semen Evaluation

Each ejaculate was strained through four layers of gauze into a graduated 600 ml. beaker as it was collected. The volume of the strained ejaculate was recorded, and the

gelatinous material was discarded. This was the only data recorded for the ejaculates from the first two collection days each week.

Duplicate 18 ml. subsamples were obtained from each ejaculate during the last collection day each week. The subsamples were poured into two 20 ml. test tubes, and these were placed in an insulated chest held at a temperature of approximately 100° F. by the addition of heated water. After all of the ejaculates had been collected, the subsamples were taken to the Veterinary Medicine Building, approximately one mile away, for further examination.

At the laboratory the specimens were removed from the chest and placed in a water bath maintained at a temperature of 100° F. The laboratory evaluations that were carried out were as follows:

1. Motility estimates were made by subjectively estimating the percent motile sperm cells in undiluted semen observed under 100 and 430 power magnification. Two drops of the neat semen were placed on a warm microscope slide, and aerated by gentle swirling with a stirring rod. A coverslip was placed over the semen, and the specimen was observed immediately by use of a biocular microscope. The estimate was recorded as a percent, and was not coded.

2. The percent abnormal spermatozoa was estimated from observation of 100 stained cells. The technique described by Wagelie et al. (1959) was used, utilizing carbol fuchsin stain held at 100° F. in the water bath.

Morphological forms were categorized on a multiple tally machine as normal, coiled tails, broken necks, misshapen heads and tailless. Spermatozoa with cytoplasmic beads were not considered abnormal forms in this study, because Hancock (1959) reported an average 29.6 percent neck and middle piece beads on spermatozoa from normal, fertile boars.

3. pH determinations were made within two hours after collection on a Leeds and Northup pH meter. Foley et al. (1964) reported that changes in the pH of boar semen were not severe until six hours post ejaculation. Poor results with Hydrion pH paper which was originally used, and mechanical failure of the meter during the trial made it impossible to obtain pH readings on every ejaculate.

4. Spermatozoa concentration was estimated as the average of counts made on two hemocytometer chambers filled from each of duplicate pipettings. Semen was diluted in a red blood cell pipette with 3.2 percent sodium citrate at a ratio of one part semen to 100 parts of diluter. A mechanical shaker was used to disperse the cells evenly in the pipette before the hemocytometer chambers were filled and the counts made. The average counts of the four chambers was converted to a concentration per milliliter.

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

The semen evaluation data that was obtained during a three week period was combined and averaged for statistical analysis and graphic presentation. This was done to reduce the effect of the large amount of variation that is inherent between ejaculates, within boars. Therefore, each value represents the average for the successful collections over a three week period. This would be three attempted ejaculations per boar for all determinations except volume which is the average of nine ejaculates.

Photoelectric Concentration Determination

This phase of the study was designed to calibrate a Coleman, Model 6A, Junior Spectrophotometer for the determination of spermatozoa concentration of boar semen. Procedures outlined in the Coleman Operating Directions 174-B (1957) were used to determine the most effective wave length and dilution ratio for boar semen.

It was determined that 625 millimicrons was the most specific wave length for absorption by boar spermatozoa which thus would minimize the effect of interfering substances. Of the dilution ratios examined, one part semen to ten parts of diluter was found to produce transmittance values falling most consistently in the more sensitive area of the galvanometer scale.

The semen was thoroughly mixed before one milliliter of the semen was diluted with ten milliliters of 3.2 percent sodium citrate in a test tube. The spectrophotometer

was adjusted to zero optical density using a Coleman 19 x 105 millimeter curvette reference blank of the sodium citrate solution.

The test tubes of diluted semen were inverted several times then emptied into curvettes and placed in the instrument. After allowing one minute for the bubbles to rise, and optical density was read directly off of a Coleman 6-401 galvanometer scale panel. This scale panel permitted readings in both percent transmittance and optical density, but the latter was used because it is logarthmic and, according to the Lambert-Beer Law, directly proportional to the concentration.

The concentration established by the direct hemocytometer count was then regressed on the optical density of the diluted sample. This regression produced a linear relationship by which the spermatozoa concentration, corresponding to any particular density reading, could be determined.

Testicular Measurements

Approximately one month after the collections were terminated, the boars were castrated. The testicles were removed from the tunica vaginalis and the pampiniform plexus was trimmed away before weighing the intact testis and epididymis on an O'Haus triple beam balance. The testis and epididymis were then separated and the testis was weighed. The difference between these two weights

was recorded as the epidiymal weight.

Two sections were removed from each testicle along the same axis, but opposite the epididymis. This is the area (B) described as representative of the testicular parenchyma by Kennelly and Foote (1964). The tissue sections were immediately immersed in Bouins fixative where they were left for thirty-six hours. The fixed specimens were imbedded in paraffin, sectioned at eight microns and the slides stained with hematoxylin and eosin by personnel of the Veterinary Medicine staff.

Two measurements were taken from the microscopic examination of the slides. The first was the average number of seminiferous tubules that could be counted in one low power (100x) microscopic field. This value was the average obtained from observations of twenty fields in both the left and right testicles. The sectioning of the testis resulted in the tubules being cut at innumerable angles, and for counting purposes any section enclosed in a complete and separate basement membrane was counted as a tubule.

The other measurement was the average diameter of the seminiferous tubule, as determined by an ocular micrometer. Ten randomly chosen, circular tubules were measured in each testis.

Data Analyses

Graphic presentation was used to follow the pattern

of semen characteristic fluctuations over the seven month period involved in this study. The changes in environmental temperatures were also illustrated graphically for the same period.

The period involved in this study was bisected by an average environmental temperature which is considered near optimum for swine of the weight involved (Heitman et al. 1949). The "t" test was used to compare mean performances of the boars during the periods when temperatures were above and below optimum.

Simple correlations were used to determine the relationships between the various semen criteria, and between testis measurements and semen production. An analysis of variance for a completely randomized design was used to determine the effect of the type of shelter on semen production. Statistical analyses used in this study were calculated according to procedures set forth by Snedecor (1956), except for Kramer's modification of Duncan's New Multiple Range Test which was used in conjunction with the analyses of variance described above, and is outlined by Steele and Torrie (1960).

RESULTS AND DISCUSSION

Collections

During the course of this study, 712 individual collections were attempted, and 634 (89.0 percent) of these were successful. The successful collections per boar ranged from 69.7 percent to 97.8 percent of those attempted as presented in Table XI. Ejaculates were obtained from 96.6, 83.1 and 85.4 percent of the collections attempted from the boars in Lots I, II and III, respectively.

Three litter mates, boars 61-6, 61-7 and 61-8, which were originally assigned to the experiment varied widely in their response to collection. Boar 61-7 was culled because he could not be trained to serve the dummy, 71.9 percent of the attempts to collect boar 61-6 were successful, and 97.8 percent of the attempts to collect boar 61-8 produced ejaculates. The failure to obtain regular collections from trained boars has also been reported by Hancock (1959), and Cox and Willham (1961).

Semen Criteria

The number of observations, means, standard deviations and coefficients of variation for the semen characteristics used in this study are given in Table II. The large volume

TABLE II

POPULATION DESCRIPTION OF SEMEN

Semen Characteristic	n[1]	Mean	S.D.	C.V.(%)[2]
Initial pH	83	7.45	0.136	18.3
Percent Motile Sperm	216	55.37	27.65	50.1
Percent Abnormal Sperm	216	11.66	13.02	111.7
Volume (ml.)	634	212.5	93.1	43.8
Concentration (Sperm/ml.)	216	213.01($X10^6$)	186.54	87.6
Total Sperm/Ejaculate	216	36.88($X10^9$)	17.73	48.1

[1]Number of observations
[2]Coefficient of variation

and long duration of ejaculation in the boar accounts for some of the variation shown in this table. The means for the semen characteristics in this study are well within the ranges established in the literature, taking into consideration the age, weight, breed and treatment of the boars used in the various studies.

The reported pH values are consistently in a range of 7.3 to 7.9 which Polge (1956) and Nalbandov (1964) present as normal values. The average percent motile spermatozoa determined in this study was close to the fifty-eight percent value reported by Turkheimer, et al. (1958), but considerably lower than the 80.2 percent motility found by Gerrits et al. (1962). Polge (1956) and Gerrits et al.

(1962) reported ten percent and 9.6 percent abnormal spermatozoa in boar semen, respectively, and this is comparable to the 11.7 percent value of this study.

The average strained ejaculate volume of 212.5 milliliters for the boars in this study is very close to the 205.5 reported by Gerrits et al. (1962). A somewhat lower volume of about 172 milliliters has been reported by both Turkheimer et al. (1957), and Baker and Dziuk (1964), but their respective total sperm per ejaculate values of 45.1 and 44.0 x 10^9 were higher than the 36.9 x 10^9 average of this study. Borton et al. (1965) presents 150 to 250 x 10^6 spermatozoa per milliliter as a normal range for the average concentration of boar semen.

Interrelationships of Semen Criteria

Table III gives the simple correlations between the semen criteria employed in this study. Four of the fifteen correlations were statistically significant, but only the one between concentration and total sperm per ejaculate accounted for more than twenty percent of the associated variance between the two characteristics involved.

The product of concentration and volume provided the total sperm per ejaculate values, and the correlations show a much more intense association between concentration and total sperm ($r = 0.68$) than between volume and total sperm ($r = 0.10$). This inequality is in accord with the coefficient of variation (Table II) being twice as large for

TABLE III

SIMPLE CORRELATION COEFFICIENTS BETWEEN SEMEN CHARACTERISTIC MEASUREMENTS

	Percent Motile Sperm	Percent Abnormal Sperm	Volume	Concentration	Total Sperm
Initial pH	-.34**	.06	-.12	-.04	-.23*
Percent Motile Sperm		-.42**	.02	-.01	.04
Percent Abnormal Sperm			.04	-.03	-.02
Volume				-.12	.10
Concentration					.68**

*Level of significance = $P<0.05$
**Level of significance = $P<0.01$

the concentration as it is for the volume (87.6 vs. 43.8), and the low correlation between these two characteristics ($r = -.12$).

The two other highly significant (P 0.01) correlations show negative relationships between initial pH and motility ($r = -.34$), and between motility and the percent abnormal cells ($r = -.42$). Both of these correlations are substantiated by the results of several bull semen studies, as summarized by Salisbury (1955).

No significant correlation existed between pH and either of the components of the total spermatozoa values, but a significant ($P<0.05$) correlation ($r = -.23$) was found between pH and total sperm per ejaculate. This correlation is consistent with both volume and concentration exhibiting

a low, negative correlation with pH, and a positive correlation with total sperm.

The coefficients of determination (r^2) range from .001 to .014 for the non significant correlations, and from .05 to .47 for the significant correlations. Because of the low correlations between these criteria, each has to be individually determined if it is considered important.

Semen Characteristic Patterns

Research has shown that semen quality values do not remain constant, but fluctuate with varying climatological conditions over time. The two part figures, Figures 1 through 5, present the patterns established for the semen criteria used in this study, for the period involved. Each point, in the single lined graph of each pair, represents the average value for all eight boars during a three week period, irrespective of the lots. The lots were separated in the second three line graphs, and each point represents the average value for the boars in the designated lot for the three week periods. The average values of the semen characteristics are presented on the ordinate axis as a percent of the respective means. Therefore, the graphs do not show quantitative values or differences, but illustrate the direction and relative magnitude of the changes.

Figure 6 illustrates the average maximum and minimum daily temperatures for each three week period. The

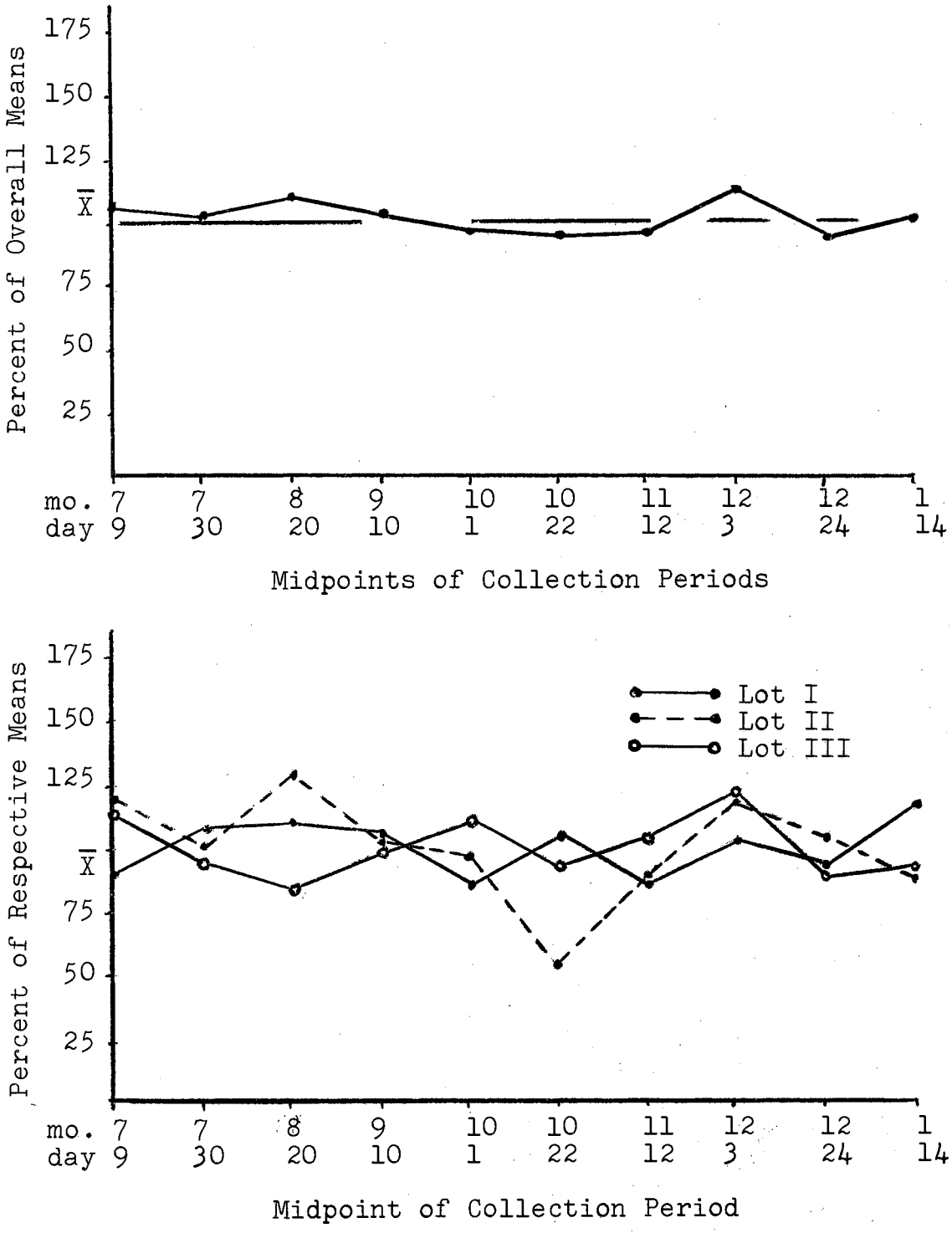

Figure 1. Pattern of Percent Motile Spermatozoa as a Percent of the Respective Means

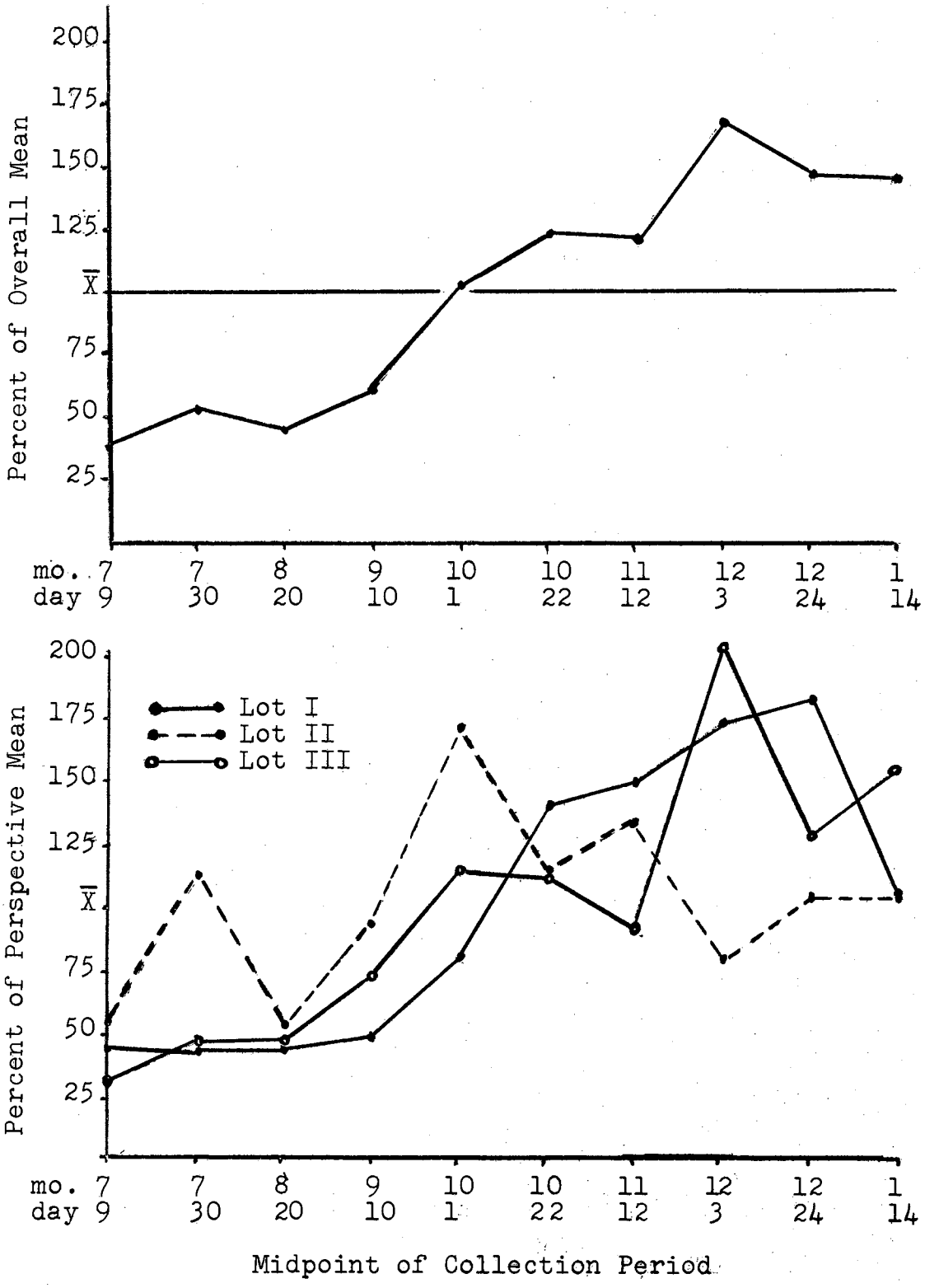

Figure 2. Pattern of Percent Abnormal Spermatozoa as a Percent of the Respective Means

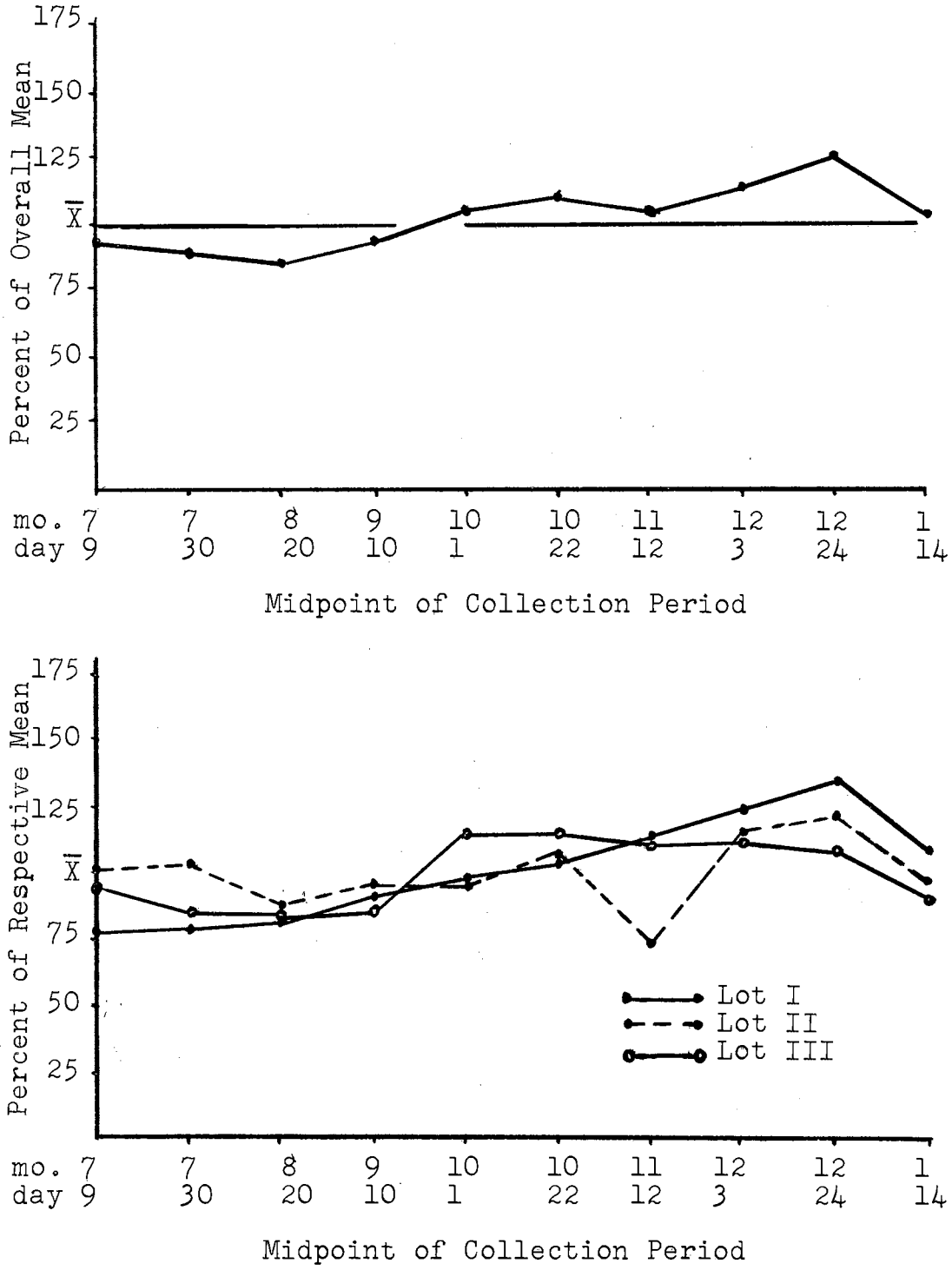

Figure 3. Pattern of Ejaculate Volume as a Percent of the Respective Means

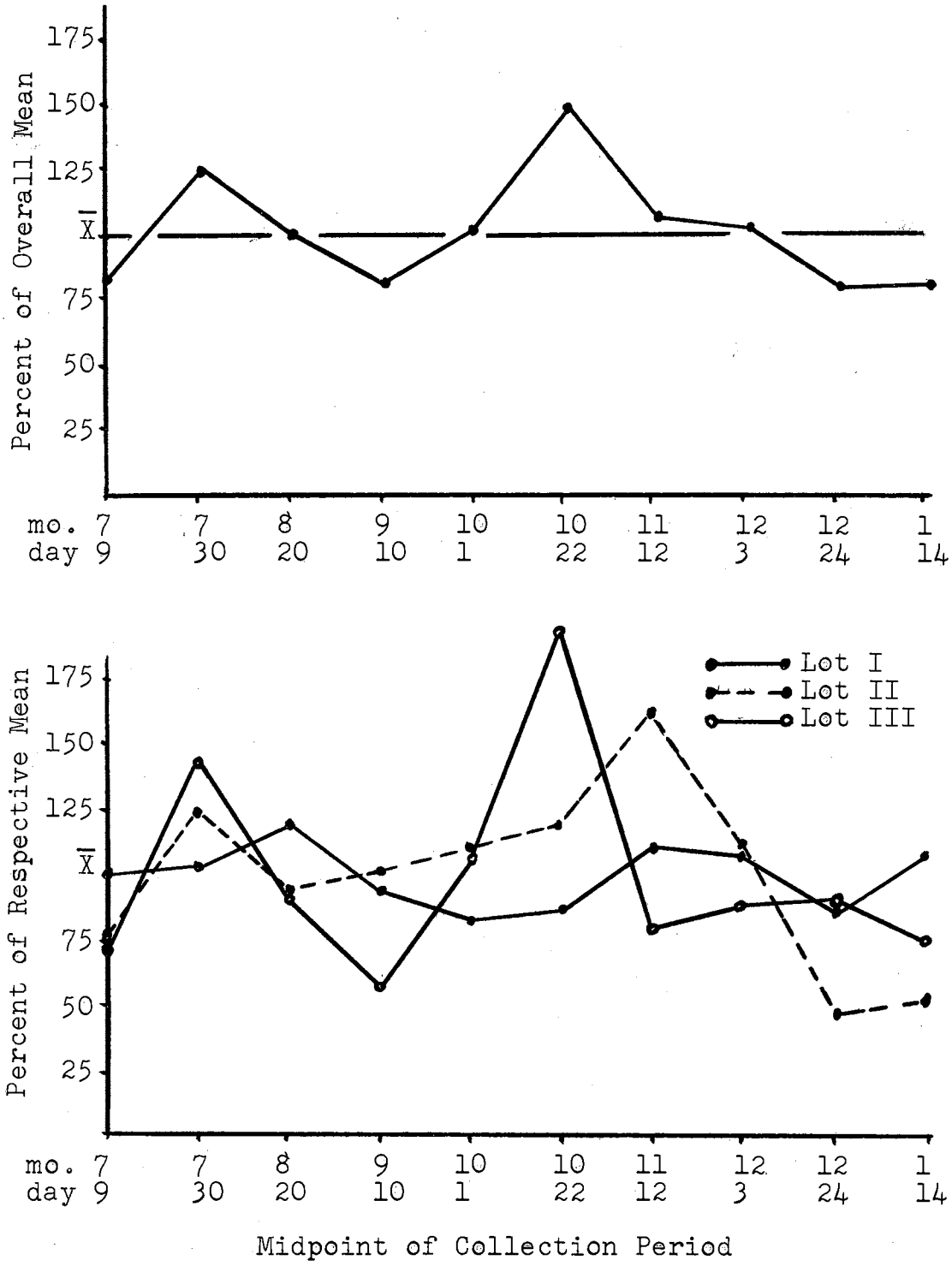

Figure 4. Pattern of Spermatozoa Concentration as a Percent of the Respective Means

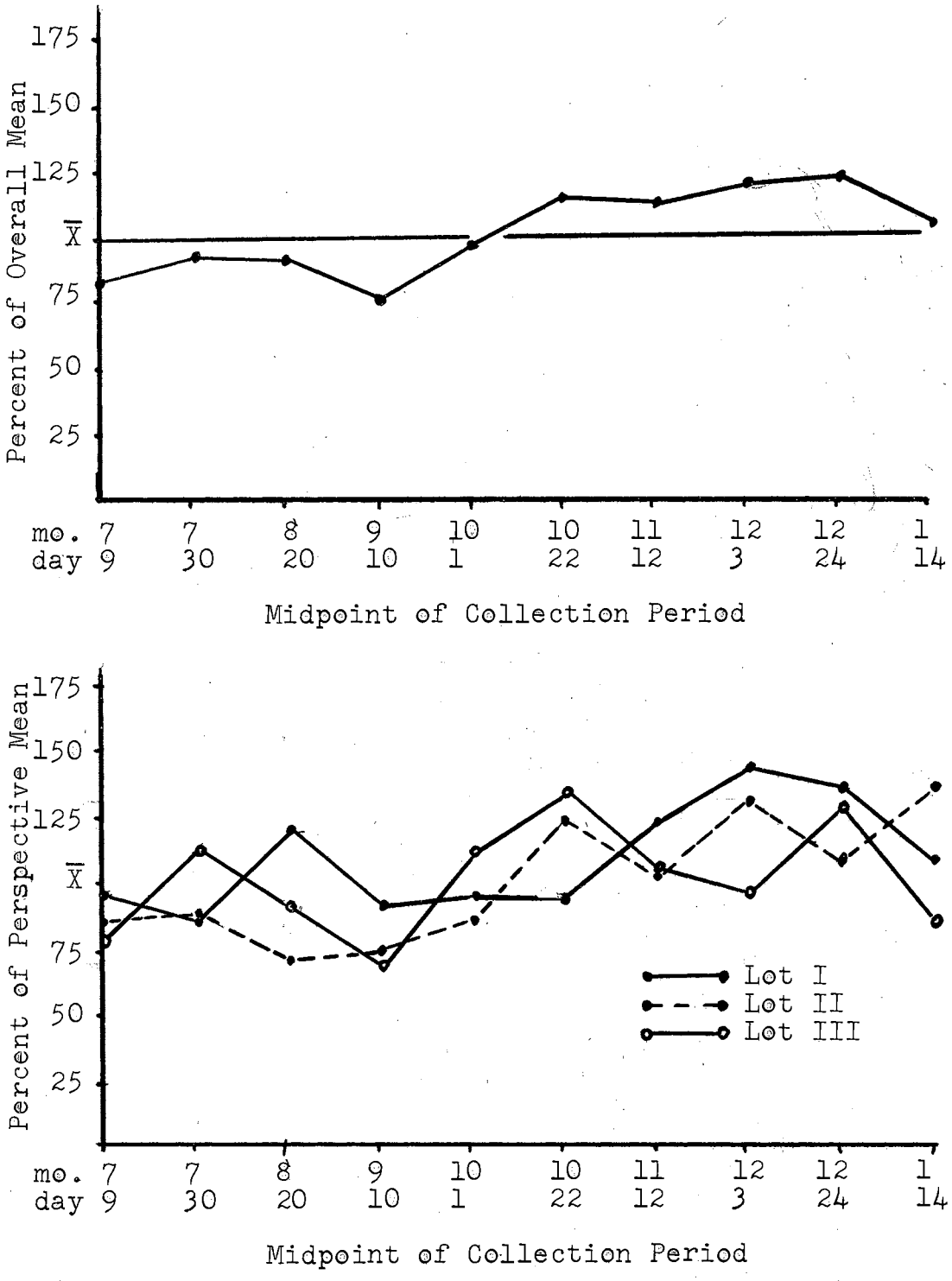

Figure 5. Pattern of Total Sperm Per Ejaculate Values as a Percent of the Respective Means

temperature graph is presented for comparison with semen characteristic patterns, but the extent to which the patterns were actually affected by the temperature changes cannot be determined.

The vertical line in Figure 6 bisects the experimental period into one half, beginning in July, during which the average of the maximum and minimum temperatures is greater than 61° F., and the second half is characterized by averages of the two extremes of less than 61° F. This division was based on the effect that the physiological mechanisms described by Winchester (1964) have on production performance, which Heitman and Hughes (1964), Heitman et al.

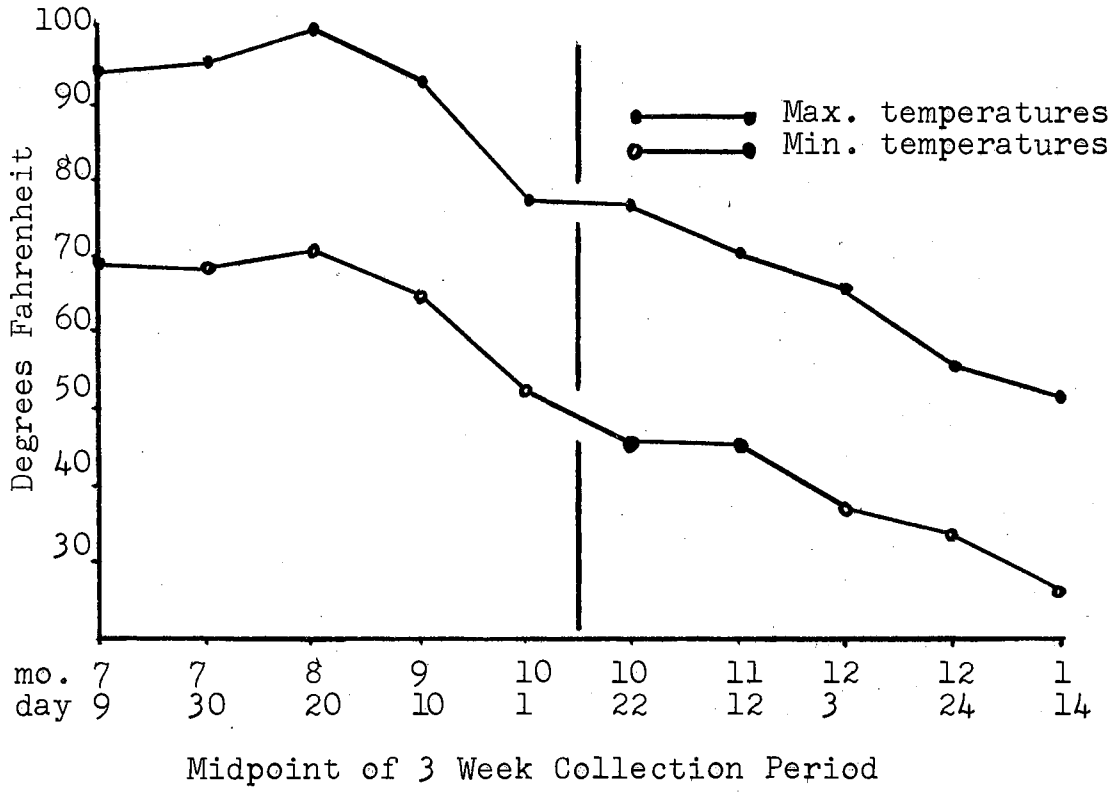

Figure 6. Average Maximum and Minimum Daily Temperatures for the 3 Week Collection Periods

TABLE IV

"t" TEST COMPARISONS OF THE MEAN SEMEN CRITERIA VALUES ESTABLISHED WHEN THE AVERAGE TEMPERATURES WERE ABOVE AND BELOW THE OPTIMUM

Criteria	Mean	"t" Value
Percent Motile Sperm	56.59 ± 2.56[a] 53.71 ± 2.72[b]	.753
Percent Abnormal Sperm	6.42 ± 1.20 15.05 ± 1.28	15.16**
Volume (ml.)	197.09 ± 5.12 238.21 ± 5.34	3.195*
Concentration ($X10^6$/ml.)	197.27 ± 17.24 216.54 ± 18.40	.747
Total Sperm ($X10^9$)	32.775 ± 1.64 42.186 ± 1.75	3.838**

[a]Mean ± standard error for period beginning in July
[b]Mean ± standard error for period beginning in October
*Level of significance = $P<.005$
**Level of significance = $P<.001$

(1954) and Heitman et al. (1958) reported as maximum for heavy pigs at this temperature.

Table IV compares, by "t" tests, the mean semen criteria values established when the average temperatures were above and below this 61° F. optimum. The percent abnormal spermatozoa, volume and total sperm per ejaculate means were all significantly greater in the cooler period. The differences in the means of initial motility and concentration, for the two periods, were not significant. The one lined graphs of Figures 2, 3 and 5 illustrate the

significant changes that occurred, and the inverse similarity between these criteria graphs and the temperature graph (Fig. 6) indicates that some relationship does exist between them. It is conceivable that the seminal trends are a carry-over effect from the preceeding collection routine, but only a longer experiment could separate this possibility from the cyclic, seasonal effect reported in earlier research.

Since only seven months were involved in this study there is no way of knowing if the highest or lowest annual levels of the semen characteristics had been obtained, and this curtails comparison with other studies. The December peak in the percent of abnormal cells in this study is in contradiction to the July, August and September maximum reported by Erb et al. (1942) for dairy bulls in New York, but this discrepancy may be due to a species or location difference.

The sensitivity of the percent abnormal spermatozoa as an index of semen quality has been demonstrated in the ram. Simpson and Dutt (1958) found this semen criterion to be the only one, of the several used, that was affected by the mildest of three thermal treatments in their study. Dutt and Simpson (1957) reported significantly more abnormals in the semen of rams maintained for four months where the highest, maximum average monthly temperature was a relatively mild 89° F., than in rams maintained at 46° F.

The December peak for abnormals observed in this study may be the result of the testis sensitivity, as demonstrated in the ram, and an interval between the appearance of maximum ejaculate symptoms from previous stress. A nine week delay has been established by Pace et al. (1959) and Cox Willham (1961) from a single exposure to irradiation. Several exposures over a period of time was shown by Ladd and Murphree (1964) to lengthen this interval and have a cummulative effect on the ejaculate symptoms. If this reasoning is valid, the high percentage of abnormals in the first part of December would have resulted from environmental influences of, and prior to, early October.

The larger volumes obtained during the cooler period of this study are substantiated by the findings of Erb et al. (1942) and Mercier and Salisbury (1946) working with bulls, by Hafez et al. (1954) and Cupps et al. (1960) with rams and by Stevermer et al. (1961) as observed with boars. The values for total sperm per ejaculate agree with the bulk of the literature which indicates highest production during cool weather as reported by Sapford (1951), Mercier and Salisbury (1946), Hafez et al. (1954) and Cupps et al. (1960) which include both sheep and cattle studies, but is opposed to the boar study by Stevermer et al. (1961). Stevermer and his associates observed the greatest sperm production in the hot weather of June through August. The same researchers all indicate that concentration, perhaps because of its negative relationship to volume, is highest

in the warmer summer months. Motility estimates are quite variable and, although they tend to be highest in the warmer periods, considerable inconsistency is found in different studies.

Lot Effect on Semen Criteria

Representative average afternoon temperatures for the three shelters, and the corresponding average maximum daily environmental temperatures for eight of the three week collection periods are presented in Table V. The shelter temperatures were taken only once each day and are not representative of the extremes that may have been reached, but they do provide a means of comparison between the lots.

Table V shows that the difference in the temperatures of Lots I and II was generally less than 2° F. while the temperatures in Lot III varied less and were considerably cooler in the hot weather, and warmer in the cool weather. Although Lots I and II appear to have been very similar in ambient temperature, there was no attempt to measure differences in ground and bedding temperatures, ventilation, or available light of the shelters.

The lot effect, on each of the five criteria, was evaluated by analyses of variance for a completely randomized experiment, and the results of these analyses are tabulated in Table VI. Duncan's New Multiple Range Test with Kramer's modification for unequal numbers (Steel and Torrie, 1960) was used to locate the differences in lot

TABLE V

CORRESPONDING MAXIMUM DAILY ENVIRONMENTAL AND AFTERNOON SHELTER TEMPERATURES[1]

Environmental Max. Aug.	Shelters in Lot I	Lot II	Lot III
99.5	93.2	91.4	74.5
92.5	84.5	84.1	71.1
76.8	73.7	73.3	69.2
76.5	69.6	70.1	69.7
70.1	62.4	63.4	72.3
65.6	54.8	55.0	64.0
55.9	46.9	48.1	56.9
51.5	42.8	42.9	55.9

[1]Averages for a three week period expressed as degrees fahrenheit.

effects which the analysis of variance had shown to exist. The results of the multiple range tests are presented in Table VII.

The facilities were designed to be applicable to production conditions, and did not impose rigid climatological controls. The air-conditioned house of Lot III should have provided the most protection from environmental extremes with the three sided shelter of Lot II providing more protection than the open shade of Lot I. It is assumed that the physiological stress on the boars was decreased as increased environmental control was exerted, but the effect, if any, of the restricted watering and wallows in Lots II and III cannot be evaluated.

The analyses of variance indicates, that of the

TABLE VI

TABULATED ANALYSES OF VARIANCE OF LOT EFFECT ON SEMEN CHARACTERISTICS

Source	D.F.	Mean Square	F Value
Percent Motile Sperm			
Between Lots	2	1,009.1	1.319
Within Lots	213	764.9	
Percent Abnormal Sperm			
Between Lots	2	980.5	5.980**
Within Lots	213	164.0	
Volume (ml.)			
Between Lots	2	43,403	4.310*
Within Lots	631	10,071	
Concentration (/ml.)			
Between Lots	2	2,228.95	7.048***
Within Lots	213	316.25	
Total Sperm			
Between Lots	2	31.850	10.789****
Within Lots	213	2.952	

*Level of significance = P<.025
**Level of significance = P<.005
***Level of significance = P<.001
****Level of significance = P<.0005

semen characteristics involved, only motility estimates were not affected by the lot treatments. Concentration and total sperm per ejaculate were significantly higher in Lots II and III, which were not significantly different, than in Lot I, and this concurs with the hypothesized stress gradient. Volume, which would also be expected to be greatest under optimum conditions, was higher in Lot I than in Lot III, while Lot II was not significantly different from

TABLE VII

DUNCAN'S NEW MULTIPLE RANGE TESTS[1] OF LOT MEANS[2] FOR SEMEN MEASUREMENTS

	Lot I	Lot II	Lot III
Percent Motile Sperm	58.0[a]	57.4[a]	51.5[a]
Percent Abnormal Sperm	12.23[a]	4.98	12.01[a]
Volume (ml.)	222.1[b]	215.6[ab]	195.9[a]
Concentration/ml.($X10^6$)	151.38	218.61[a]	253.71[a]
Total Sperm($X10^9$)	30.50	43.72[a]	39.53[a]

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

[2]Lot means of the same measurement with the same superscripts do not differ significantly (tested at $P<.05$ level of significance).

either of them. Lots I and III were characterized by about twelve percent abnormal spermatozoa and were significantly higher than the five percent value for Lot II.

Dutt and Simpson (1957) reported that cooling rams during hot weather had a favorable effect on semen quality as measured by spermatozoa concentration, motility and the percent abnormals. When males were exposed to increased temperatures the semen measurements used by Casady et al. (1953), Simpson and Rice (1957) and Simpson and Dutt (1958) were observed to decline, with the exception of volume which was not effected. The boars in Lot III were both cooler in the hot weather and warmer in the cold weather

than the other treatments, and although it may be reflected in the sperm production values it is not evidenced in the other semen criteria. Comparison of the treatment means in Table VII reveals that the overall semen quality was higher for the boars housed in the three sided shelter than those housed in either of the other shelters.

The validity of the analysis for the fixed lot effects depends upon the basic assumption that the observations may be presented as a linear model in which they equal a constant population mean, plus the true effect of the treatments, plus a normal and independently distributed experimental error. The error term in this instance is the effect of a boar being subjected to a specific treatment, and it also includes the effects of extraneous factors. These extraneous factors could contaminate the results of the experiment, and randomization was relied upon to prevent this contamination.

Population Description of Testicular Measurements

The number of observations, means, standard deviations and coefficients of variation for testicular related measurements are presented in Table VIII. The mean testis and epididymis weights both represent only one of the paired organs and is the average of the sixteen specimens available from the study.

The left testicle was heavier than the right in seven of the eight boars, and in boar, 55-7 this difference was

TABLE VIII

POPULATION DESCRIPTION OF TESTICULAR MEASUREMENTS

Measurement	N[1]	Mean	S.D.	C.V. (%)[2]
Testis Weight (grams)	16	474.2	95.5	20.1
Epididymis Weight (grams)	16	113.2	19.8	17.5
Tubule Number/100 x field	320	16.85	2.68	15.9
Tubule Area[3] (Sq. microns)	160	36,063.7	5,154.0	14.3

[1]Number of observations
[2]Coefficient of variation
[3]Cross section area of a single seminiferous tubule

337 grams. The smaller testicle weighed only 153 grams compared to an overall right testicle average of 444 grams, and was considered abnormal even though abnormalities were not observed in the semen of that boar. The left testicle was an average of 20.8 $\pm$ 17.2 grams heavier than the right from the seven normal boars, and a paired "t" test indicated that this was a significant ($P<0.01$) difference. Hauser et al. (1952), Holet (1952) and Hancock (1959) have reported similar observations on the within pair differences of boar testes.

The tubule diameter, which was used to calculate the tubule area, averaged 214.0 microns, and is comparable to the 165.7 micron diameter reported by Philip and Andrews

TABLE IX

CORRELATION COEFFICIENTS INVOLVING MICROSCOPIC TESTIS MEASUREMENTS

	Tubule[1] Area	Volume	Concentration	Total Sperm/ Ejaculate
Tubule No./ 100 x Field	-.90**	-.01	.09	-.07
Total Tubule Area/Field[2]		.49	-.32	.05

[1]Cross section area of a single seminiferous tubule
[2]Product of a single tubule area and the tubule number per field
**Level of significance = $P < .01$

(1936) for 147 day old boars. Table IX shows a highly significant correlation of -.90 between tubule area and tubule number per field, and suggests that testes with large tubules would have fewer, or less convoluted tubules. Testes with larger but fewer tubules should have more spermatogenic area than in an inverse situation, but the low correlation with total sperm values in Table IX does not reflect such a relationship. The 0.49 correlation between total tubule area per field and volume of the ejaculate, though non significant with the numbers involved, suggests that some unexplained relationship might exist between them.

The relationship of testis weight with six other measurements was considered, and the results are presented in Table X. The only significant correlation was between

TABLE X

CORRELATION COEFFICIENTS WITH TESTIS WEIGHT

Body Wt.	Epididymis Wt.	Tubule Area	Tubule No. 1(100X) Field	Tubule Area 1 Field	Total Sperm/ Ejaculate
.04	.66**	.19	-.19	-.18	.45

**Level of significance = P<.01

testis and epididymis weights (r = .66), and is comparable to the .71 correlation obtained from boars up to 158 days of age by Hauser et al. (1952). The .54 correlation between tubule diameter and testis weight, which Hauser et al. reported in the same study, is much larger than any found for the histological measurements from the mature boars of this study.

Willett and Ohms (1957) established a 0.94 correlation between scrotal circumference and excised testis weight in bulls, and found this easily attainable measurement to have a 0.43 correlation with the sperm production of routine service. This adds credence to the 0.452 correlation between the testis weights and total sperm values found for boars in this study. The apparent lack of any relationship between a boar's testes and body weight was also found by McKenzie et al. (1938).

The mean testis and semen measurements are presented together, for each individual boar, in Table XI.

TABLE XI

MEAN MEASUREMENTS FOR INDIVIDUAL EXPERIMENTAL UNITS

Measurement	Boar							
	55-7	61-8	39-5	61-6	54-13	73-1	54-12	53-11
pH	7.51	7.37	7.49	7.49	7.39	7.27	7.56	7.49
Percent Motile Sperm	50.8	69.9	54.3	71.2	43.6	87.4	46.3	18.4
Percent Abnormal Sperm	13.7	7.1	14.6	2.3	7.2	1.7	6.0	28.4
Volume (ml.)	124.3	254.3	310.8	146.5	259.9	251.4	130.6	190.8
Concentration/ml.($X10^6$)	170.7	174.1	108.6	310.7	139.8	192.8	410.6	177.9
Total Sperm/Ejac. ($X10^9$)	24.22	39.10	29.85	52.38	36.35	44.84	40.92	33.37
Tubule Dia. (microns)	221.54	232.41	198.79	224.50	216.59	210.66	214.61	192.85
Tubule No./(10X)Field	15.30	13.80	19.35	16.32	13.80	18.52	17.12	20.62
Paired Testis Wt.(gr.)	643	1095	1001	1051	1045	866	869	1017
Paired Epididymis Wt. (gr.)	157	241	251	237	276	230	231	188
Percent Successful Collections	94.4	97.8	97.8	71.9	94.4	91.0	69.7	95.5

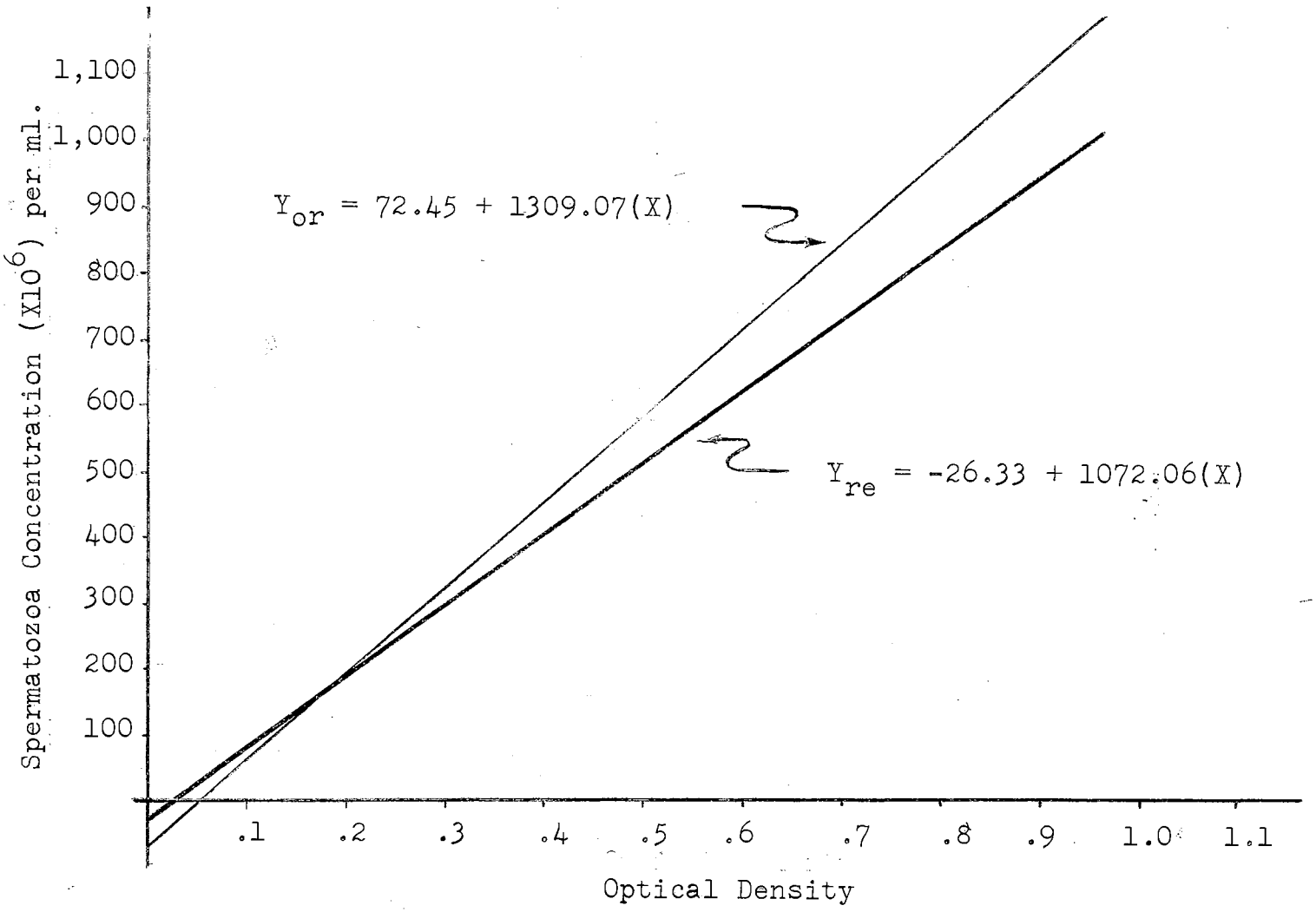

Figure 7. Spermatozoa Concentration Regressed on Optical Density as Established from the Original (or) and Revised (re) Data

TABLE XII

COMPARISON OF THE ORIGINAL AND REVISED CONSTITUENTS OF THE REGRESSION OF SPERMATOZOA CONCENTRATION (Y) ON OPTICAL DENSITY (X)

	Original	Revised
Estimate of Y	-72.45+1309.07(X)	-26.33+1072.06(X)
Number of Observations	233	228
Correlation Coefficient (XY)	.956	.959
Coefficient of Determination	.916	.920
Standard Error of the Estimate	56.27	35.69
Mean Concentration (Sperm/ml.)X10^6	222.09	200.931
Standard Error (of $\bar{Y}$)	11.20	8.083

Boar Spermatozoa Concentration Determination

This phase of the experiment was designed to establish a regression equation for the estimation of spermatozoa concentration from the optical density, as measured in a colorimeter, of a semen sample. The data collected for this purpose were the optical density reading and a corresponding hemocytometer count of the spermatozoa concentration of 233 individual semen samples.

The number of spermatozoa per milliliter ranged from 56.25 X 10^6 to 1,720.00 X 10^6, and the optical density

readings ranged from .05 to 1.00 (the latter two values correspond to 89.5 and 9.5 percent light transmittance, respectively). When all 233 observations were plotted in a graph, it was evident that five of the concentration values of 927.50×10^6 sperm per milliliter and higher were considerably greater and deviated much farther from the regression line than any of the other values. These five observations were eliminated in the calculation of a revised regression equation which included the 228 remaining observations, and involved a maximum concentration of 787.50×10^6 spermatozoa per milliliter.

The original and revised regression equations are compared graphically in Figure 7 and statistically in Table XII. The standard errors of the estimates indicate that a considerable increase in accuracy is realized with the revised equation, and although the numerical values of both are relatively large, the actual interval about the regression line is reduced by the steep slope of the line. Acceptance and use of the revised equation, over the original, should be justified in as much as it included 97.9 percent of the original observations, and the original mean of the concentration values plus two standard deviations is equal to 607.59×10^6, or less than the maximum value used in the calculation of the revised equation.

The 0.96 correlation coefficients obtained between the direct count, concentration determinations and photoelectric readings in this study, are in accord with the

0.978, 0.97, 0.95 and 0.92 values calculated from the data of Comstock et al. (1943), Willett and Buckner (1951), Young et al. (1960), and Foote and Boucher (1964), respectively. Willett and Buckner (1951) established a regression line with twenty-five bull ejaculates and expressed the standard error of the estimate as twelve percent of the mean concentration or five percent of the range in sperm numbers, and the comparable values for the revised equation in this study were 17.7 and 4.9 percent, respectively.

A direct comparison of regression equations for spermatozoa concentration requires that the same species and dilution ratio be involved in each study, but according to Willett and Buckner (1951), and Foote and Boucher (1964) the kind of photoelectric meter used may vary. Two equations established by Young et al. (1960), for the estimation of boar spermatozoa in millions per milliliter, at dilution ratios of 1:9 and 1:19 were -72.4 + 862.1(X) and -21.0 + 1328.9(X) respectively. The 1:10 dilution ratio used in establishing the equations reported here, produced a "Y" intercept and slope value similar and intermediate to those reported by Young et al.

Willett and Buckner (1951) reported that the removal of seminal plasma from bull semen did not improve photoelectric estimates of concentration, but such a procedure would be expected to move the "Y" intercept to zero from its normally negative position. In Figure 7 the point

where the regression line crosses the abscissa corresponds to the estimated spermatozoa concentration of zero, and serves as an estimate of the optical density of the seminal plasma.

The regression equation (-26.33 + 1072.06X) results in a number which is multiplied by 10^6 to obtain the estimated number of spermatozoa in a milliliter of gel free boar semen. The application of this equation requires that the neat semen be diluted at a ratio of 1:10 with a suitable diluter, preferrably in a 19 X 105 milliliter cuvette, and with the instrument's light source set at a wave length of 625 millimicrons.

SUMMARY

Semen evaluations were used to follow quantitative and qualitative semen characteristic changes of eight boars over a seven month period. Three shelter modifications were used to house the boars and their effect on reproductive function was also analyzed. At the termination of the trial the boars were castrated and gross and histological observations were made on the testis to examine the relationships of these measurements to semen production.

The results indicated that the quantitative values of volume and total sperm per ejaculate were higher during cool weather, but the semen quality, as measured by the percent abnormal sperm, was lower during this period. The analyses for shelter effect suggests that the shelters which provided the most protection from the environment, the three sided shelter and the air-conditioned house, may have been the most conducive to spermatogenic function as measured by concentration and total numbers of spermatozoa.

The greatest ejaculate volumes were obtained in the coolest part of the trial period, but they were not associated with the coolest shelter, and there is no immediate explanation for this apparent contradiction. Nor is there any available explanation for the percentage of abnormal

spermatozoa being significantly higher in the air-conditioned house and shade lots than in the lot with the three sided shelter. There is a need for more research in this area, first to see if the results are repeatable, and secondly to initiate procedures from which causative factors could be assertained.

The relationships established between testicular and semen characteristic measurements were to low to have any predictive value. Normal semen values were obtained from one boar which had one abnormally small testis, and a very high percentage of abnormal sperm was associated with another boar that had what appeared to be normal testicular development.

The semen collected for evaluation was also used to develop a regression equation for the estimation of spermatozoa concentration, in gel free boar semen, by photoelectric measurement. The spermatozoa concentrations, determined by direct count, was regressed on corresponding optical density readings and a .96 correlation was found between the two measurements. The resulting equation (-26.33 + 1070.60X) had a standard error which was $\pm$18 percent of the mean concentration value. The photoelectric method of concentration estimation is faster and subject to fewer technique errors than the direct count, and may be of considerable use in future studies.

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