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The Influence of the Thyroid Gland and Ambient Temperatures on Fertility of Rams

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CONTENTS

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JERRY R. BROOKS AND C. V. ROSS

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INTRODUCTION

Among farm animals, the marked seasonal breeding pattern of sheep is unique. For a considerable portion of the year the ewe exhibits little interest in and will not accept the ram. With the advent of the cool nights of late summer, a resurgence of the mating urge is manifested. It is of great practical importance to the flock owner to breed as many ewes as early as possible in order for the lambs to reach marketable size and condition by May and June when Spring lambs traditionally command the highest prices.

In some areas, particularly the central and southern states, sheepmen have experienced difficulty in getting ewes to settle early in the breeding season. Part of this early infertility is traceable to the lowered fertility of many rams during and following periods of high environmental temperature. Such a reproductive deficiency is reflected in delayed lambing dates with a consequent economic loss to the producer.

Methods of preventing or alleviating "summer sterility" would be of obvious benefit to sheep producers.

REVIEW OF LITERATURE

A. Effect of Temperature on Spermatogenesis and Semen Quality

Spermatozoa are a product of the testis an organ which is quite responsive to a variety of influences that may exert an adverse effect upon spermatogenesis, thus limiting reproductive efficiency. Such an influence is high temperature, both environmental and, under certain conditions, the animal's own.

To illustrate, it has been demonstrated with dogs, Griffiths (1893); rats, Moore (1922); guinea pigs, Moore (1924); rams, Moore and Oslund (1924); apes, Brummelkamp (1933); and rabbits, Asdell and Salisbury (1941), that return of the testes from the scrotum to the abdominal cavity will be followed closely by a loss of spermatogenic activity. A gradual return to normal functioning upon replacement of the testes in the scrotal sac led Moore (1922) to suggest that a temperature differential might be the factor involved. A similar theory was advanced by Crew (1922) who stated the temperature regulating mechanism of the scrotum allowed a lower temperature to exist there than in the body cavity, a condition essential for spermatozoa formation. This is in agreement with observations by Moore and Quick (1924) and Moule and Knapp (1950). The latter two workers inserted fine wire thermocouples into the testes and were able to show that testicular temperatures in resting rams were uniformly lower than rectal temperatures. The magnitude of these temperature differences was critically studied by Foote *et al.* (1957) who kept both sheared and unsheared rams in a room in which temperature and humidity were varied. Both rectal and testis temperatures were increased by temperature changes from 70 to 82° F. and from 82 to 95° F. In addition, the overall averages for rectal and testis temperatures showed that these were increased with high (78-88 percent), as contrasted to low (57-69 percent), humidity. Shearing was without noticeable effect on rectal temperatures, but sheared rams had lower testis temperatures.

Moule and Knapp (1950) reported that insulating the scrotum brought about increased testicular temperature. This had been indicated in previous work, Moore and Oslund (1924); Brummelkamp (1933); Phillips and McKenzie (1934), showing an inhibition of spermatogenesis following scrotal insulation. Webster (1952) noted that a heavily wooled scrotum may provide sufficient insulation to reduce spermatogenesis. Recent work (Austin *et al.,* 1961) has demonstrated that in bulls, insulation injury is primarily to those cells more highly developed than spermatogonia.

Heller (1929) removed the testes of guinea pigs and rats while leaving the epididymis intact. He compared the viability of spermatozoa from epididymides left in the scrotum and those returned to the abdominal cavity. The capacity for motility in those cells from the normally located epididymis persisted much longer. This result was felt to show that the thermoregulating function of the scrotum encompasses formed cells as well as the germinal epithelium of the testis.

The effect of the direct application of heat to the external surface to the scrotum was studied by Moore (1924). Water at 47° C. was applied to the scrotal surface of the guinea pig for ten minutes. Within two weeks the seminiferous tubules near the point of water application had undergone marked degeneration. Seminiferous tubules on the testis side away from the site of application appeared practically normal. He further showed that a five minute submersion of the exposed guinea pig testis in water at 47° C. was sufficient to cause degeneration in all tubules. Submersion in 43° C. water for five minutes did not noticeably affect the exposed testis. This work is in accord with similar studies by Young (1927) who showed, in addition, that a recovery of spermatogenic function may take place if living spermatogonia are present. Such a return to spermatozoa formation was said to require about forty-five days in the guinea pig.

The effect on semen quality of high environmental temperatures has been studied by several workers. Dutt and Hamm (1955) conducted winter trials in which both sheared and unsheared rams were placed for one week into a room heated to 90° F. Control rams were kept in unheated quarters. Semen from the rams subjected to heat did not differ significantly from that of controls during pre-test collections or immediately at the completion of the test. However, five weeks after the test period sperm motility was 85, 80 and less than ten percent for the controls, hot room sheared and hot room unsheared rams, respectively. Percentages of abnormal sperm were 10.0, 8.1 and 71.0, respectively. Eight weeks after the test, semen from all the rams was again similar, indicating a temporary impairment of spermatogenesis in the hot room unsheared rams. El-Sheikh and Casida (1955) subjected male rabbits to a temperature of 110° F. and a relative humidity of 30-40 percent for one hour. They observed that spermatozoan motility was not affected by the high temperature although the ratio of fertilized to unfertilized ova in test females was significantly lowered. Some recovery was made three weeks to a month after treatment. Impairment of spermatogenesis was noted in Guernsey bulls following their exposure to approximately 100° F. for two weeks or to 86° F. for five weeks. Libido was not affected in these animals, Casady *et al.* (1953).

Gunn and associates (1942) showed that temperature and humidity conditions comparable to those normally found in New South Wales was sufficient to cause seminal degeneration in the ram. Dutt and Bush (1955) observed improved breeding performance, as measured by services required per conception, in rams quartered in an air-conditioned room at 45-48° F. from May 26, until October 8. This work was continued by Dutt and Simpson (1957) who again kept rams at 45-48° F. during the summer months. From August 20, to September 24, the average motility rating of sperm from treated rams was 70.3 percent versus 41.8 percent for controls. At the same time, semen from the treated rams contained 6.4 percent abnormal cells while that from the controls contained 36.9 percent. Sperm concentration was significantly higher for treated rams. Conception rate and percent of ewes lambing was highly significantly improved by the use of semen from treated versus control rams.

In a summer experiment conducted by Simpson *et al.* (1959), two groups of crossbred rams were kept in similar surroundings with the exception that one group was allowed free access to an air-conditioned room. A 15° F. temperature differential was realized in this room on hot days. Weekly semen studies revealed a higher volume, motility, concentration and percent normal cells in the treated rams. Statistically, these differences were all either significant or highly significant.

B. Male Fertility in Relation to Hyperthyroidism

The existence of a thyroid-gonad relationship has been recognized for some time. This linkage appears rather complex in certain aspects and experimental results have often differed markedly between investigators.

Induced hyperthyroidism in the male rat was shown by Smelser (1939) to be reflected in decreased testis weight and sperm production. He also noted that there was apparently either a marked decrease in the ability of the testis to respond to gonadotropic hormones or an increase in its threshold to them. Such an explanation was based on the fact that normal testicular function in hyperthyroid males was maintained by injections of gonadotropic extracts.

The feeding of one or two grams of thyroprotein in the daily ration for fifteen weeks was found by Eaton *et al.* (1948) to adversely affect the semen quality of rams. Semen from control rams was superior in concentration, percent normal sperm and motility. Semen volume was greatest in those rams receiving two grams of thyroprotein. Similarly, Black and co-workers (1949) reported that daily oral doses of one gram of thyroprotein per 100 pounds of body weight caused a significant loss of weight and a decrease in sperm concentration. Warwick and associates (1948) fed thyroprotein to rams in amounts sufficient to bring about noticeable weight loss and reported that this treatment generally reduced semen quality. However, in those rams which had the ability to tolerate the dosage given, semen quality was equal or superior to that of controls. Differences in tolerance to high levels of thyroprotein were also found in family groups of poultry by Kheireldin and Shaffner (1957). Somewhat in contrast to the reports just mentioned are those of Berliner and Warbritton (1937) and Bogart and Mayer (1946) indicating that under certain conditions the administration of thyroid-active materials to rams increased reproductive efficiency.

Semen studies with poultry have generally failed to show any beneficial effects from thyroprotein administration. Shaffner (1948), on the basis of fertility data and laboratory examinations, reported a definite lowering of semen quality in cocks fed ten grams of thyroprotein per 100 pounds of feed for sixteen weeks. Relatively little change was observed in semen quality of cocks fed thyroprotein as .02 percent of the diet (Huston and Wheeler, 1949). Wilwerth *et al.,* (1954) found that .04 percent thyroprotein significantly increased sperm concentration but not volume. In this experiment, both volume and concentration were significantly decreased by levels of .08 percent and .16 percent.

Studies with the guinea pig, Young *et al.* (1952), and man, Werner (1955)

have given little indication that their reproductive function is affected by hyperthyroidism. Large amounts of orally administered thyroprotein were found by Maqsood and Reineke (1950) to inhibit testis development in the mouse. Hurst and Turner (1948), on the other hand, failed to observe any effect on reproductive ability in the male mouse when experimental hyperthyroidism was produced by thyroxine injections.

In summary, the preponderance of experimental evidence appears to indicate that hyperthyroidism is deleterious to an optimal fertility status.

C. Hypothyroidism as Related to Male Reproductive Functions

With regard to the effect of hypothyroidism on reproductive phenomena much species and individual variation has been reported. Zalesky and Wells (1937) were able to show that complete surgical thyroidectomy of ground squirrels during the non-breeding season prevented most of them from attaining breeding capacity, as measured by spermatogenesis or testis hormone production. However, in several animals with no detectable thyroid tissue remaining, normal sperm production and male hormone secretion was seen. Even so, it was concluded that the thyroids are necessary for gonadal growth and function in this animal.

Hypothyroidism in the human male was believed by Rose (1942) to be associated with loss of libido and impotence. This viewpoint was accepted by Werner (1955) who added that physical examination of testes, accessory sex organs and secondary sex characteristics is usually non-revealing.

Young and co-workers (1952) observed the effect of thyroidectomy on strength of the sex drive in adult male guinea pigs and found that it was not diminished by the operation. They stated that fertility appeared to be somewhat lowered in these animals. A somewhat different response was observed by Peterson *et al.* (1941) in a thyroidectomized immature Jersey bull. Gonadal development proceeded normally, ejaculates obtained by ampullar massage were normal but libido was completely absent. Sexual desire was awakened in the bull by the feeding of twenty-five grams of desiccated thyroid over a three-day period. The effects of thyroid feeding were noticeable in twenty-four hours and lasted for ten days after being discontinued.

The thyroids of adult male rats were removed by Smelser (1939) who reported this resulted in regression of the accessory reproductive organs, a marked lowering of sperm production and a probable decrease in secretion of gonadotropic hormones into the blood stream. This latter finding, if true, may explain the other two results. Smelser's (1939) work was substantiated by the experiments of Leblond and Eartly (1952) in which male rats of about fifty grams weight were thyroidectomized and fed a low-iodine diet. The rats were sacrificed and autopsied five weeks after the operation. The sex organs were seen to be lacking in development, the testes were small and the accessory organs were much atrophied. Seminal vesicles of these rats had a mean weight of only twenty-seven mg. as compared to 750 mg. in thyroxine treated thyroidectomized

animals. Jones *et al.* (1946) reported that thiouracil induced hypothyroidism in the male rat had no effect on its ability to sire litters.

In mice, both Gorbman (1950) and Bruce and Sloviter (1957) have reported that destruction of the thyroid by radioactive iodine is apparently without effect upon the male reproductive system. Rugh (1953) treated mice with sufficient radioiodine (four μc per week for six months) to bring about certain changes in the thyroids of both sexes. Whereas the males responded by producing what was described as a cytologically hyperactive but otherwise normal thyroid, that of females were severely damaged. Too, it was stated that the ovaries, but not testes, showed considerable degeneration after such long term administration of radioiodine.

D. The Thyroid—Testis—Temperature Interrelationship

The work of Berliner and Warbritton (1937) strongly suggested a linkage between fertility in the ram, thyroid activity and environmental temperature. These workers made simultaneous histological studies of the testis and thyroids in Shropshire and Hampshire rams and correlated their results with sperm production. It was found that Shropshires responded to heat by a very marked decrease in sperm production whereas Hampshires under like conditions were little affected. Testes of Shropshires were quite deranged with much destruction of seminiferous epithelium, fibrosis and edema, those of the Hampshires were only slightly disordered. Thyroids of Hampshires were moderately active, those of Shropshires generally inactive. Thyroxine injections were shown to promote normal sperm production in a sterile thyroidectomized Hampshire and to bring Shropshire rams to a high breeding level in September. Control Shropshires required until October to reach a comparable fertility status. These findings were strengthened by those of Bogart and Mayer (1946) who, by administering thyroid-active substances to rams, were able to prevent the fluctuations in semen quality seen with the onset of high summer temperatures. These workers went on to show that the detrimental changes in semen quality of rams during hot weather could be duplicated in the breeding season by thiouracil administration and that adequate amounts of thyro-active material would counteract such changes.

It was felt that temperature changes influenced reproductive performance of the ram indirectly through the thyroid gland. Furthermore it seemed that the thyroid affects the spermatogenic tissues of the testes and has little or no effect upon the interstitial tissue or the accessory organs which are dependent upon androgens produced by the interstitial tissue. A somewhat similar conclusion was reached by Lee (1950).

Work with rabbits led Oloufa *et al.* (1949) to state that continuous high temperatures were more detrimental to fertility than intermittent high and low temperatures. Thyroxine therapy tended to accentuate the deterioration in fertility status of rabbits kept under conditions of alternating high and low temperatures. A major conclusion arising from this experiment was that thyroxine

addition would not alleviate the direct effect of high temperature on the testis.

E. The Determination of Thyroxine Secretion Rates

Recently, a great deal of effort has been expended in attempts to closely evaluate thyroid status in terms of its hormone production. Such information is sought in order that the relationship between the productive processes and the thyroid may be understood.

Dempsey and Astwood (1943) described a successful technique for determining thyroid secretion rate. In this method, a goitrogen was given to cause thyroid enlargement and, simultaneously, thyroxine was administered to ascertain at what hormone level normal thyroid weight could be maintained or restored. Such an amount of thyroxine was held to be equivalent to the normal endogenous thyroid hormone secretion rate. Although useful, this procedure required the sacrifice of the subject and consequently was applicable mainly to small laboratory animals. This limitation was overcome when Pipes *et al.* (1950) were able to estimate the thyroid secretion rate of the live, intact animal. These workers found that tracer doses of radioiodine could be followed in the body from the time of its collection by the thyroid to its appearance in the blood as protein bound I^{131}, presumably thyroxine. Release of the hormone by the thyroid is largely dependent upon the thyroxine level of the blood. The injection of thyroxine in amounts to equal the thyroid secretion rate will inhibit the release of thyroid stimulating hormone from the pituitary and, as a consequence, secretory activity of the thyroid itself will essentially cease. Thus, it was found possible to determine the secretion rate by finding at what point a cessation of thyroid function occurred, as indicated by a slower release of protein bound I^{131} into the bloodstream.

Henneman *et al.* (1952) demonstrated that radioactivity counts secured directly over the thyroid could be used to study the activity of the gland. Graded doses of injected thyroxine were used to supplant that normally released by the gland. As the doses of exogenous thyroxine increased the relative percent of remaining radioactivity of the thyroid also increased from one counting period to the next. This indicated that less and less thyroidal I^{131}, representing iodine incorporated into the thyroglobulin (thyroxine) moiety of the thyroid, was being released into the bloodstream. The dosage of thyroxine capable of bringing about cessation of endogenous hormone release was felt to represent the thyroid secretion rate. Much more detailed explanations of the use of radioiodine in determining the thyroid secretion rate of animals have been made available by Pipes (1955) and Premachandra (1958).

With various modifications of the radioiodine technique, secretion rates have been determined for sheep (Henneman, *et al.*, 1952, 1955; Singh *et al.*, 1956; Falconer and Robertson, 1961; Hoersch *et al.*, 1961) dairy cattle (Pipes and Ruppert, 1955; Lodge *et al.*, 1957; Pipes *et al.*, 1958; Premachandra *et al.*, 1958) goats (Flamboe and Reineke, 1957, 1959) and rats (Reineke and Singh, 1955).

As might be expected, such studies have revealed considerable differences between species and individuals in the amount of thyroid hormone produced under similar circumstances. Premachandra *et al.* (1957) observed a three-fold individual variation in thyroid secretion rate of dairy animals during both the winter and summer months. It was noted by Lewis and co-workers (1957) that dairy calves showed less variation between breeds than individuals within a breed in secretion rate. However, a large seasonal difference in secretion rate as had been found for sheep, Henneman *et al.* (1955), and dairy cattle, Premachandra *et al.* (1957), was not apparent in that work.

The experiments of Henneman and his associates (1955) demonstrated the much lower thyroid secretion rate of ewes in mid-summer (July) than at other times of the year. Also, in that study breed, age and lactation, but not pregnancy, all had a marked effect upon secretion rate. Hampshire two year-old and lactating ewes versus Shropshire four year-old and non-lactating ewes all had significantly higher secretion rates. Work with dairy goats, Flamboe and Reineke (1957), was reported in which non-lactating animals had somewhat higher secretion rates than did a similar, lactating group. However, that study did confirm the pronounced summer decline in thyroid secretion rate observed in sheep by Henneman *et al.* (1955).

THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON THE THYROXINE SECRETION RATE OF RAMS

The objective of this experiment was to determine how, and to what extent, the thyroxine secretion rate of rams is affected by environmental temperature.

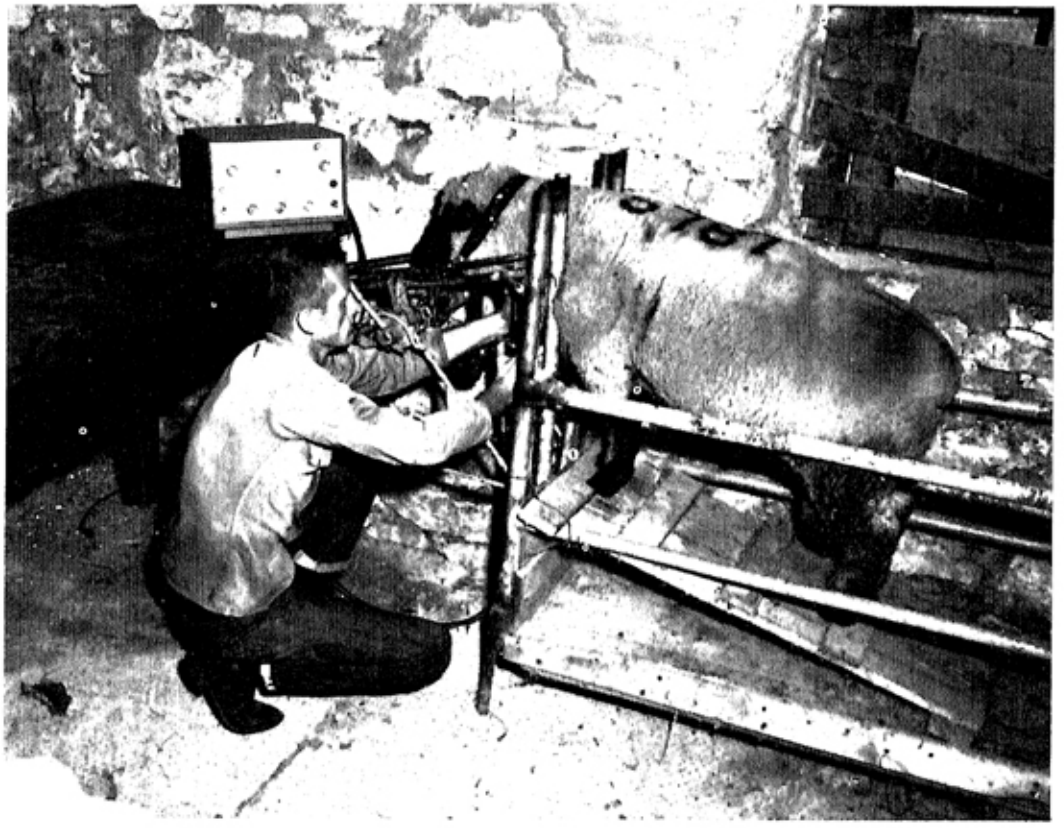
A. Experimental Procedure

Purebred Hampshire rams ranging from one to three years of age and in weight from about 100 to 200 pounds were used in the experiment. They were group-fed a ration of alfalfa hay and about one half pound of a concentrate mixture per head daily. Fresh water and a simple mineral supplement composed of equal parts of plain salt and steamed bone meal were available. They were housed in two similar 14 ft. by 14 ft. by 10 ft. rooms which were insulated with a single layer of two in. rock wool batting to facilitate temperature control. One room contained a thermostatically controlled steam radiator and an exhaust fan for ventilation. The second room was equipped with two air conditioners which held the temperature to a maximum of about 70° F. Continuous temperature readings were taken by recording thermometers located in each room. To minimize any possible experimental variation due to light, the rooms were made essentially light-proof and artificial light was provided between the hours of approximately 8:00 a.m. and 5:00 p.m.

Thyroxine secretion rates were determined using the general procedure described by Pipes *et al.* (1957) for dairy cattle. Individual rams were given a subcutaneous injection of either 200 or 300 microcuries of radioiodine. In given

trials, the I^{131} dosage was similar for all rams. Standards were prepared with each trial. They consisted of an aliquot of the I^{131} solution used for injecting the rams and provided a basis for calculating the extent of radioactive decay as well as the percent of injected dose remaining in the thyroid gland.

The instrument used for the reception of radioactivity was a scintillation detector containing a one inch NaI thallium activated crystal. It was attached to a Nuclear-Chicago analytical count rate meter Model 1620A.



Radioactivity counts were taken with the animal in a holding chute equipped with a head holder to which the head was fastened in a stationary position.

The scintillation detector was fixed by its holder to a jointed movable arm which allowed it to be maneuvered firmly into position below the thyroid region. A steel rod spacer on the holder insured that counts would be made at the same distance between the scintillation crystal and neck of each animal. In given trials the crystal was located either 15 or 30 cm. from the neck.

On the third day following the I^{131} injection the initial determinations of thyroidal radioactivity were made. Other measurements followed at three day intervals and as nearly as possible at the same time each day. This procedure was continued until the secretion rate had been obtained.

Early in the first trial, it became obvious that a goitrogen was necessary in order to prevent the recycling of I^{131}.

Recycling is that process in which organically bound iodine, such as is present in thyroxine, is freed through metabolic pathways, circulates back to the thyroid and, is again organically bound. If organification is blocked by goitrogens, the iodine can be excreted. In the case of I^{131}, radioactivity determinations will then necessarily reflect a much faster I^{131} release rate. This allows more precise measurements of the rate of thyroidal release of I^{131} labeled thyroxine. In turn, the extent of inhibition of thyroxine release which occurs in the presence of exogenous thyroxine can be calculated more closely. With these facts in mind, in all trials the animals were given daily doses of tapazole as an aqueous drench. The dosage level in the first trial was 800 milligrams of tapazole per 100 pounds body weight. This amount was reduced in succeeding trials to 400 milligrams per 100 pounds and the desired sharp I^{131} release curve was maintained on the lower level. In all trials except the first the administration of tapazole was begun on the third day following radioiodine injection.

The method of determining thyroxine secretion rate outlined by Pipes *et al.* involves replacement therapy with graded subcutaneous injections of L-thyroxine. Administration of the hormone in amounts equalling that normally secreted by the thyroid is known to inhibit endogenous thyroxine release. Thus, in order to determine the thyroxine secretion rate, it was necessary to find the level of injected thyroxine which caused such a suppression. To accomplish this, replacement therapy was begun at a level considered to be below the animal's own rate of secretion. Administration of thyroxine was customarily begun on the third day following I^{131} injection. The dosage level was increased every three days. Counts of remaining thyroidal radioactivity were made at the end of each three day period.

The point was eventually reached where such remaining radioactivity was within 95 percent or above that present at the preceding count, after correction for radioactive decay. This plateau in the measurement indicated that sufficient exogenous thyroxine was present in the system to inhibit the release of thyroxine by the thyroid gland. The amount of injected thyroxine just capable of causing this cessation of thyroidal I^{131} (thyroxine) release was presumed to be equivalent to that normally secreted by the animal under similar circumstances and to constitute its thyroxine secretion rate.

B. Results

Thyroxine secretion rates were estimated on twenty-one different Hampshire rams. From one to seven secretion rates were determined on individual rams. This was due to certain animals being used in all trials while others were introduced into the experiment as it progressed.

In the interests of making trial results due to temperature effects more evident, the secretion rates were divided into two groups. The first included those secretion rates obtained in trials where the average temperature was between 46°

and 66° F. The second contained those found in trials in which the average temperature was between 67° and 87° F.

A frequency distribution of twenty three secretion rates obtained from ten rams at average temperatures of 46° to 66° F. is shown in Figure 1. The range of secretion rates was from 0.20 to 0.70 milligrams of L-thyroxine daily with an average of 0.43 milligrams. Individual secretion rates and the trial temperatures at which each was obtained are presented in Table I. Forty-one thyroxine secretion rates, estimated on twenty-one rams in trials where temperatures averaged between 67° and 87° F., are illustrated as a frequency distribution in Figure 2. The range of these secretion rates was from 0.20 to 0.50 milligrams with an average of 0.31 milligrams. Table II lists these secretion rates singly and the temperatures at which each was obtained. To illustrate graphically the effect of increasing temperature on thyroxine secretion rate, a regression line was plotted using all secretion rates and the average temperature for each. This showed (Figure 3) that temperature increases were reflected in lowered secretion rates. The slope of the regression line was best described by the equation Y .620 (−.0038) X with Y representing thyroxine secretion rate and X temperature between 46° and 87° F. The regression coefficient of the −.0038 was highly significant ($P < .01$).

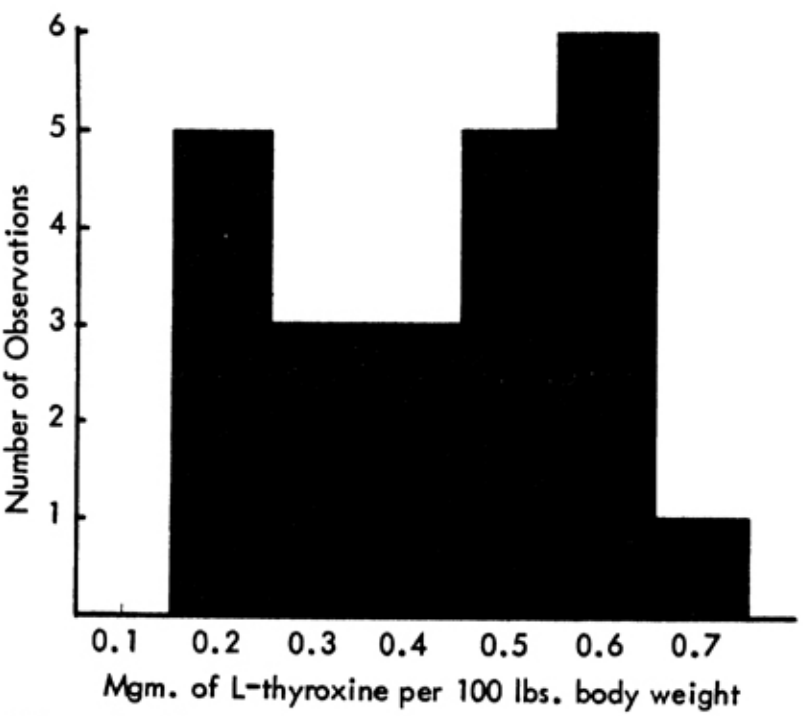


Figure 1. Thyroxine Secretion Rates of Ten Hampshire Rams Determined at Environmental Temperatures of 46° to 66°F.

TABLE I-THYROXINE SECRETION RATES OBTAINED WITH HAMPSHIRE RAMS AT TEMPERATURES AVERAGING 46° TO 66° F.

Ram	Temperature (°F.) Avg. (Min.-Max.)	Thyroxine Secretion Rate[1]
5786	46°(29°-57°)	0.40
	51°(37°-62°)	0.60
	59°(54°-66°)	0.40
	59°(53°-66°)	0.50
	52°(38°-73°)	0.30
5772	48°(38°-58°)	0.20
	56°(42°-70°)	0.60
5864	51°(37°-62°)	0.60
	59°(54°-66°)	0.50
5868	51°(37°-62°)	0.60
	59°(54°-66°)	0.50
5800	51°(37°-62°)	0.40
	59°(54°-66°)	0.50
	59°(53°-66°)	0.30
	52°(38°-73°)	0.20
5787	56°(42°-70°)	0.60
5810	56°(42°-70°)	0.60
	59°(53°-66°)	0.30
	52°(38°-73°)	0.20
5864	56°(42°-70°)	0.70
5824	59°(54°-66°)	0.50
5944	59°(54°-66°)	0.20
	52°(38°-73°)	0.20

[1]Expressed as milligrams L-thyroxine per 100 pounds body weight.

C. Discussion

Comparisons of thyroxine secretion rates obtained at temperatures averaging between 46° and 66° F. and those found at temperatures averaging 67° and 87° F. support the premise that a lowering of thyroxine secretion occurs as temperature increases. This is indicated by the mean secretion rates under the two temperature regimens; that of the cooler being 0.43 milligrams of L-thyroxine daily per 100 pounds body weight that of the warmer only 0.31 milligrams. The highly significant ($P<.01$) regression coefficient plotted between secretion rate and temperature also supports this conclusion. On the basis of other reports (Henneman *et al.* (1955), Premachandra *et al.* (1958), Flamboe and Reineke (1959), such a finding was expected.

The ratio between the lowest (0.20 milligram) and the highest (0.70 milligram) secretion rate is somewhat less than that found in ewes by Henneman *et al.* (1955) who reported a mean daily secretion of 0.05 milligram of L-thyroxine in a group of 2 year old Hampshire ewes (dry) during July, 0.28 milligram in the same ewes in January, (pregnant) and 0.33 milligram in March (lactating). Premachandra *et al.* (1958) observed a range of 0.20 to 1.0 milli-

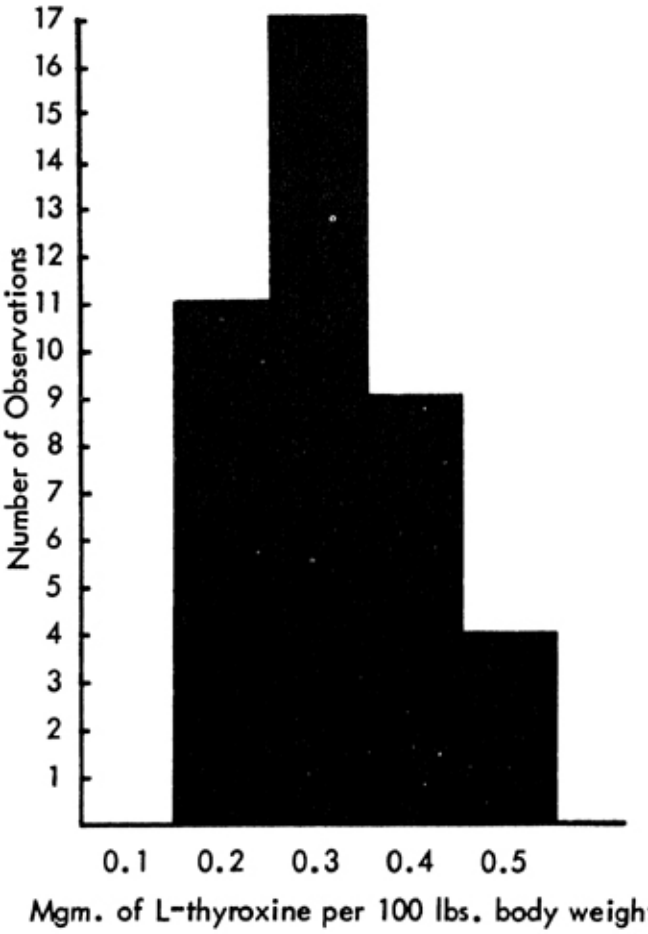


Figure 2. Thyroxine Secretion Rates of Twenty-one Hampshire Rams Determined at Environmental Temperatures of 67° to 87°F.

grams of L-thyroxine daily per hundred weight in dairy cattle during winter. It should be noted that the work of the latter two groups was conducted under uncontrolled environmental conditions, a factor which undoubtedly contributed to more climatic variation than was true for the present study.

Recently, Falconer and Robertson (1961) reported the thyroxine secretion rate of a small group of aged (5-7 years) Cheviot ewes (dry) to vary from .132 to .562 milligram of L-thyroxine daily per hundred weight with a mean of 0.322 milligrams. Although these figures correspond well with those reported here, they cannot be compared directly since secretion rates are usually higher for the female, Schultze and Turner (1945), Singh *et al.* (1956). In addition, breed, Henneman *et al.* (1955), and age, Flamboe and Reineke (1959), Falconer and Robertson (1961), affect thyroxine secretion.

Experiments, Kenyon, (1933), Starr and Roskelley (1940), Schmidt and Schmidt (1938), have suggested there is a rapid response by the thyroid gland to conditions occasioning the need for greater or lesser amounts of thyroxine. Ac-

TABLE II-THYROXINE SECRETION RATES OBTAINED WITH HAMPSHIRE RAMS AT TEMPERATURES AVERAGING 67° TO 87° F.

Ram	Temperature (°F.) Avg. (Min.-Max.)	Thyroxine Secretion Rate[1]
5786	86°(77°-93°)	0.30
	74°(69°-82°)	0.40
5772	87°(82°-92°)	0.30
	68°(62°-76°)	0.50
	74°(69°-82°)	0.40
5846	86°(77°-93°)	0.30
	71°(69°-73°)	0.20
5868	86°(77°-93°)	0.30
	72°(69°-77°)	0.40
	80°(76°-82°)	0.30
5800	86°(77°-93°)	0.30
5787	87°(82°-92°)	0.40
	68°(62°-76°)	0.40
	71°(69°-73°)	0.20
	72°(69°-77°)	0.40
	80°(76°-82°)	0.30
5810	87°(82°-92°)	0.40
5864	74°(69°-82°)	0.50
	72°(69°-77°)	0.50
	80°(76°-82°)	0.30
5824	86°(77°-93°)	0.30
5944	79°(73°-83°)	0.20
5961	72°(69°-77°)	0.20
	80°(76°-82°)	0.40
	79°(73°-83°)	0.20
5952	72°(69°-77°)	0.30
	80°(76°-82°)	0.40
	71°(65°-73°)	0.20
5937	72°(69°-77°)	0.30
	80°(76°-82°)	0.50
	71°(65°-73°)	0.40
5971	80°(76°-82°)	0.20
	79°(73°-83°)	0.30
McPike	71°(65°-73°)	0.20
5969	71°(65°-73°)	0.20
5964	79°(73°-83°)	0.20
5963	79°(73°-83°)	0.30
5956	79°(73°-83°)	0.40
5915	71°(65°-73°)	0.40
5987	72°(69°-77°)	0.20
	80°(76°-82°)	0.30

[1]Expressed as milligrams L-thyroxine per 100 pounds body weight.

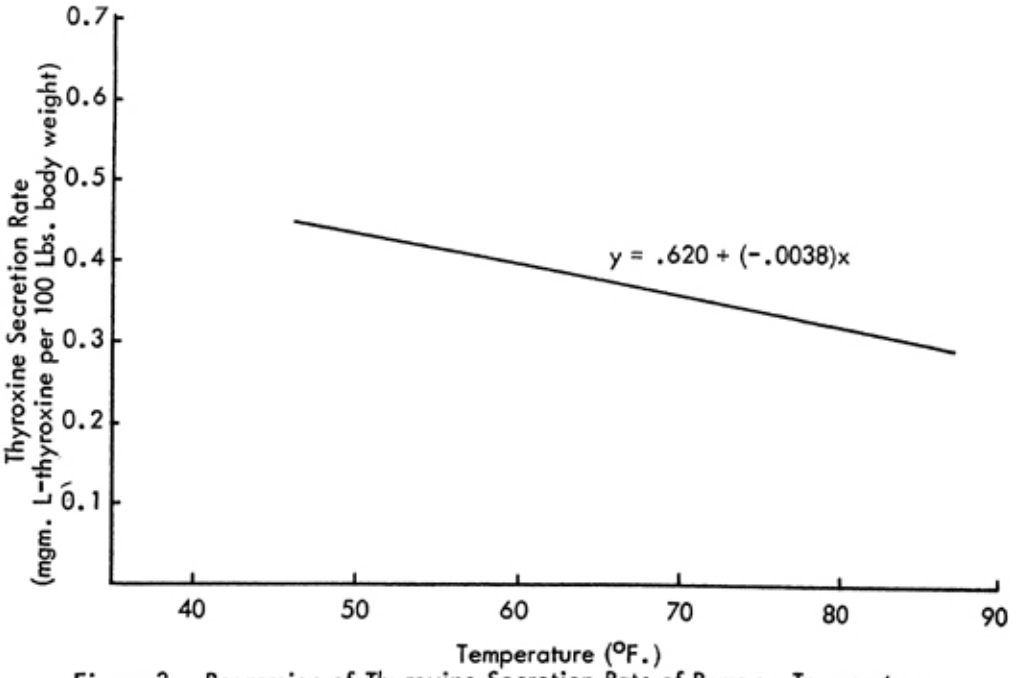


Figure 3. Regression of Thyroxine Secretion Rate of Rams on Temperature

cordingly, in the trials reported here the assumption was made that little time was required for the secretion of thyroxine to reach a relatively constant level in the rams rotated between the temperature control rooms. Even so, except for one trial in which only eight days were allowed, at least two weeks were given as a period of thyroidal adjustment to a particular set of temperature conditions before thyroxine secretion rate estimations were made. However, Stahl *et al.* (1961) found that under controlled lowered temperatures, the secretion rate of chickens continued on a rising plane over a considerable period of time, in instances as long as six months. In the light of such results, there is the interesting possibility that the secretion rates determinations reported here may differ from those which might be found if rams were to be kept under controlled environmental temperatures for longer periods before estimations were made of their thyroxine secretion rates.

THE EFFECT OF ENVIRONMENTAL TEMPERATURE AND THYROXINE THERAPY ON SEMEN QUALITY OF RAMS

A. Objective

Four trials were conducted to test the theory that "summer sterility" might be caused not only by higher ambient temperatures than are compatible with normal spermatogenesis but also, indirectly, by decreased thyroid function at such high temperatures. Accordingly, the effect of thyroxine therapy on the semen quality of rams subjected to different temperature levels was determined.

B. Experimental Procedure

The rams which were used for the thyroxine secretion rate studies were also used in this work. These animals and their care have been described.

Beginning the latter part of June, 1960, collections of semen were made at approximately weekly intervals. At that time, the rams were being handled as one group under uncontrolled temperature conditions. Semen collections were continued until termination of the experiment in February, 1961. The artificial vagina technique was used. At least two pre-trial collections were made from all but two rams. During this time all rams from which semen was secured appeared to be of a satisfactory fertility status. On the 11th of July the rams were randomly divided into two groups of eight. One group was placed in the air-conditioned room and the other in a similar but uncooled room. The period from the 11th of July to the 17th of September comprised trial one. During that time the average temperature in the cooled room was 69° F. and humidity averaged over 90 percent. Meanwhile, in the uncooled room the average temperature was 80° F. and the mean humidity was 73 percent.

On the 17th of September a reversal of rams between rooms was made. In addition, four rams from each room were randomly chosen to receive thyroxine therapy. The thyroxine secretion rate studies had indicated that 0.40 milligrams of L-thyroxine per 100 pounds body weight should furnish a physiological dosage to a ram. For trial two the hormone was given daily as a single subcutaneous injection from September 18, until October 18. Temperatures during that time averaged 67° F. in the cool room and 80° F. in the heated room. Relative humidity was constantly above 90 percent in the cooled room and averaged 63 percent in the heated chamber.

Trial three was begun on October 26 as a second reversal was made between rooms. Rams chosen in the previous trial to receive thyroxine therapy were again used for this purpose. The thyroxine dosage level was lowered to 0.30 milligrams per 100 pounds body weight. This regimen was continued until November 20. Temperatures for this trial averaged 62° F. in the cool room and 81°F. in the heated room. Mean humidity for this trial was above 90 percent in the cooled room and was 64 percent in the heated room.

A third reversal between rooms was made on December 5, and trial four was started. Daily thyroxine injections, for the same rams treated in the previous two trials, were again begun. The thyroxine dosage level was further lowered to 0.20 milligrams per 100 pounds body weight. The trial was terminated on February 23. Temperatures during the experimental period averaged 50° F. in the cool room and 83° F. in the heated room. Relative humidity again averaged above 90 percent in the cooled room while in the heated room the mean humidity was 59 percent.

Each semen sample was evaluated for volume, motility, concentration and percentages of live and normal spermatozoa. Volume was measured directly to

the nearest 0.1 c.c. from the graduated vials used for collection. Motility was estimated within three minutes after the semen collection was made. Motility was estimated by inserting the tip of a glass rod into the sample and removing the drop of semen adhering to it. The drop was transferred to the surface of a clean glass slide and examined immediately under low power. Each sample was rated from 0 to 5 with 0 indicating no motility and 5 maximum motility. In cool weather the entire artificial vagina except for the open end was wrapped in a warmed cloth. This precaution was taken to insure that the semen remained warm until the motility estimation had been made. Also, all estimations were made in a steam heated room. In this way a spurious, lowered motility rating due to cooling of the sample was prevented.

The percentages of live and normal spermatozoa were calculated from the same slides. These were made by placing one drop of the live-dead differential stain recommended by Mayer et al. (1951) on a clean dry glass slide and mixing with this stain the semen clinging to a glass rod which had been dipped into the sample. A second glass slide was then placed over the stain-semen mixture. The slides were gently drawn apart and dried quickly under a portable electric hair dryer. The procedure was carried out as rapidly as possible and the stain was used at room temperature since experience had shown that both speed in making the slides and the temperature of the stain contributed greatly to the success of the process. Spermatozoa which were alive when mixed with the stain were seen to be unstained when examined microscopically. Those which were dead when the slide was made absorbed the stain and appeared reddish-blue under the microscope. At least 200 spermatozoa were counted in arriving at the percentage of live cells. Similarly, 200 were counted in estimating the percentage of normal spermatozoa present. The oil immersion lens was used in determining percentages of the live and normal spermatozoa.

A hemacytometer was used in making concentration determinations. The semen was diluted in a 1:200 ratio with five percent NaCl solution in a red blood cell pipette. The pipette was agitated thoroughly for about two minutes, the first five drops were allowed to escape and then diluted semen was used to fill the two sides of an improved Levy counting chamber. The cells were allowed to settle for five minutes. Those included in a diagonally situated five block group, each containing sixteen squares, were counted. The number counted multiplied by 10,000 equalled the number of cells per cubic millimeter.

C. Results

In order to obtain a valid comparison of the effect of treatments and temperatures, sufficient time was allowed in all four trials for semen quality to stabilize. Then, data used in comparing such effects were obtained from the last two semen collection periods of each trial. This largely eliminated the early periods when apparent fertility was changing due to adjustment to trial conditions.

Results of trial one are shown in Table III. There was a marked response

TABLE III-EFFECT OF ENVIRONMENTAL TEMPERATURE ON SEMEN QUALITY OF RAMS[1]

	Treatment	
	Cooled	Heated
Avg. Trial Temperature (°F.)	69	80
Avg. Volume of Semen (ml.)	0.92 (11)[2]	1.20 (8)*
Avg. Motility	3.5 (14)**	1.8 (12)
Avg. Percentage Live Sperm	67 (14)*	35 (12)
Avg. Percentage Normal Sperm	73 (14)*	43 (12)
Avg. Sperm Concentration	3.48 (14)**	1.43 (12)

[1]Semen data of the last two collection periods of Trial I
[2]Numbers in parentheses refer to the number of observations
[3]Concentration expressed in millions/mm.[3]
*(P .05) **(P .01)

by the rams to cool temperatures. Semen from rams kept in the air-conditioned room was superior to that from ones held in the heated room in motility ($P<.01$), concentration ($P<.01$), percentage of live spermatozoa ($P<.05$) and percentage of normal cells ($P<.05$), but greater volume ($P<.05$) was observed in rams from the hot room.

Data from trial two are presented in Table IV. A daily injection of 0.4 milligram of L-thyroxine per 100 pounds body weight apparently had no significant effect on the semen quality of rams in either the heated or cooled rooms. However, there was an indication that thyroxine therapy was detrimental to semen quality under the higher environmental temperature. Motility, percentage of live sperm and concentration seemed to be affected rather drastically and the percentage of normal sperm was influenced to a lesser extent. Semen volume appeared little affected. In the cooled room, thyroxine therapy seemed to have little effect with the exceptions that concentration was considerably higher for the treated rams and volume of semen was greater for untreated ones.

TABLE IV-EFFECT OF ENVIRONMENTAL TEMPERATURE AND THYROXINE ON SEMEN QUALITY OF RAMS[1]

	Treatment			
	Cooled		Heated	
	No Thyroxine	Thyroxine[4]	No Thyroxine	Thyroxine
Avg. Trial Temperature (°F.)	67	67	80	80
Avg. Volume of Semen (ml.)	1.2 (6)[2]	0.6 (6)	0.8 (4)	0.7 (8)
Avg. Motility	3.8 (6)	4.0 (6)	3.1 (6)	1.1 (8)
Avg. Percentage Live Sperm	76 (6)	70 (6)	46 (6)	18 (8)
Avg. Percentage Normal Sperm	83 (6)	77 (6)	62 (6)	48 (8)
Avg. Sperm Concentration[3]	2.79 (6)	4.30 (6)	1.93 (6)	1.77 (8)

[1]Semen data of the last two collection periods of Trial II
[2]Numbers in parentheses refer to the number of observations
[3]Concentration expressed in millions/mm[3]
[4]Thyroxine administered at rate of 0.4 mgm. per 100 pounds body weight

The results of trial three are shown in Table V. Although it appeared the daily injection of 0.3 milligram of L-thyroxine per 100 pounds body weight was without a significant effect on semen quality of rams in either of the experimental rooms, it was again seen that semen from treated rams in the heated room seemed to be inferior to that of the unheated group. The data indicated that all indices of semen quality were higher for the rams receiving no thyroxine. In the cooled room there seemed to be only slight differences between the treated and untreated groups. The treated group was a bit superior in motility, percentage of normal sperm and sperm concentration.

The semen quality data from trials two and three were strikingly similar. In both experiments the treated group in the heated room appeared to have a poorer semen quality picture than did the others. Overall, semen from the heated group of rams was of lower quality than was that from rams kept in the cooled room. This followed rather closely the trend set in trial one.

The results of trial four, which are presented in Table VI, did not seem to fit the pattern of the preceding three trials. Among the heated group of rams,

TABLE V-EFFECT OF ENVIRONMENTAL TEMPERATURE AND THYROXINE ON SEMEN QUALITY OF RAMS[1]

	Treatment			
	Cooled		Heated	
	No Thyroxine	Thyroxine[4]	No Thyroxine	Thyroxine
Avg. Trial Temperature (°F.)	62	62	81	81
Avg. Volume of Semen (ml.)	0.8 (5)	0.8 (7)	1.2 (6)	0.7 (3)
Avg. Motility	2.9 (6)	3.4 (8)	1.6 (6)	0.6 (4)
Avg. Percentage Live Sperm	64 (6)	64 (8)	33 (6)	6 (4)
Avg. Percentage Normal Sperm	66 (6)	73 (8)	69 (6)	54 (4)
Avg. Sperm Concentration[3]	2.26 (6)	2.94 (8)	4.47 (6)	2.71 (4)

[1]Semen data of the last two collection periods of Trial III
[2]Numbers in parentheses refer to the number of observations
[3]Concentration expressed in millions/mm^3
[4]Thyroxine administered at rate of 0.3 mgm. per 100 pounds body weight

no significant differences were found which could be attributed to hormone treatment. However, those rams receiving thyroxine, injected at levels of 0.2 milligram per 100 pounds body weight in this trial, had a higher sperm concentration and also a higher percentage of normal sperm than did the untreated animals. Conversely, the untreated rams produced a greater volume of semen and the semen had a higher motility and percentage of live sperm than was true for that produced by the treated group. In the cooled room, the semen from the untreated group was of higher quality in all indices than was that from the treated group. In was surprising to find that, apparently, the hot room untreated rams also surpassed the cool room thyroxine treated group in all indices of semen quality, except concentration.

Pooled data representing the last two collection periods of trials two, three,

TABLE VI-EFFECT OF ENVIRONMENTAL TEMPERATURE AND THYROXINE ON SEMEN QUALITY OF RAMS[1]

	Treatment			
	Cooled		Heated	
	No Thyroxine	Thyroxine[4]	No Thyroxine	Thyroxine
Avg. Trial Temperature (°F.)	50	50	83	83
Avg. Volume of Semen (ml.)	0.8 (6)[2]	0.6 (6)	1.2 (2)	0.8 (7)
Avg. Motility	4.3 (6)	2.8 (6)	3.8 (4)	2.6 (7)
Avg. Percentage Live Sperm	84 (6)	58 (6)	66 (4)	56 (7)
Avg. Percentage Normal Sperm	98 (6)	71 (6)	80 (4)	89 (7)
Avg. Sperm Concentration[3]	6.40 (6)	4.97 (6)	3.05 (4)	5.13 (7)

[1]Semen data of the last two collection periods of Trial IV
[2]Numbers in parentheses refer to the number of observations
[3]Concentration expressed in millions/mm[3]
[4]Thyroxine administered at rate of 0.2 mgm per 100 pounds body weight

and four are shown in Table VII. The data show that treated rams in the heated room produced semen of low quality. They were superior to rams on other treatments in that they produced semen of slightly higher concentration than did the hot room untreated group and volume was a bit greater than was found for the treated group in cooled room. The untreated rams in the hot room produced the greatest semen volume in these trials just as they did in trial one, but their sperm was lower in motility, percentage of live or normal sperm, and concentration, than any other group in the cooled room. The untreated rams in the cooled room produced a greater volume of semen than did the treated rams. Semen motility and percentages of live and normal sperm were also higher for the untreated rams. Sperm concentration was a bit in favor of the treated group.

In general, the effect of temperature on semen quality of rams was essentially the same for trials two, three, and four as it was in trial one. The effect of cooling rams was especially noticeable on motility and percentage of live sperm ($P<.01$). Concentration was also higher ($P<.05$) for rams subjected to the cooler temperature.

Semen volume, which was significantly greater for rams in the heated room in trial one, showed no consistent pattern in the last three trials and no significant differences due to temperatures were noted.

Thyroxine therapy influenced only semen volume in trials two, three, and four. Volume was lower ($P<.05$) for rams which received thyroxine.

Interaction between temperature and thyroxine therapy affected only semen volume ($P<.01$) and the greatest volume of semen on any combination of treatments was that produced by untreated rams in the heated room.

In Figures 4 through 23, comparisons are shown of the various indices of semen quality for all collections in each of the four trials.

Semen collection data for individual rams used in these experiments are presented in Tables XIX through XXXVIII.

Analyses of variance for these data were conducted according to methods proposed by Snedecor (1956).

TABLE VII-EFFECT OF ENVIRONMENTAL TEMPERATURE AND THYROXINE ON SEMEN QUALITY OF RAMS[a, b]

Item	Mean Temperature 60° F.						81° F.					
	No Thyroxine		Thyroxine		Pooled		No Thyroxine		Thyroxine		Pooled	
	Obs.	Avg.	Obs.	Avg.	Obs.	Avg.	Obs.	Avg.	Obs.	Avg.	Obs.	Avg.
Volume of Semen (ml.)	17	0.9	19	0.7	36	0.8	12	1.1	18	0.8	30	0.9
Motility	18	3.6	20	3.4	38	3.5	16	2.7	19	1.6	35	2.1**
Percent Live Sperm	18	75	20	64	38	69	16	46	19	29	35	37**
Percent Normal Sperm	18	82	20	74	38	78	16	69	19	64	35	66
Sperm Concentration[c]	18	3.82	20	3.96	38	3.89	16	3.16	19	3.20	35	3.18*

*P .05
**P .01
[a]Pooled semen data of the last two collections of Trials II, III, and IV.
[b]Relative humidity in Trials II, III and IV averaged 61 percent in the heated room and above 90 percent in the cooled room.
[c]Concentration expressed in millions/mm.^{3}

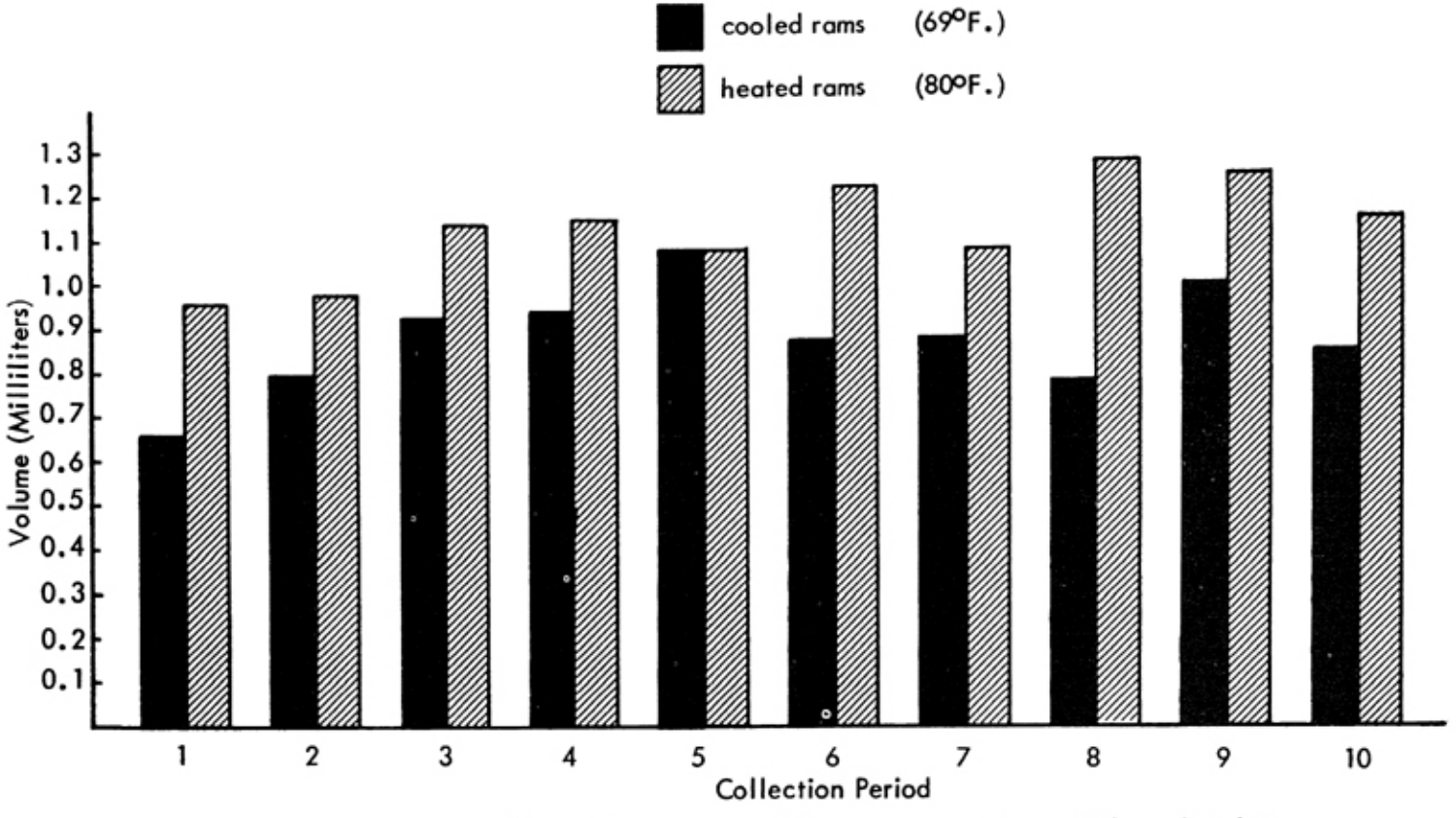


Figure 4. Effect of Environmental Temperature on Semen Volume (Trial I).

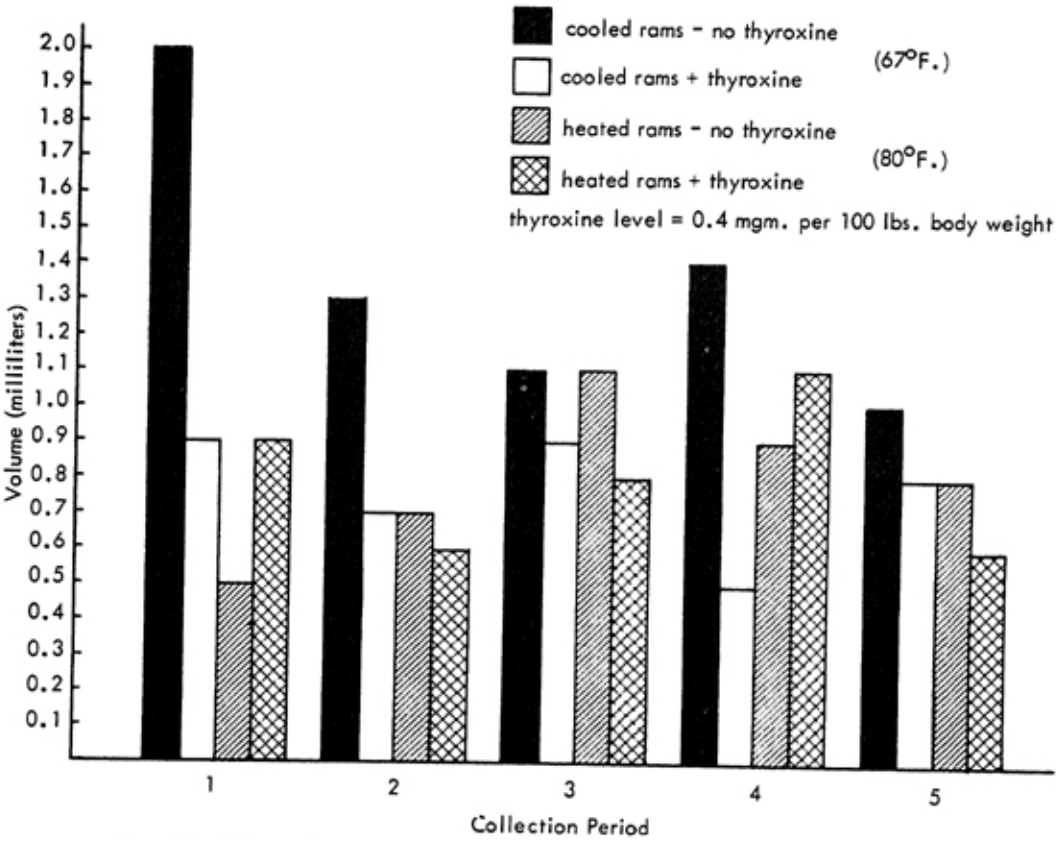


Figure 5. Effect of Environmental Temperature and Thyroxine on Semen Volume (Trial II).

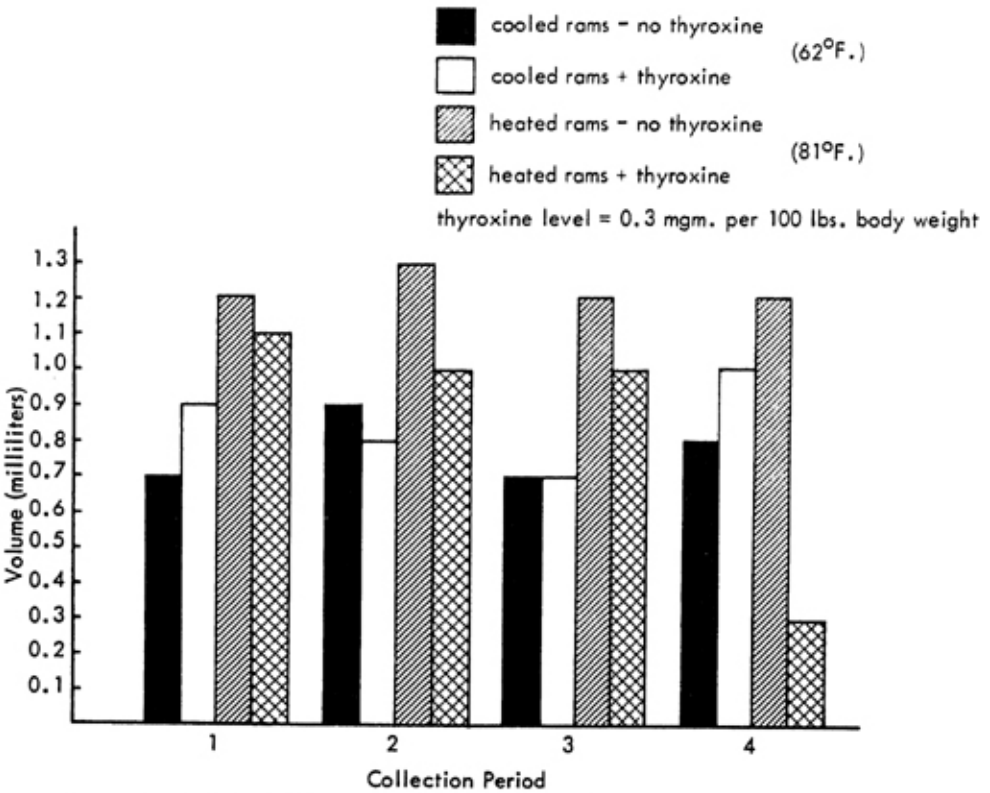


Figure 6. Effect of Environmental Temperature and Thyroxine on Semen Volume (Trial III).

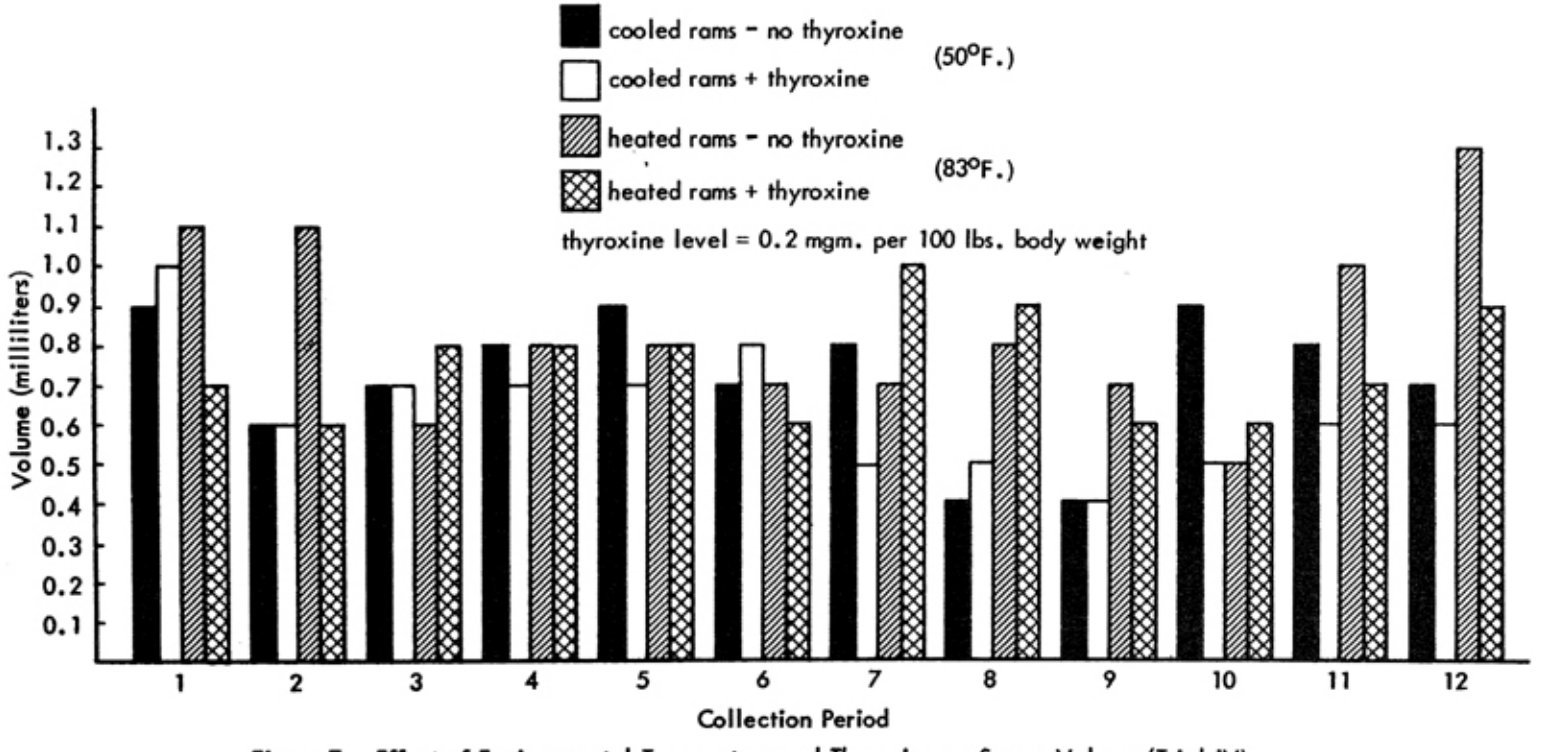


Figure 7. Effect of Environmental Temperature and Thyroxine on Semen Volume (Trial IV).

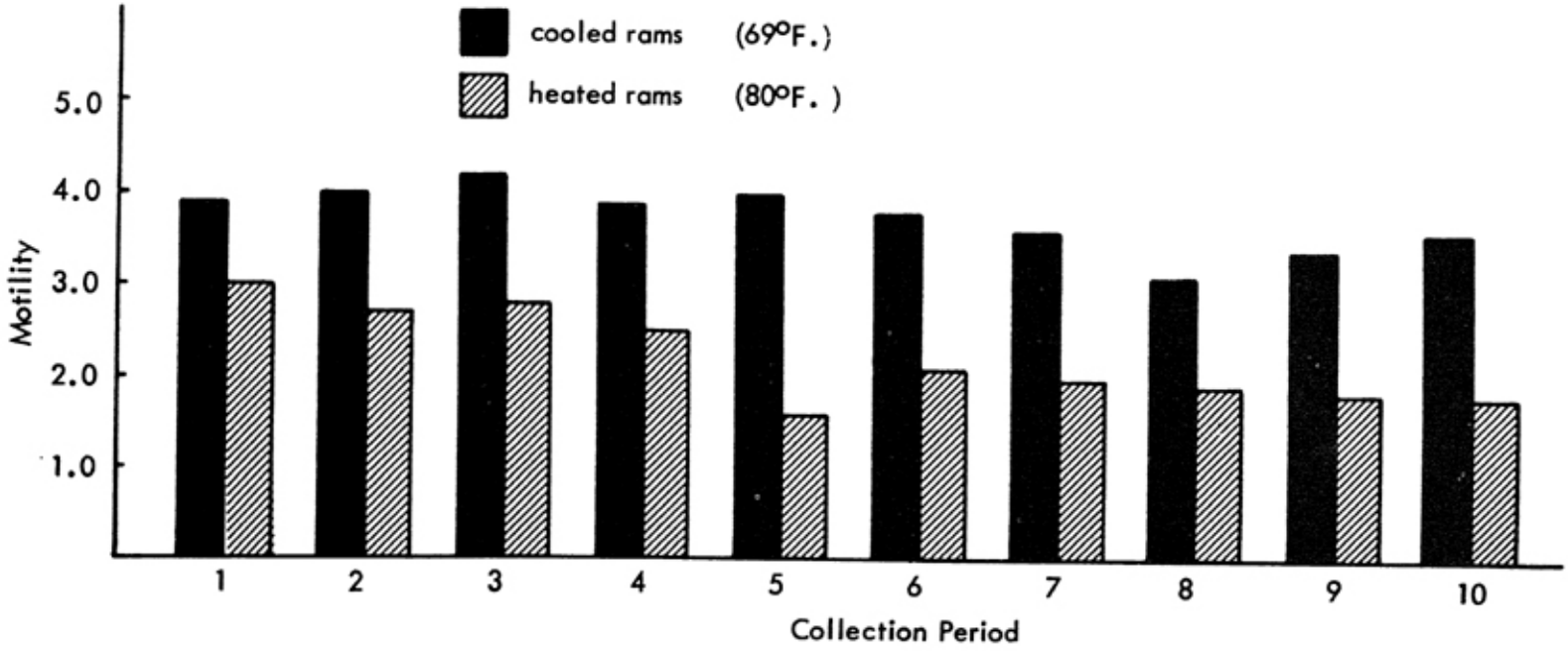


Figure 8. Effect of Environmental Temperature on Motility of Semen (Trial I).

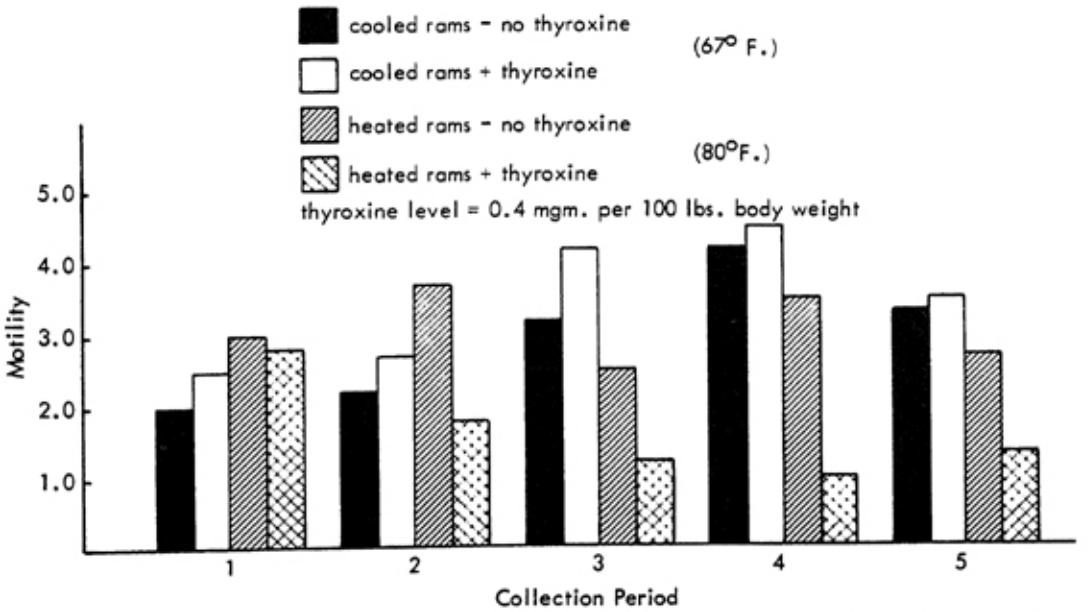


Figure 9. Effect of Environmental Temperature and Thyroxine on Motility of Semen (Trial II).

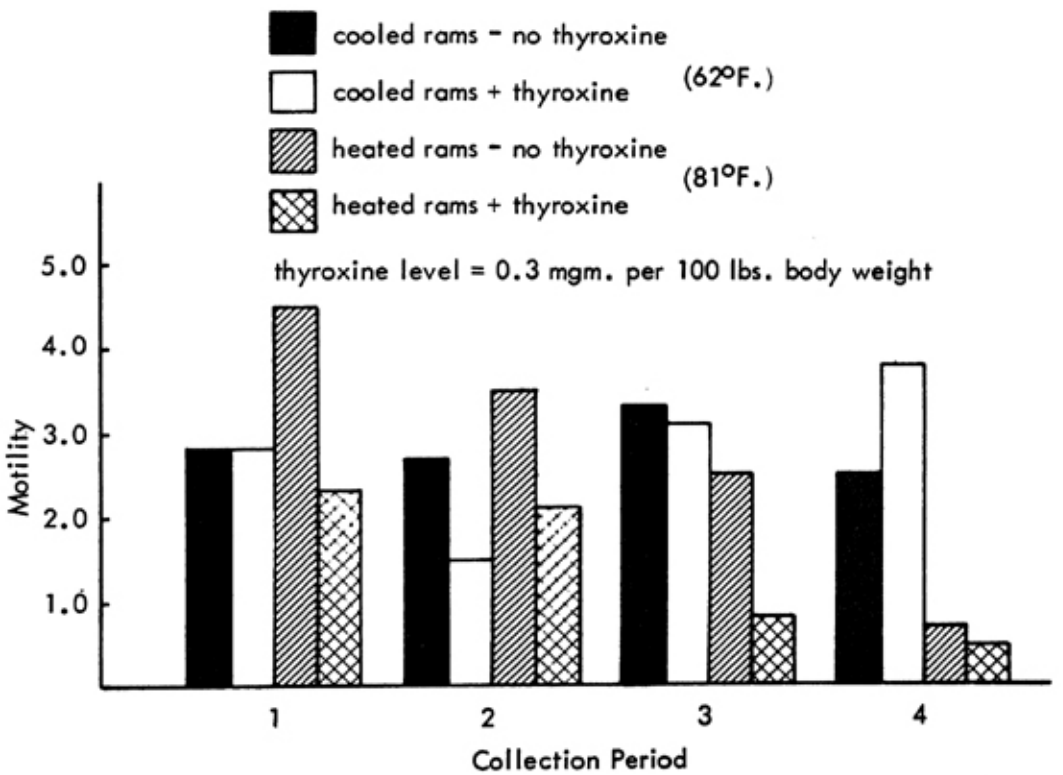


Figure 10. Effect of Environmental Temperature and Thyroxine on motility of Semen (Trial III).

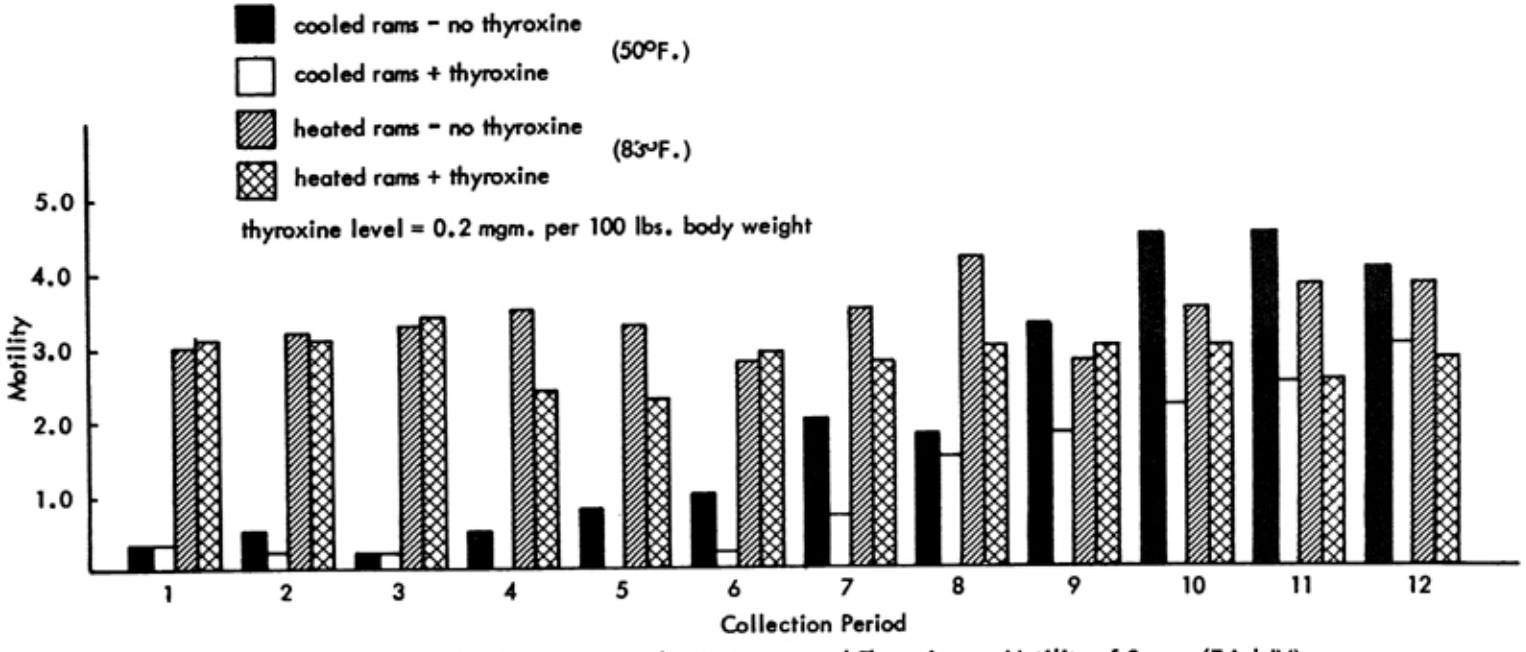


Figure 11. Effect of Environmental Temperature and Thyroxine on Motility of Semen (Trial IV).

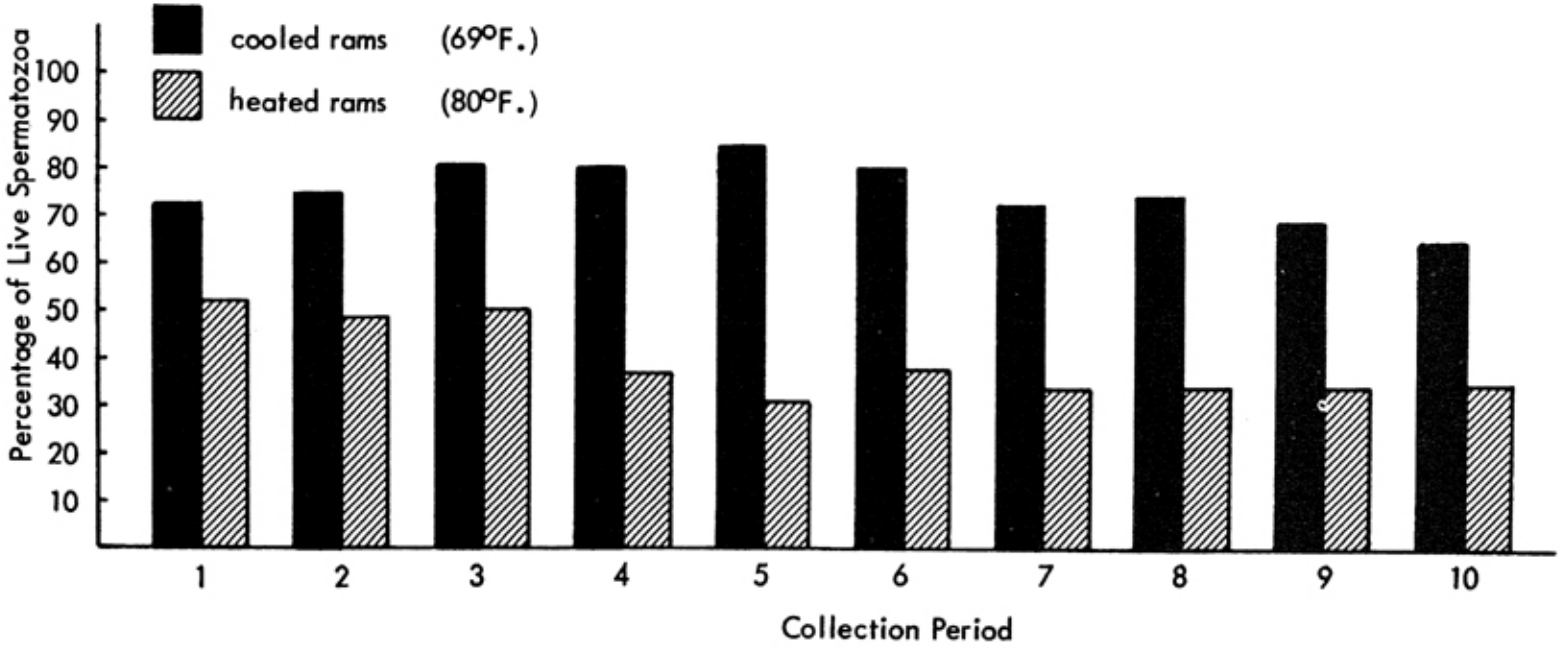


Figure 12. Effect of Environmental Temperature on Percentage of Live Spermatozoa (Trial I).

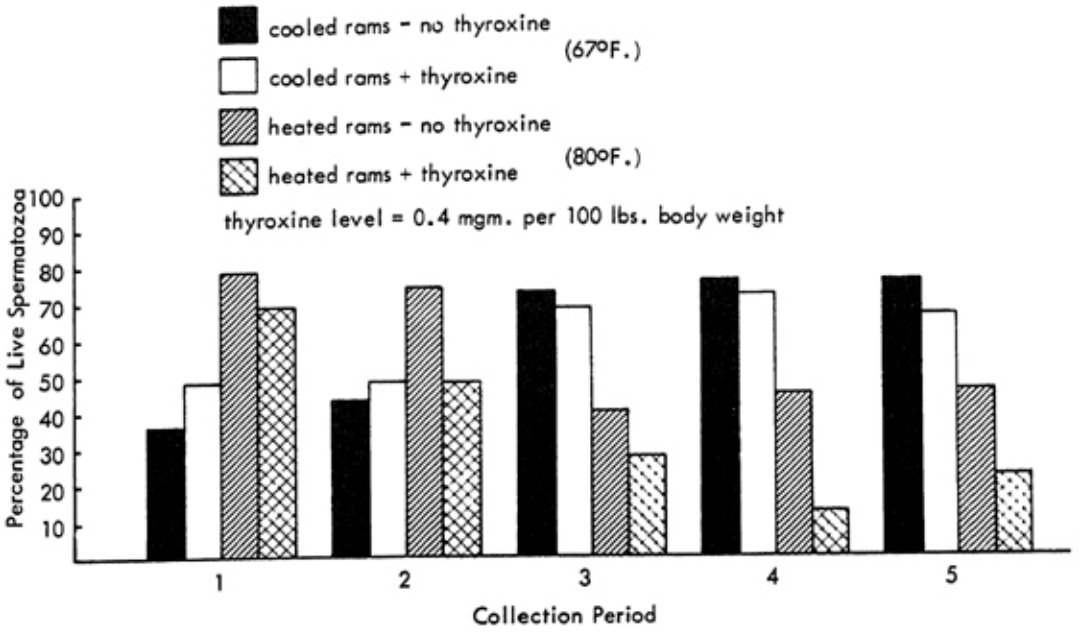


Figure 13. Effect of Environmental Temperature and Thyroxine on Percentage of Live Sperm (Trial II).

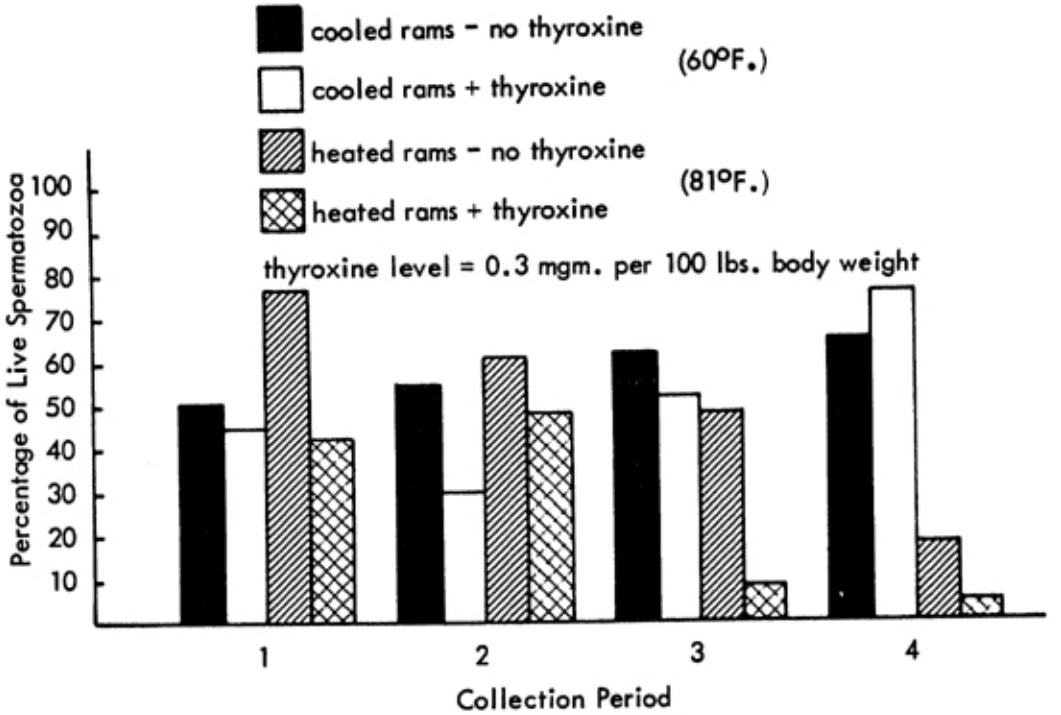


Figure 14. Effect of Environmental Temperature and Thyroxine on Percentage of Live Sperm (Trial III).

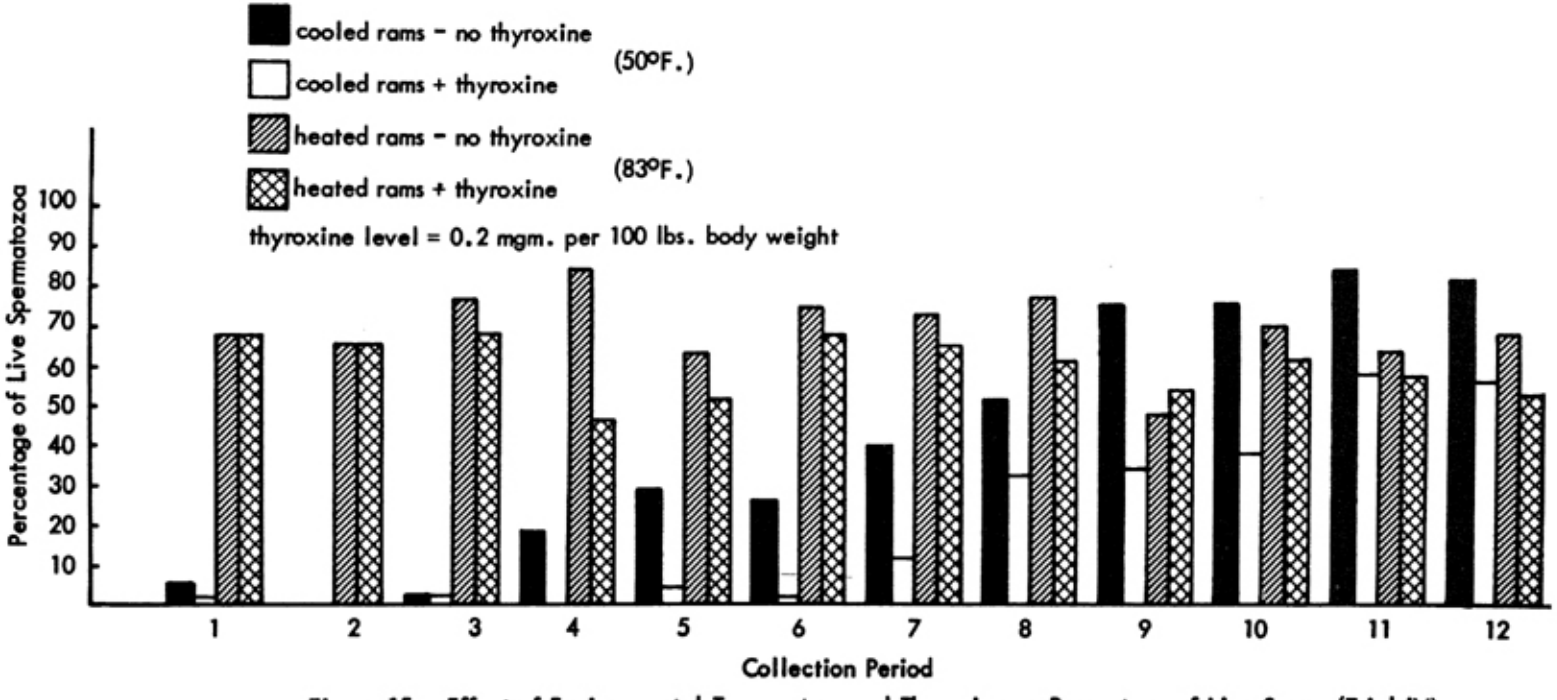


Figure 15. Effect of Environmental Temperature and Thyroxine on Percentage of Live Sperm (Trial IV).

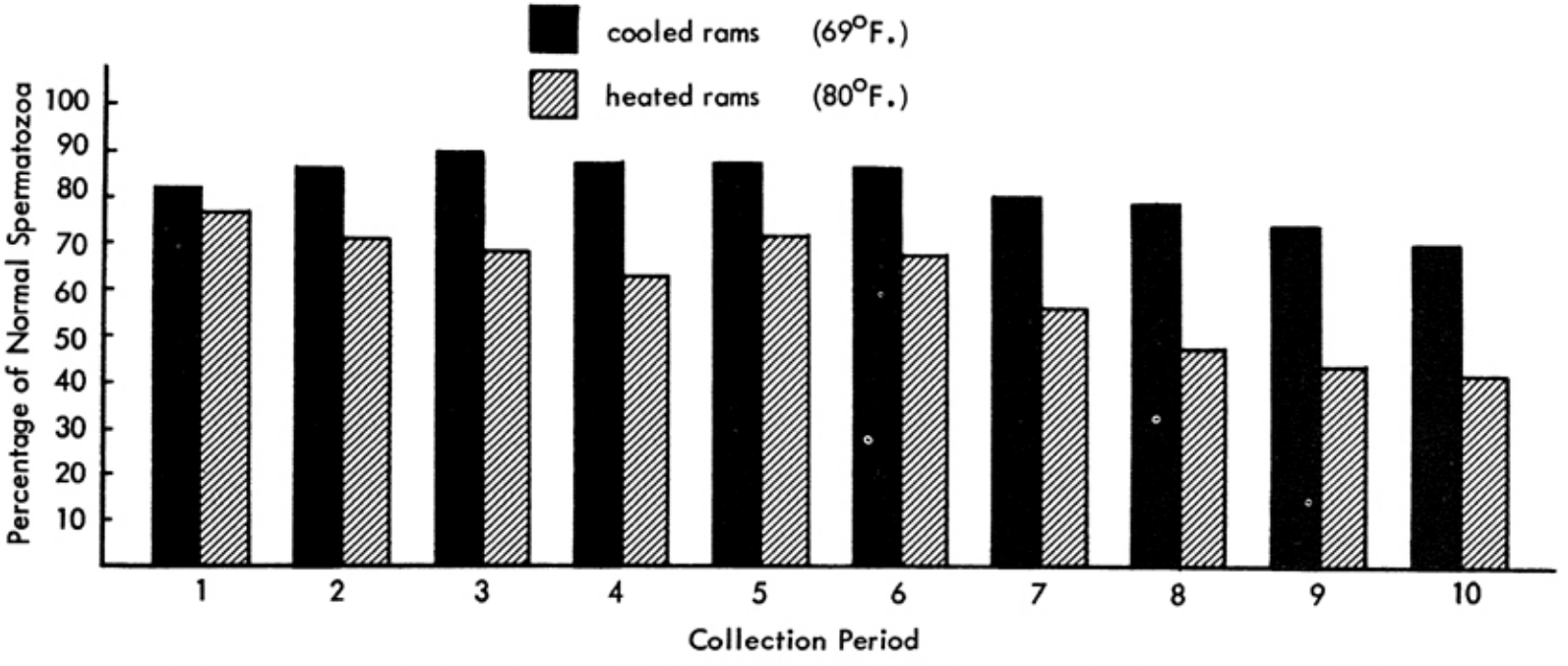


Figure 16. Effect of Environmental Temperature on Percentage of Normal Spermatozoa (Trial I).

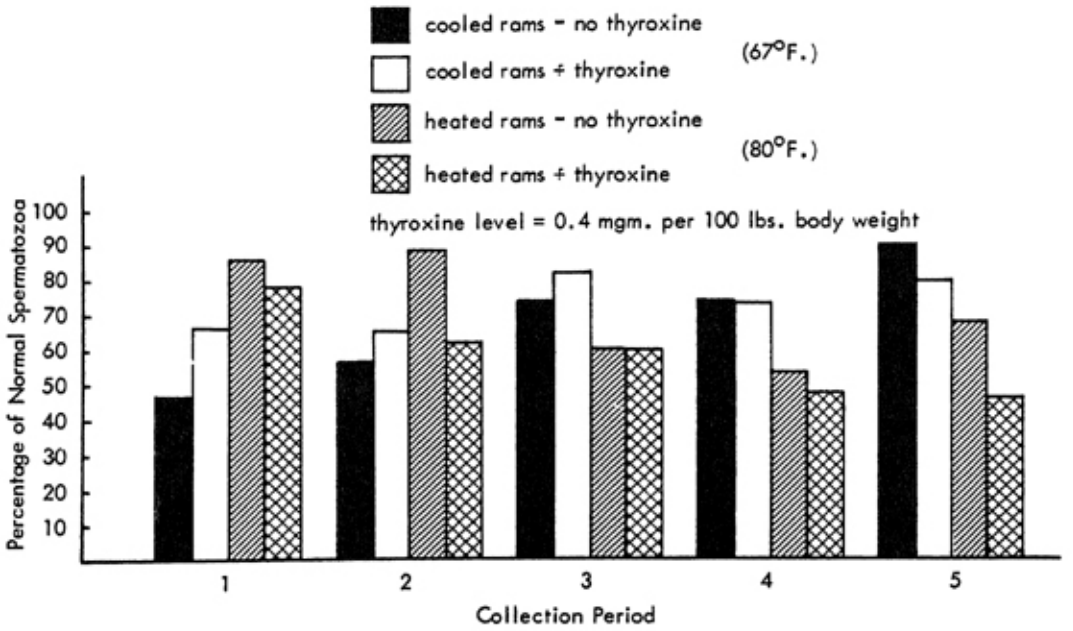


Figure 17. Effect of Environmental Temperature and Thyroxine on Percentage of Normal Sperm (Trial II).

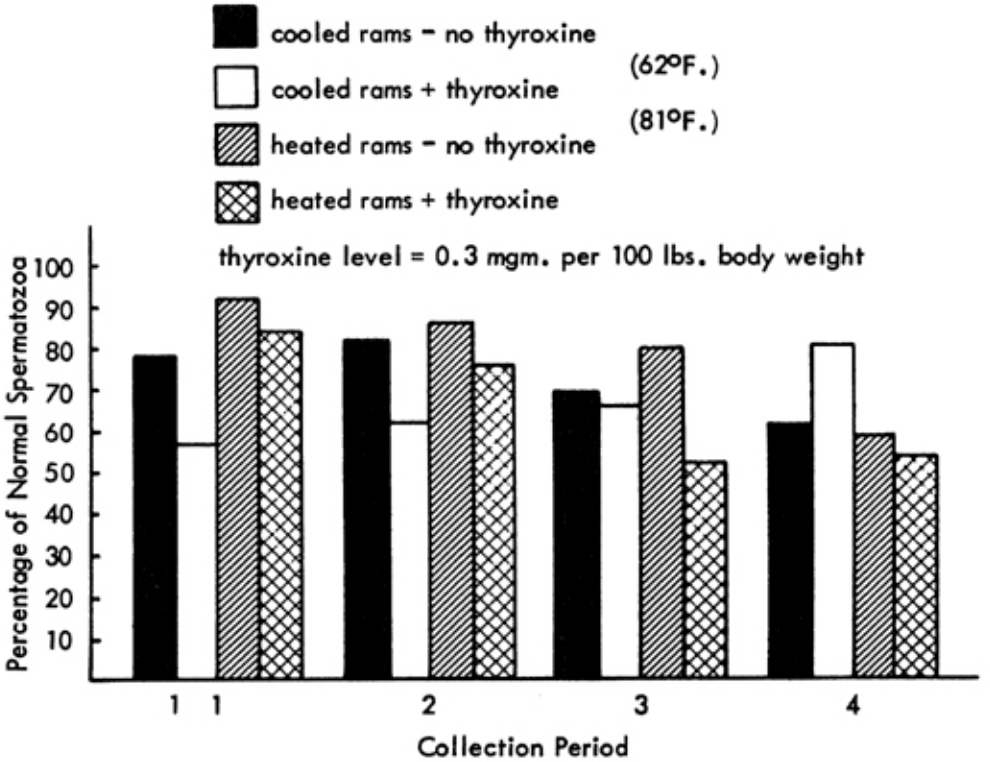


Figure 18. Effect of Environmental Temperature and Thyroxine on Percentage of Normal Sperm (Trial III).

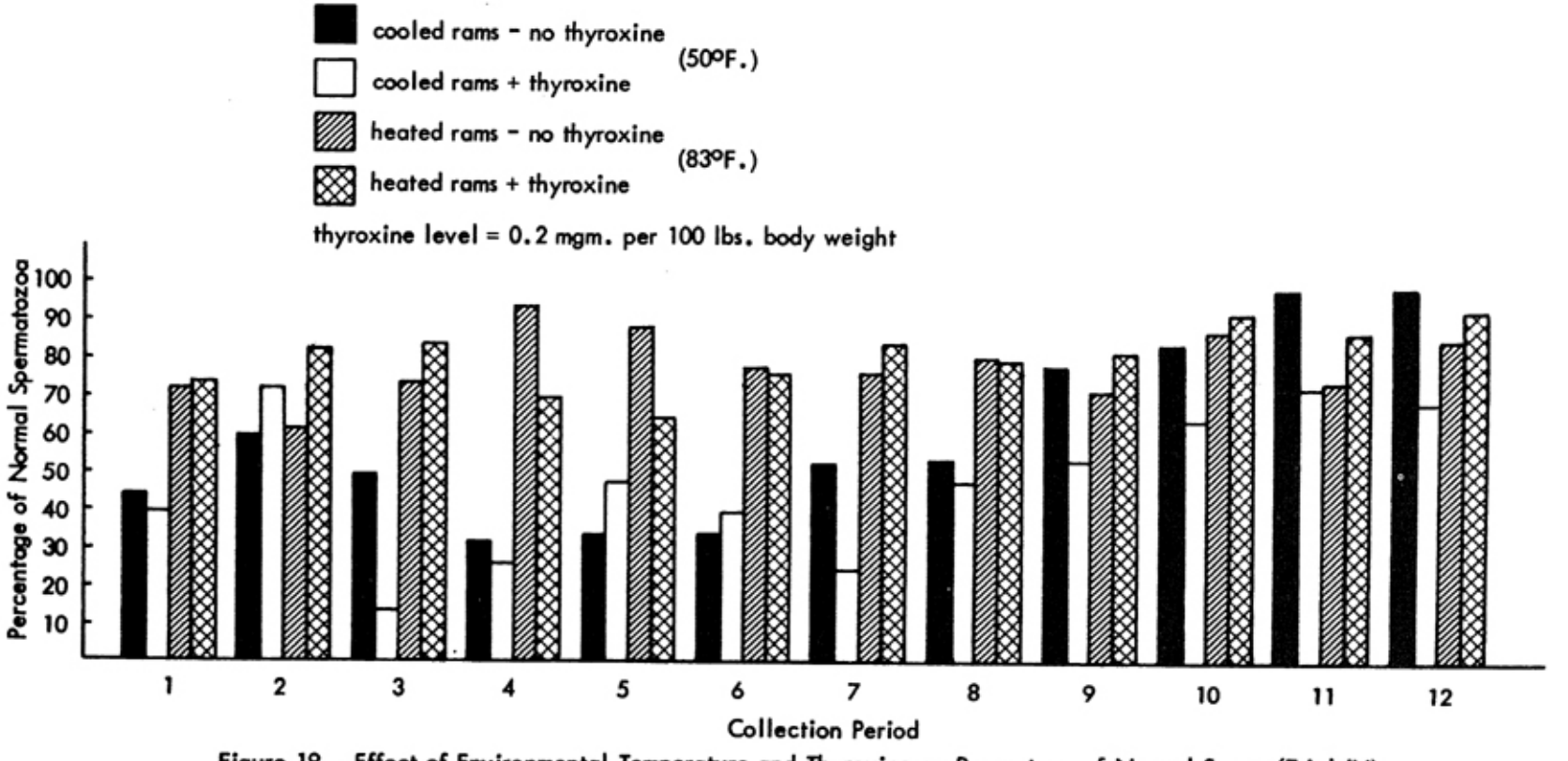


Figure 19. Effect of Environmental Temperature and Thyroxine on Percentage of Normal Sperm (Trial IV).

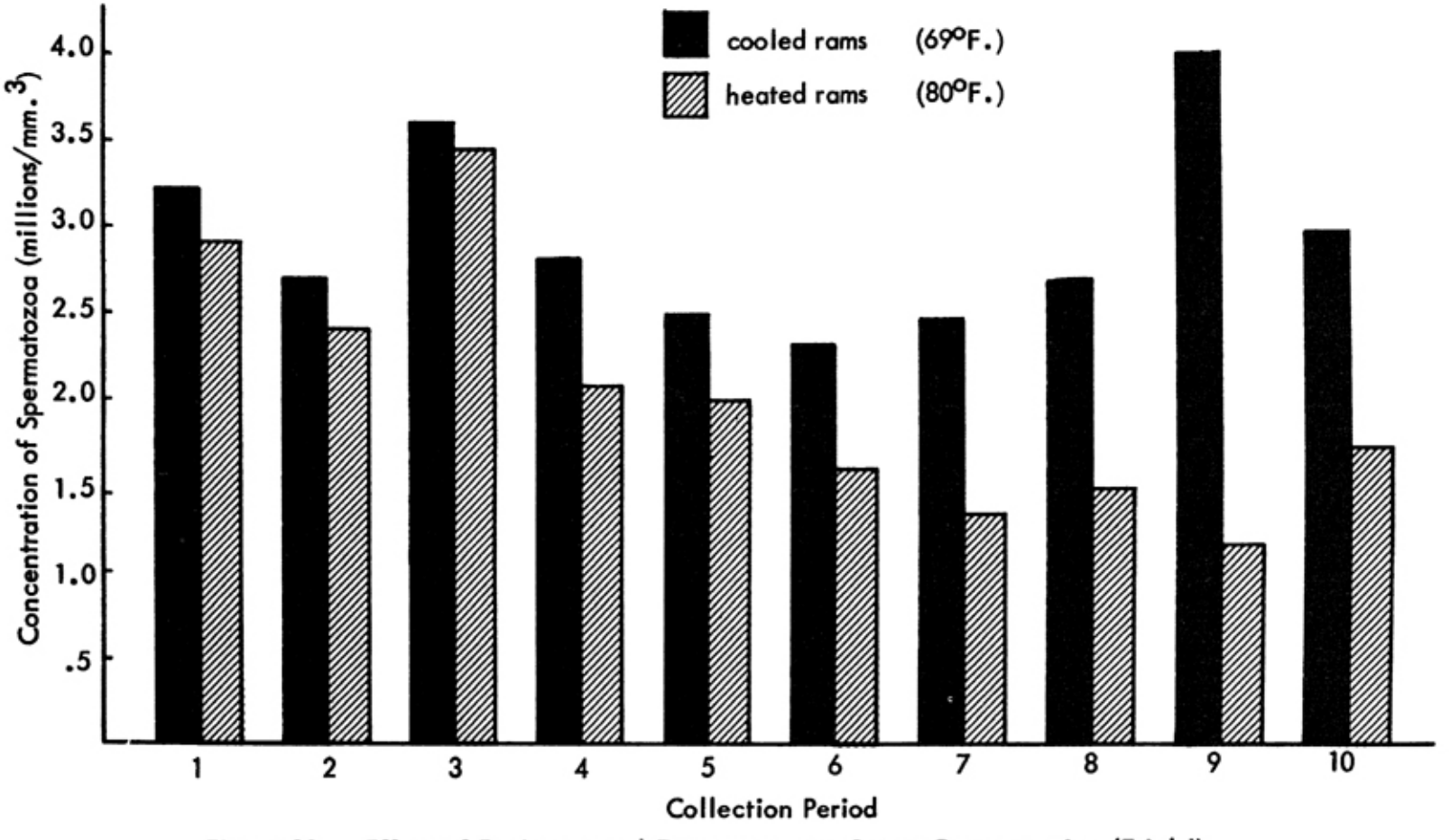


Figure 20 . Effect of Environmental Temperature on Sperm Concentration (Trial I).

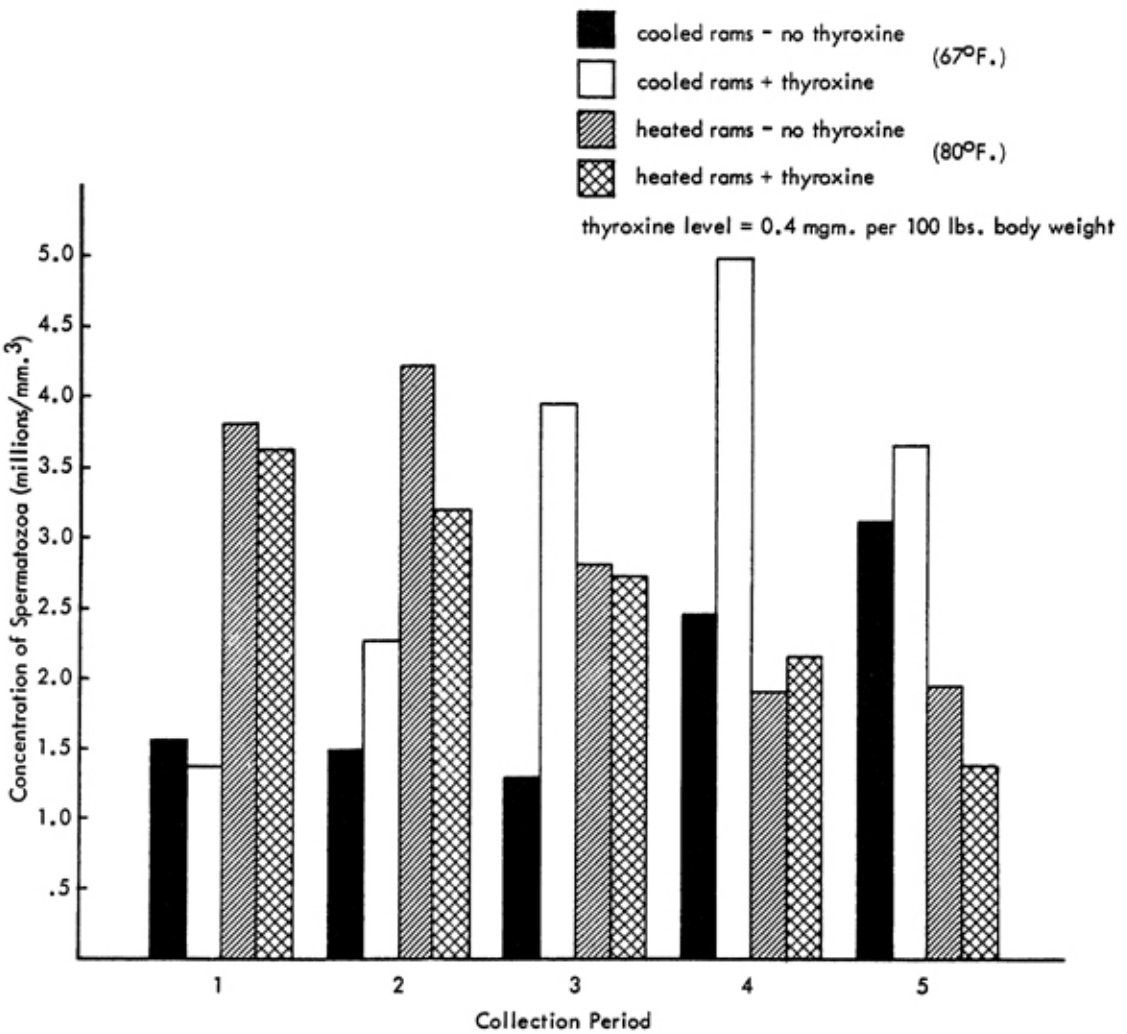


Figure 21. Effect of Environmental Temperature and Thyroxine on Sperm Concentration (Trial II).

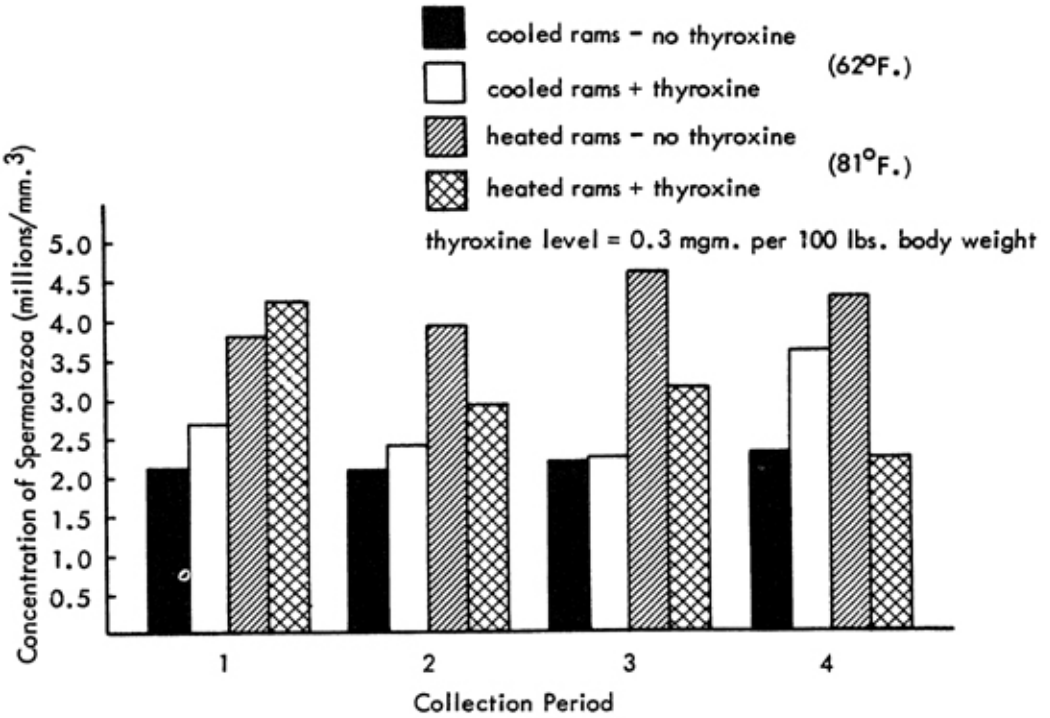


Figure 22. Effect of Environmental Temperature and Thyroxine on Sperm Concentration (Trial III).

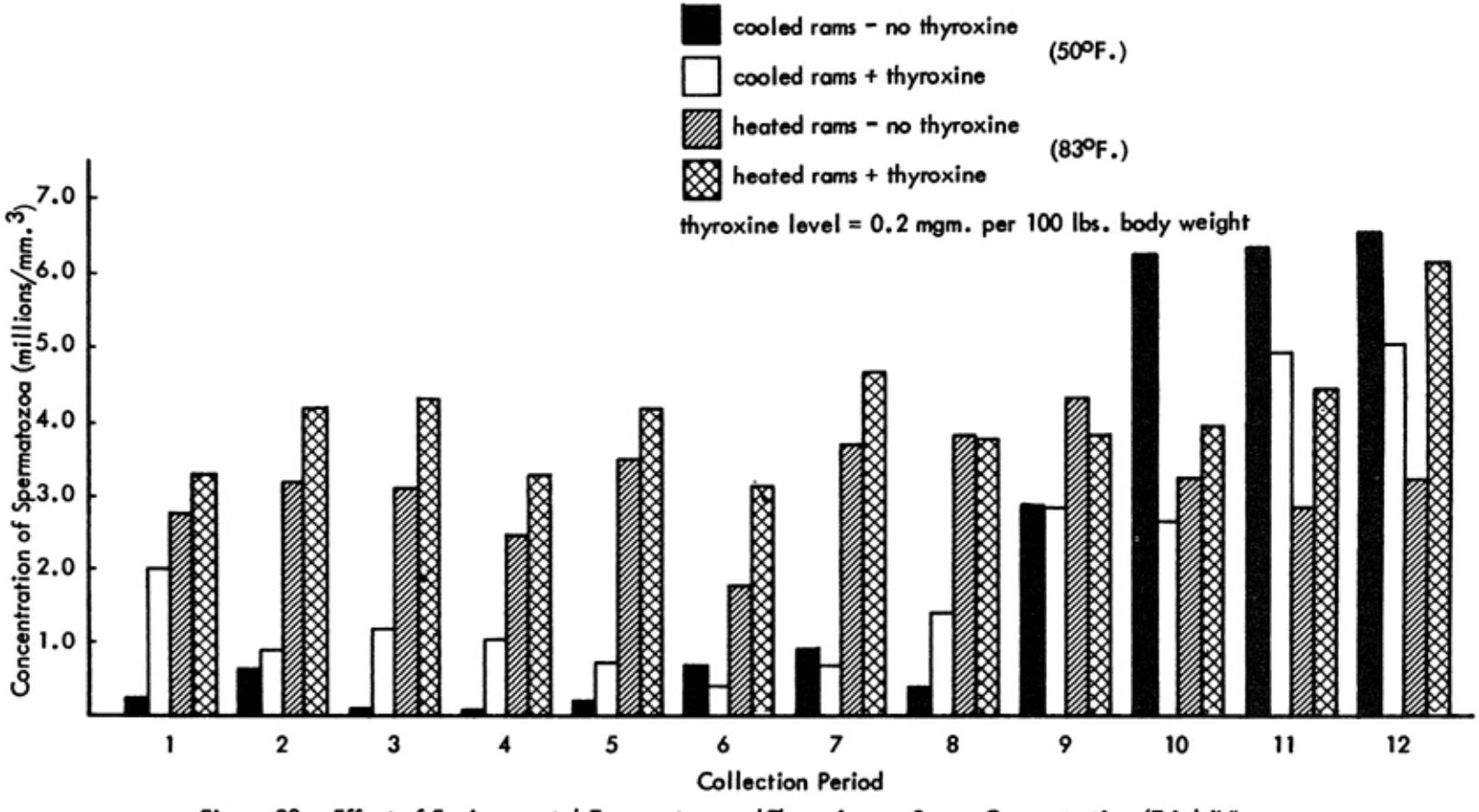


Figure 23. Effect of Environmental Temperature and Thyroxine on Sperm Concentration (Trial IV).

TABLE VIII-EFFECT OF TAPAZOLE ON VOLUME[1] OF SEMEN PRODUCED AT EACH COLLECTION PERIOD (PHASE I).

Date	12/2/ 60	12/9/ 60	12/16/ 60	12/23/ 60	12/30/ 60	1/6/ 61	1/12/ 61	1/20/ 61	1/28/ 61	2/3/ 61	2/10/ 61	2/17/ 61	3/1/ 61	3/22/ 61
Ram														
1[2]	0.4*	0.4*	0.5*		0.7*	0.6*	0.6*	0.6*	0.2*	0.6*	1.0*	0.4*	0.6*	0.5*
2		1.1*	0.5*	0.7*	0.6*	0.4*	1.1*							
3	0.5*	0.6	0.4	0.7	0.5	0.3	0.5	0.5	0.7	0.4	0.3	0.2		
4		0.7	0.4	0.2	1.0	0.4			2.0	0.8	0.7			
5[2]			0.4	0.3	0.3		0.3	0.2	0.6	0.2	0.3	0.4	0.2	0.2
6			0.2	0.8	0.6	0.4	0.3	0.9	1.0	0.9	0.5	0.5	0.8	
7							0.2	0.2	0.3					
8													0.3*	0.3*
9													0.9*	1.1
10													0.2	0.4
11													0.3	0.3

[1]Volume expressed in milliliters

[2]Control ram

*Samples collected using artificial vagina. Remainder were collected by electro-ejaculation.

TABLE IX-EFFECT OF TAPAZOLE ON MOTILITY OF SEMEN PRODUCED AT EACH COLLECTION PERIOD (PHASE I).[3]

Date	12/2/ 60	12/9/ 60	12/16* 60	12/23/ 60	12/30/ 60	1/6/ 61	1/12/ 61	1/20/ 61	1/28/ 61	2/3/ 61	2/10/ 61	2/17/ 61	3/1/ 61	3/22/ 61
Ram														
1[2]	4.0*	5.0*	5.0*	5.0*	5.0*	5.0*	5.0*	5.0*	4.5*	4.0*	4.0*	4.5*	4.5*	4.5*
2		4.5*	4.5*	5.0*	4.5*	4.5*	4.5*							
3	5.0*	4.0	3.0	3.0	2.5	4.0	3.0	4.0	4.0	3.0	4.0	3.0		
4		2.5	3.5	2.5	4.5	4.0	2.5	1.0	2.0	4.0	3.5			
5[2]			4.0	2.5	1.5		2.0	2.5	3.5	2.5	3.5	3.0	3.5	4.0
6			3.0	4.0	4.0	3.0	3.5	4.5	3.5	4.0	4.0	3.0	4.0	
7							1.5	2.5	3.0					
8													3.0*	4.0*
9													2.5*	3.0
10													3.5	1.5
11													0.0	0.0

[3]For explanation of symbols see Table VIII.

TABLE X-EFFECT OF TAPAZOLE ON PERCENTAGE OF LIVE SPERM PRODUCED AT EACH COLLECTION PERIOD (PHASE I).

Date	12/2/ 60	12/9/ 60	12/16/ 60	12/23/ 60	12/30/ 60	1/6/ 61	1/12/ 61	1/20/ 61	1/28/ 61	2/3/ 61	2/10/ 61	2/17/ 61	3/1/ 61	3/22/ 61
Ram														
1[2]	93*	75*	84*	88*	83*	78*	88*	88*	78*	90*	88*	85*	89*	93*
2		93*	55*	56*	88*	96*	97*							
3	88*		87	96	98	88	97	95	97	92	95	94		
4			90	83	95	99	95	91	95	92	95			
5[2]			97	92	94	89	95	92	93	92	94	90	89	96
6			95	98	99	98	98	97	93	94	95	92	91	
7							94	84	90					
8													63*	61*
9													52*	80
10													77	56
11													1	

[3] For explanation of symbols see Table VIII.

TABLE XI-EFFECT OF TAPAZOLE ON PERCENTAGE OF NORMAL SPERM PRODUCED AT EACH COLLECTION PERIOD (PHASE I).[3]

Date	12/2/ 60	12/9/ 60	12/16/ 60	12/23/ 60	12/30/ 60	1/6/ 61	1/12/ 61	1/20/ 61	1/28/ 61	2/3/ 61	2/10/ 61	2/17/ 61	3/1/ 61	3/22/ 61
Ram														
1[2]	93*	99*	86*	97*	99*	97*	98*	96*	97*	97*	98*	98*	96*	96*
2		93*	85*	97*	97*	99*	99*							
3	93*	99	96	97	96	95	98	98	100	98	99	93		
4		90	95	96	99	99	98	95	100	96	95			
5[2]			96	95	94	89	93	87	81	86	93	91	93	97
6			96	99	95	99	99	100	98	100	99	100	93	
7							93	65	81					
8													98*	76*
9													95*	88
10													84	57
11													18	

[3]For explanation of symbols see Table VIII.

TABLE XII-EFFECT OF TAPAZOLE ON SPERM CONCENTRATION[4] AT EACH COLLECTION PERIOD (PHASE I).[3]

Date	12/2/ 60	12/9/ 60	12/16/ 60	12/23/ 60	12/30/ 60	1/6/ 61	1/12/ 61	1/20/ 61	1/28/ 61	2/3/ 61	2/10/ 61	2/17/ 61	3/1/ 61	3/22/ 61
Ram														
1[2]	2.88*	6.00*	5.50*	4.64*	5.74*	4.90*	4.41*	3.98*	4.74*	4.69*	5.77*	5.22*	5.31*	5.51*
2		4.77*	6.61*	4.88*	5.04*	3.55*	4.71*							
3	3.67*	3.05	.24	1.58	1.81	3.18	1.38	1.68	1.05	.97	2.83	2.11		
4		3.44	2.54	1.40	3.97	3.97	1.84	3.27	.71	4.97	3.82			
5[2]			4.05	.67	.34	.38	.53	.99	1.19	.39	1.02	1.47	1.22	.50
6			2.24	2.48	3.40	1.80	2.50	3.84	3.70	6.37	2.72	7.02	4.74	
7							.33	.75	.56					
8													4.95*	4.21*
9													4.55*	2.90
10													.88	.06
11													.08	.00

[3]For explanation of symbols see Table VIII.
[4]Concentration expressed as millions/mm^3.

TABLE XIII-EFFECT OF TAPAZOLE OR THYROIDECTOMY ON VOLUME[1] OF SEMEN PRODUCED AT EACH COLLECTION PERIOD (PHASE II)

Date	5/7/ 61	5/14/ 61	5/22/ 61	5/29/ 61	6/6/ 61	6/14/ 61	6/21/ 61	6/28/ 61	7/4/ 61
Ram									
1	0.7				0.8*	0.9*	0.9*	1.5*	
2[2]		0.3*	0.8*			1.3*			1.3*
3[2]	0.6	0.5	0.6		0.5	0.2	0.4*	0.4*	0.3*
4	0.4	0.4		0.7	0.4	0.6			
5	0.4		0.3		0.3		0.3		
6[2]	0.5*	0.8*	1.0*		1.4*	1.3*	1.2*	1.2*	1.0*
7	0.8	0.8	1.0	1.5	0.8	0.7	1.5	0.4	
8	0.6	0.7	0.5	0.7	0.2		0.4	1.2	
9		0.5	1.9	1.8	1.2	1.5			
10	0.3	0.5							
11	0.5			0.4	0.6	0.7	0.6	1.0	
12[2]	0.7*	0.7*	0.8*	1.3*	1.0*	0.8*	0.6*	1.1*	1.0*
13[3]	0.6		1.0	0.6	1.2		1.1	0.6	
14[3]	1.0	1.4	0.4	1.4	0.8	1.5	1.8	0.6	
15[3]	0.5						0.4	0.2	
16[3]	0.3				0.3	0.2	0.4	0.4	

[1]Volume expressed in milliliters
[2]Thyroidectomized rams
[3]Control rams
*Sample collected by artificial vagina

TABLE XIV-EFFECT OF TAPAZOLE OR THYROIDECTOMY ON MOTILITY OF SEMEN PRODUCED AT EACH COLLECTION PERIOD (PHASE II).[4]

Date	5/7/ 61	5/14/ 61	5/22/ 61	5/29/ 61	6/6/ 61	6/14/ 61	6/21/ 61	6/28/ 61	7/4/ 61
Ram									
1	1.5				3.0*	4.5*	4.5*	5.0*	
2[2]	2.5*	3.0*	3.0*	4.0*	2.5*	2.5*	3.5*	3.5*	3.5*
3[2]	4.0	3.5	3.0		3.0	1.5	4.0*	4.5*	3.5*
4	4.5	1.5		2.5	2.5	1.0			
5	1.5		0.5		2.0		1.0	1.0	
6[2]	2.5*	4.0*	4.0*		4.0*	4.5*	4.5*	3.0*	3.5*
7	3.0	2.5	1.5	4.0	4.0	4.0	2.5	3.5	
8	2.5	3.5	3.5	1.5	0.5	2.5	1.5	0.5	
9		3.0	3.5	4.0	4.0	0.5	0.5		
10	2.5	2.5							
11	1.5			1.0	2.0	1.5	1.5	2.0	
12[2]	3.5*	4.0*	3.5*	4.5*	4.0*	3.0*	4.0*	4.5*	3.5*
13[3]	4.0		4.0	3.5	4.0	3.5	3.5	3.0	
14[3]	4.0	3.5	2.5	3.5	4.0	3.5	4.0	4.0	
15[3]	4.0						2.0	1.0	
16[3]	2.0				2.5	1.0	4.0	1.5	

[4]For explanation of symbols see Table XIII.

TABLE XV-EFFECT OF TAPAZOLE OR THYROIDECTOMY ON PERCENTAGE OF LIVE SPERM PRODUCED AT EACH COLLECTION PERIOD (PHASE II)[4]

Date	5/7/ 61	5/14/ 61	5/22/ 61	5/29/ 61	6/6/ 61	6/14/ 61	6/21/ 61	6/28/ 61	7/4/ 61
Ram									
1	95				58*	93*	94*	86*	
2[2]	51*	48*	76*	89*	77*	86*	94*	83*	84*
3[2]	81	92	94		96	87	91*	92*	92*
4	84	88		92	84				
5	68		77		87		92		
6[2]	71*	90*	69*		84*	93*	82*	60*	78*
7	75	75	85	89	88	90	86	86	
8	57	92	97	94	0	85	59	4	
9		86	84	87	92	0	0		
10	82	62							
11	77			85	94	82	85	59	
12[2]	59*	75*	84*	81*	88*	86*	91*	87*	87*
13[3]	76		87	91	77	73	92	81	
14[3]	72	86	73	90	83	85	90	80	
15[3]	87						88	23	
16[3]					93		84		

[4]For explanation of symbols see Table XIII.

TABLE XVI-EFFECT OF TAPAZOLE OR THYROIDECTOMY ON PERCENTAGE OF NORMAL SPERM PRODUCED AT EACH COLLECTION PERIOD (PHASE II)[4]

Date	5/7/ 61	5/14/ 61	5/22/ 61	5/29/ 61	6/6/ 61	6/14/ 61	6/21/ 61	6/28/ 61	7/4/ 61
Ram									
1	99				98*	99*	98*	97*	
2[2]	72*	80*	88*	98*	96*	93*	85*	79*	90*
3[2]	92	97	90		95	96	98*	91*	99*
4	98	85		85	47				
5	98		99		96		94		
6[2]	67*	98*	95*		97*	99*	96*	92*	96*
7	95	96	98	98	98	96	96	95	
8	54	89	96	99	89	87	74	67	
9		96	95	98	95	21	48		
10	93	59							
11	95			99	95	95	98	95	
12[2]	95*	97*	98*	96*	99*	96*	97*	98*	98*
13[3]	89		88	96	92	96	81	88	
14[3]	93	98	98	98	99	97	99	98	
15[3]	98						95	58	
16[3]	84				92		96		

[4]For explanation of symbols see Table XIII.

TABLE XVII-EFFECT OF TAPAZOLE OR THYROIDECTOMY ON SPERM CONCENTRATION[5] AT EACH COLLECTION PERIOD (PHASE II)[4]

Date	5/7/ 61	5/14/ 61	5/22/ 61	5/29/ 61	6/6/ 61	6/14/ 61	6/21/ 61	6/28/ 61	7/4/ 61
Ram									
1					4.82*	5.48*	4.83*	5.05*	
2^2	2.62*	6.73*	3.28*	3.15*	2.22*	3.01*	2.32*	2.63*	4.58*
3^2							5.38*	5.76*	5.25*
6^2	6.12*	6.18*	6.41*		4.79*	5.23*	5.70*	5.51*	4.67*
13^2	4.50*	6.23*	3.92*	4.97*	4.89*	5.28*	3.46*	4.71*	5.28*

[4]For explanation of symbols see Table XIII.
[5]Concentration expressed as millions/mm^3.

TABLE XVIII-SEVENTY-TWO HOUR I^{131} UPTAKE IN CONTROL, THYROIDECTOMIZED AND TAPAZOLE TREATED RAMS[1]

Ram	Treatment	Counts Per Minute	Percent of Injected Dose
13	Control	11000	12.7
14	Control	15000	17.4
15	Control	8200	9.4
16	Control	14600	16.9
2	Thyroidectomized	3700	4.1
3	Thyroidectomized	1500	1.5
6	Thyroidectomized	9400	10.8
12	Thyroidectomized	1150	1.1
1	Tapazole	1050	1.0
5	Tapazole	2100	2.3
7	Tapazole	6700	7.6
8	Tapazole	6800	7.8
9	Tapazole	4000	4.5
10	Tapazole	1100	1.1
11	Tapazole	2500	2.7

[1]Tapazole administered daily at rate of 400 mgm./100 pounds body weight.

TABLE XIX-VOLUME OF SEMEN PRODUCED BY RAMS IN TRIAL I

Collection Period	Volume (Milliliters)										Treatment
	1	2	3	4	5	6	7	8	9	10	
Ram No.											
5810	0.9	0.5	1.0	0.8		0.7	0.6	0.8	0.6	1.2	Cooled
5969	0.3	1.0	1.3			1.2	0.8		1.3	0.4	Cooled
5952	0.5	0.6	0.3	1.0	1.1	1.1	1.0	0.9	1.2	0.7	Cooled
5915		1.4	1.5	1.0	1.2	0.8	1.3	1.1	1.1	1.2	Cooled
5864	0.8	0.8	0.7		1.1	0.5	0.5	0.4		0.7	Cooled
5868				1.1	1.0						Cooled
5937	0.8	0.5	0.8	0.8	1.0	0.9	1.0	0.7	0.8	0.9	Cooled
5964	0.9	1.4	1.1	1.1	1.0	1.6	1.3	1.1	1.1	0.9	Heated
5963	0.6	0.6	1.1	0.7	0.7	0.9	0.7	0.3	0.2	0.8	Heated
5787	1.3	1.2	1.0	1.5	1.5	1.6	1.0	1.5	1.4	1.4	Heated
5786				1.1							Heated
5944	1.4	1.0	1.3	1.6	0.9			1.5	1.4	1.5	Heated
5961	0.6	0.7	1.2	0.9	1.3	0.8	1.3	1.0			Heated

TABLE XX-MOTILITY OF SEMEN FROM RAMS IN TRIAL I

Collection Period	Motility										Treatment
	1	2	3	4	5	6	7	8	9	10	
Ram No.											
5810	3.5	3.0	3.5	3.0		1.5	0.5	1.0	1.0	3.5	Cooled
5969	4.0	5.0	5.0	4.0		5.0	5.0	5.0	5.0	5.0	Cooled
5952	3.0	3.0	3.0	3.0	3.0	4.0	3.5	2.5	3.0	3.0	Cooled
5915		3.5	4.0	3.5	3.5	3.0	2.5	1.0	1.0	0.5	Cooled
5864	4.5	5.0	5.0	4.5	5.0	4.5	4.5	4.0	5.0	4.5	Cooled
5868			4.5	4.5	4.0	4.0	4.5	3.0	5.0	4.0	Cooled
5937	4.5	4.5	4.5	5.0	4.5	4.5	4.5	5.0	4.0	5.0	Cooled
5964	3.0	5.0	4.0	3.5	3.0	4.5	4.0	2.5	3.5	3.5	Heated
5963	3.0	1.0	3.5	5.0	3.5	3.0	4.5	4.5	4.0	3.5	Heated
5787	1.5	2.5	0.5	1.5	0.5	0.5	0.5	1.0	0.5	1.0	Heated
5786				1.0				2.5	2.0	1.5	Heated
5944	5.0	4.5	5.0	3.0	0.5		0.5	0.5	0.5	0.5	Heated
5961	2.5	0.5	1.0	1.0	0.5	0.5	0.5	0.5	0.5	0.5	Heated

TABLE XXI-PERCENTAGE OF LIVE SPERM PRODUCED BY RAMS IN TRIAL I

Collection Period	Percentage of Live Sperm										Treatment
	1	2	3	4	5	6	7	8	9	10	
Ram No.											
5810	59	42	55	61		47	17	28	22	47	Cooled
5969	68	85	84	89		87	91	99	93	96	Cooled
5952	61	72	74	66	74	74	78	54	56	62	Cooled
5915		80	86	81	78	91	50	77	28	8	Cooled
5864	89	77	90	95	97	91	96	84	94	79	Cooled
5868			86	81	88	94	90	86	93	79	Cooled
5937	90	93	94	90	90	85	90	94	95	93	Cooled
5964	66	86	74	60	62	72	65	48	70	70	Heated
5963	50	24	70	86	79	68	79	92	88	66	Heated
5787	14	28	7	16	7	5	8	11	7	11	Heated
5786				10				24	28	58	Heated
5944	79	93	91	34	33		7	18	8	0	Heated
5961	51	13	10	16	6	6	9	10	4	5	Heated

TABLE XXII-PERCENTAGE OF NORMAL SPERM PRODUCED BY RAMS IN TRIAL I

Collection Period	Percentage of Normal Sperm										Treatment
	1	2	3	4	5	6	7	8	9	10	
Ram No.											
5810	73	78	83	63		77	51	53	40	78	Cooled
5969	85	90	90	98		96	94	95	96	92	Cooled
5952	60	59	70	70	67	85	67	39	63	34	Cooled
5915		90	92	90	92	59	59	71	25	2	Cooled
5864	93	99	99	100	98	97	99	94	100	100	Cooled
5868			95	97	99	98	96	98	98	88	Cooled
5937	98	98	99	100	96	99	100	100	100	99	Cooled
5964	96	96	92	72	80	85	85	76	84	85	Heated
5963	68	58	79	87	80	65	90	93	95	60	Heated
5787	73	62	22	45	50	63	32	28	10	27	Heated
5786				32				30	31	40	Heated
5944	96	95	94	71	79		60	41	26	4	Heated
5961	53	42	58	69	70	57	20	18	17	34	Heated

TABLE XXIII-SPERM CONCENTRATION IN SEMEN PRODUCED BY RAMS IN TRIAL I

	Concentration (Millions of sperm / mm^3.)										
Collection Period	1	2	3	4	5	6	7	8	9	10	Treatment
Ram No.											
5810	3.57	3.62	5.49	3.06		2.46	3.05	4.00	3.42	2.93	Cooled
5969	3.61	3.60	3.80	2.81		2.22	4.09	1.43	4.24	3.46	Cooled
5952	.77	1.17	1.66	2.28	2.10	2.72	2.52	4.11	4.23	3.47	Cooled
5915		.74	1.54	1.30	1.00	1.16	.90	.15	.23	.07	Cooled
5864	4.27	3.79	5.18	2.80	3.57	3.73	2.15	4.87	5.47	4.65	Cooled
5868			3.79	3.24	3.20	.81	1.81	1.13	6.05	3.25	Cooled
5937	3.91	3.18	3.91	4.38	2.49	3.31	2.76	2.99	4.29	2.89	Cooled
5964	4.86	4.33	3.87	2.88	2.61	3.54	2.94	1.67	2.07	3.87	Heated
5963	3.79	2.24	5.03	2.65	3.10	2.19	3.66	4.34	3.09	3.85	Heated
5787	1.05	1.70	2.75	1.10	1.68	.36	.05	.50	.30	1.08	Heated
5786				1.19				1.63	.91	1.00	Heated
5944	3.56	2.71	2.48	2.81	1.73		.02	.21	.12	.15	Heated
5961	1.32	1.07	3.19	1.86	.78	.23	.06	.58	.45	.27	Heated

TABLE XXIV-VOLUME OF SEMEN PRODUCED BY RAMS IN TRIAL II

Collection Period	Volume (milliliters) 1	2	3	4	5	Treatment
Ram No.						
5944	1.8	1.9	1.5	0.7	0.7	Cooled
5964	1.9	1.2	0.9	1.9	1.2	Cooled
5787	2.2	0.8	0.9	1.5	1.2	Cooled
5963		0.4	1.1	0.4	0.9	Cooled and Thyroxine[1]
5786		1.0	1.3	0.6	0.8	Cooled and Thyroxine
5961	0.9	0.6	0.4	0.5	0.6	Cooled and Thyroxine
5868			1.4			Heated
5969	0.5	0.5	1.2	1.0	0.9	Heated
6008		0.8	0.8	0.8	0.6	Heated
5810	1.1	0.7		0.7	0.9	Heated and Thyroxine
5937		0.7	0.5	1.1	0.6	Heated and Thyroxine
5952	1.0		1.2	1.2	0.6	Heated and Thyroxine
5864	0.5	0.3	0.6	0.4	0.4	Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.4 mg. per 100 lb. body weight.

TABLE XXV-MOTILITY OF SEMEN FROM RAMS IN TRIAL II

Collection Period	Motility 1	2	3	4	5	Treatment
Ram No.						
5944	0.5	0.5	2.0	4.5	4.0	Cooled
5964	4.0	4.5	5.0	5.0	2.0	Cooled
5787	1.5	1.5	2.5	3.0	4.0	Cooled
5963	4.5	4.0	4.5	4.0	3.5	Cooled and Thyroxine[1]
5786		3.5	4.0	4.5	3.0	Cooled and Thyroxine
5961	0.5	0.5	4.0	5.0	4.0	Cooled and Thyroxine
5868	3.0	3.0	1.0	3.0	0.0	Heated
5969	1.5	3.5	1.5	3.0	3.5	Heated
6008	4.5	4.5	5.0	4.5	4.5	Heated
5810	3.0	1.0		0.5	0.0	Heated and Thyroxine
5937	5.0	3.0	3.5	3.0	4.5	Heated and Thyroxine
5952	1.0	0.0	0.0	0.0	0.0	Heated and Thyroxine
5864	2.0	3.0	0.0	0.5	0.5	Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.4 mg. per 100 lb. body weight.

TABLE XXVI-PERCENTAGE OF LIVE SPERM PRODUCED BY RAMS IN TRIAL II

Collection Period	Percentage of Live Sperm 1	2	3	4	5	Treatment
Ram No.						
5944	0	4	60	81	81	Cooled
5964	74	84	91	80	69	Cooled
5787	34	42	67	68	79	Cooled
5963	88	75	65	62	72	Cooled and Thyroxine[1]
5786		63	69	76	63	Cooled and Thyroxine
5961	7	9	74	78	67	Cooled and Thyroxine
5868	61	43	6	14	3	Heated
5969	78	86	20	31	58	Heated
6008	88	93	97	90	78	Heated
5810	64	47		0	1	Heated and Thyroxine
5937	96	82	86	43	85	Heated and Thyroxine
5952	51	2	0	7		Heated and Thyroxine
5864	65	60	1	0	4	Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.4 mg. per 100 lb. body weight.

TABLE XXVII-PERCENTAGE OF NORMAL SPERM PRODUCED BY RAMS IN TRIAL II

Collection Period	Percentage of Normal Sperm 1	2	3	4	5	Treatment
Ram No.						
5944	3	7	60	63	81	Cooled
5964	95	94	98	98	98	Cooled
5787	43	69	63	63	93	Cooled
5963	94	89	93	59	78	Cooled and Thyroxine[1]
5786		73	77	79	79	Cooled and Thyroxine
5961	39	35	75	84	85	Cooled and Thyroxine
5868	90	74	22	35	64	Heated
5969	72	95	66	34	48	Heated
6008	96	99	95	94	95	Heated
5810	81	61		67	42	Heated and Thyroxine
5937	96	97	87	88	97	Heated and Thyroxine
5952	47	19	19	4		Heated and Thyroxine
5864	89	74	73	34	48	Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.4 mg. per 100 lb. body weight.

TABLE XXVIII-SPERM CONCENTRATION IN SEMEN PRODUCED BY RAMS IN TRIAL II

Collection Period	Concentration (millions of sperm / mm^3.) 1	2	3	4	5	Treatment
Ram No.						
5944	.14	.12	.75	1.96	3.46	Cooled
5964	4.20	3.24	2.53	4.24	4.25	Cooled
5787	.33	1.09	.56	1.14	1.66	Cooled
5963	2.34	3.54	3.96	3.41	3.09	Cooled and Thyroxine
5786		2.15	3.09	4.34	4.44	Cooled and Thyroxine
5961	.42	1.10	4.76	7.14	3.38	Cooled and Thyroxine
5868	6.15	6.83	3.94	1.60	.65	Heated
5969	2.71	3.17	2.49	.99	1.95	Heated
6008	2.60	2.63	1.91	3.11	3.29	Heated
5810	3.13	2.48		2.49	.99	Heated and Thyroxine
5937	3.96	3.35	2.37	4.02	3.76	Heated and Thyroxine
5952	3.46	2.26	.88	.27	.07	Heated and Thyroxine
5864	3.89	4.61	4.93	1.81	.71	Heated and Thyroxine

[1] L-thyroxine injected daily at level of 0.4 mg. per 100 lb. body weight.

TABLE XXIX-VOLUME OF SEMEN PRODUCED BY RAMS IN TRIAL III

Collection Period	Volume (milliliters) 1	2	3	4	5	Treatment
Ram No.						
5868	0.7			0.5		Cooled
5969	1.0	1.1	0.9	1.2	1.2	Cooled
6008	0.4	0.6	0.5	0.7	1.0	Cooled
5810	0.6	1.0	0.7	1.4	0.8	Cooled and Thyroxine[1]
5937	0.6	0.9	0.7	0.8	0.7	Cooled and Thyroxine
5952	0.7	0.6	0.4		0.5	Cooled and Thyroxine
5864	1.7	0.8	1.0	0.8	0.7	Cooled and Thyroxine
5944	0.4	1.5	1.0	1.5	1.4	Heated
5964	1.6	1.2	1.3	1.2	0.5	Heated
5787	1.5	1.1	1.3	0.8	0.7	Heated
5963	1.5	0.8	0.8	0.3	0.7	Heated and Thyroxine
5786		1.6			1.2	Heated and Thyroxine
5961	0.6	0.6	1.1			Heated and Thyroxine

[1] L-thyroxine injected daily at level of 0.3 mg. per 100 pounds body weight.

TABLE XXX-MOTILITY OF SEMEN FROM RAMS IN TRIAL III

Collection Period	Motility 1	2	3	4	5	Treatment
Ram No.						
5868	0.0	0.0	0.5	0.5	0.5	Cooled
5969	4.0	3.0	4.5	4.5	4.5	Cooled
6008	4.5	5.0	5.0	2.5	4.0	Cooled
5810	3.0	2.0	4.0	4.5	4.0	Cooled and Thyroxine[1]
5937	5.0	3.0	5.0	4.5	4.5	Cooled and Thyroxine
5952	0.5	0.5	1.0	1.5	1.5	Cooled and Thyroxine
5864	2.5	0.5	2.5	4.5	2.5	Cooled and Thyroxine
5944	4.0	3.0	1.0	0.0	0.0	Heated
5964	5.0	4.0	2.5	1.0	0.0	Heated
5787	4.5	3.5	4.0	1.0	1.0	Heated
5963	3.5	3.0	1.0	0.5	0.5	Heated and Thyroxine
5786		4.0			0.0	Heated and Thyroxine
5961	1.0	1.0	0.5	0.5		Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.3 mg. per 100 pounds body weight.

TABLE XXXI-PERCENTAGE OF LIVE SPERM PRODUCED BY RAMS IN TRIAL III

Collection Period	Percentage of Live Sperm 1	2	3	4	5	Treatment
Ram No.						
5868	0	1	9	27	33	Cooled
5969	57	68	81	79	82	Cooled
6008	96	96	97	89	89	Cooled
5810	53	29	66	76	77	Cooled and Thyroxine[1]
5937	84	74	86	81	88	Cooled and Thyroxine
5952	2	10	22	65	36	Cooled and Thyroxine
5864	39	5	33	83	69	Cooled and Thyroxine
5944	70	36	17	1	1	Heated
5964	84	74	55	13	0	Heated
5787	77	73	72	40	14	Heated
5963	73	59	11	4	3	Heated and Thyroxine
5786		76			0	Heated and Thyroxine
5961	10	8	5	5		Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.3 mg. per 100 pounds body weight.

TABLE XXXII-PERCENTAGE OF NORMAL SPERM PRODUCED BY RAMS IN TRIAL III

Collection Period	Percentage of Normal Sperm 1	2	3	4	5	Treatment
Ram No.						
5868	58	71	16	20	33	Cooled
5964	79	76	95	89	90	Cooled
6008	99	98	99	76	95	Cooled
5810	63	73	93	96	97	Cooled and Thyroxine[1]
5937	96	93	91	97	98	Cooled and Thyroxine
5952	12	37	19	41	20	Cooled and Thyroxine
5864	58	44	60	88	82	Cooled and Thyroxine
5944	84	65	53	67	56	Heated
5964	98	98	94	46	53	Heated
5787	93	98	92	63	25	Heated
5963	92	77	32	43	47	Heated and Thyroxine
5786		96			32	Heated and Thyroxine
5961	75	55	74	66		Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.3 mg. per 100 pounds body weight.

TABLE XXXIII-SPERM CONCENTRATION IN SEMEN PRODUCED BY RAMS IN TRIAL III

Collection Period	Concentration (millions of sperm / mm^3.) 1	2	3	4	5	Treatment
Ram No.						
5868	1.35	.43	.06	.24	.10	Cooled
5969	3.11	2.75	2.34	3.05	4.13	Cooled
6008	1.92	3.11	4.19	3.70	4.10	Cooled
5810	3.79	3.25	2.44	6.13	2.43	Cooled and Thyroxine[1]
5937	0	5.29	3.97	4.09	5.28	Cooled and Thyroxine
5952	.24	.44	.42	.55	2.43	Cooled and Thyroxine
5864	2.40	.63	2.20	3.70	3.18	Cooled and Thyroxine
5944	4.37	3.71	4.78	2.95	.18	Heated
5964	4.20	5.00	5.15	4.23	.23	Heated
5787	2.99	3.18	2.98	5.70	.40	Heated
5963	5.37	3.59	3.73	3.46	3.97	Heated and Thyroxine
5786		3.00			.11	Heated and Thyroxine
5961	3.15	2.13	2.54	1.09		Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.3 mg. per 100 pounds body weight.

TABLE XXXIV-VOLUME OF SEMEN PRODUCED BY RAMS IN TRIAL IV

Collection Period	Volume (milliliters)												Treatment
	1	2	3	4	5	6	7	8	9	10	11	12	
Ram No.													
5944	1.4	0.4				0.4				0.8	0.7	0.7	Cooled
5964	0.5	0.5	0.3	0.2	0.2	0.2	0.6	0.3	0.2	0.3	0.8	0.4	Cooled
5787	0.7	1.0	1.0	1.3	1.6	1.5	0.9	0.4	0.6	1.6	0.9	1.1	Cooled
5963	0.7	0.4	0.5	0.7	0.6	1.1	0.5	0.4	0.4	0.6	0.6	0.8	Cooled and Thyroxine[1]
5786	1.2	1.2	0.9		0.8		0.6	0.8			0.7	0.6	Cooled and Thyroxine
5961		0.2	0.6			0.4	0.4	0.3		0.3	0.6	0.5	Cooled and Thyroxine
5868			0.3	0.8	0.4	1.7			0.4				Heated
5969	1.2	1.3		0.9	1.5			0.9	1.2	0.5	1.0	1.3	Heated
6008	1.0	0.8	0.8	0.7	0.6	0.4	0.7	0.6	0.4				Heated
5952	0.5	0.3	1.0	0.8	0.5	0.6	1.0	0.7	0.4	0.5	0.1		Heated and Thyroxine
5864	0.7	0.4	0.7	0.6	0.7	0.8	0.8	0.7	0.5	0.4	0.6	0.9	Heated and Thyroxine
5810	0.8	1.1	1.1	0.9	1.1	0.4	1.6	1.4	0.8	0.8	1.6	1.4	Heated and Thyroxine
5937	0.7	0.7	0.4	0.8	0.8	0.4	0.5	0.8	0.7	0.5	0.4	0.5	Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.2 mg. per 100 pounds body weight.

TABLE XXXV-MOTILITY OF SEMEN FROM RAMS IN TRIAL IV

Collection Period	Motility 1	2	3	4	5	6	7	8	9	10	11	12	Treatment
Ram No.													
5944	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.0	3.5	4.5	4.5	4.5	Cooled
5964	0.0	0.5	0.0	0.5	1.0	1.5	3.0	2.5	3.5	4.5	4.5	4.0	Cooled
5787	1.0	1.0	0.5	1.0	1.5	1.5	2.5	2.0	3.0	4.5	4.5	3.5	Cooled
5963	0.5	0.5	0.5	0.0	0.0	0.0	1.0	0.0	0.0	0.5	0.5	0.0	Cooled and Thyroxine[1]
5786	0.0	0.0	0.0	0.0	0.0		0.5	2.5	4.0	4.0	3.0	4.5	Cooled and Thyroxine
5961		0.0	0.0	0.0	0.0	0.5	0.5	2.0	1.5	2.0	4.0	4.5	Cooled and Thyroxine
5868	0.5	1.0	1.5	2.5	2.5	2.0	3.0	3.5	1.5	2.5	3.0	3.5	Heated
5969	4.5	4.0		4.5	4.5			5.0	4.5	4.5	4.5	4.0	Heated
6008	4.0	4.5	5.0	3.5	3.0	3.5	4.0	4.0	2.5				Heated
5952	1.5	3.0	1.0	1.0	0.5	1.5	1.0	1.0	0.5	0.5	0.0		Heated and Thyroxine
5864	2.5	3.0	4.0	3.0	3.0	3.0	3.0	3.0	3.5	4.5	3.0	3.5	Heated and Thyroxine
5810	4.0	3.0	4.0	1.0	1.5	3.0	2.5	4.0	3.0	3.0	2.5	2.0	Heated and Thyroxine
5937	4.5	3.5	4.5	4.5	4.0	4.0	4.5	4.0	4.5	4.0	4.5	3.0	Heated and Thyroxine

[1] L-thyroxine injected daily at level of 0.2 mg. per 100 pounds body weight.

TABLE XXXVI-PERCENTAGE OF LIVE SPERM PRODUCED BY RAMS IN TRIAL IV

Collection Period	Percentage of Live Sperm												Treatment
	1	2	3	4	5	6	7	8	9	10	11	12	
Ram No.													
5944	1		2				11	32	76	72	89	91	Cooled
5964	0		0	13	23	27	74	76	78	85	77	69	Cooled
5787	14		3	23	34	27	39	47	75	72	89	87	Cooled
5963	3		4	0	4	2	6	1	1	2	2	0	Cooled and Thyroxine[1]
5786	0		0				18	51	83	87	88	86	Cooled and Thyroxine
5961							12	47	22	25	86	86	Cooled and Thyroxine
5868	33	33	70	92	48	77	77	72	20	52	40	64	Heated
5969	82	70		82	72			89	91	90	87	53	Heated
6008	89	94	84	80	71	74	71	73	37				Heated
5952	36	68	36	10	3	18	33	19	7	16	0		Heated and Thyroxine
5864	69	76	79	70	79	84	84	73	86	84	75	79	Heated and Thyroxine
5810	77	68	69	20	45	79	72	65	48	76	76	30	Heated and Thyroxine
5937	88	60	93	88	81	94	76	02	77	81	91	51	Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.2 mg. per 100 pounds body weight.

TABLE XXXVII-PERCENTAGE OF NORMAL SPERM PRODUCED BY RAMS IN TRIAL IV

Collection Period	Percentage of Normal Sperm												Treatment
	1	2	3	4	5	6	7	8	9	10	11	12	
Ram No.													
5944	56	67					35	23	75	69	98	99	Cooled
5964	53	51	43	30	22	31	79	83	91	95	97	98	Cooled
5787	25	52	59	33	46	39	46	55	70	89	98	99	Cooled
5963	47	73	16	27	49	41	53	51	32	60	28	21	Cooled and Thyroxine[1]
5786	32						6	34	75	83	96	89	Cooled and Thyroxine
5961							17	58	54	51	96	96	Cooled and Thyroxine
5868	33	17	51	93	89	92	81	78	55	78	49	77	Heated
5969	90	73		97	87			97	96	96	99	93	Heated
6008	95	95	99	93	92	64	73	67	65				Heated
5952	20	55	50	35	37	34	46	41	56		63		Heated and Thyroxine
5864	82	82	96	93	84	95	96	90	95	97	95	97	Heated and Thyroxine
5810	97	98	94	60	41	85	93	87	76	89	91	88	Heated and Thyroxine
5937	98	98	98	97	98	98	99	100	99	89	98	94	Heated and Thyroxine

[1] L-thyroxine injected daily at level of 0.2 mg. per 100 pounds body weight.

TABLE XXXVIII-SPERM CONCENTRATION IN SEMEN PRODUCED BY RAMS IN TRIAL IV

Collection Period	Concentration (millions of sperm/mm.^{3})												Treatment
	1	2	3	4	5	6	7	8	9	10	11	12	
Ram No.													
5944	.18	.08	.03	.01	.01	.03	.03	.10	3.19	6.54	5.59	6.65	Cooled
5964	.23	1.46	.18	.18	.24	1.65	1.01	.69	2.38	5.87	6.47	7.56	Cooled
5787	.40	.45	.15	.04	.50	.49	1.66	.39	3.04	6.19	6.85	5.28	Cooled
5963	3.97	2.69	4.17	3.17	2.30	.90	1.80	1.63	2.17	2.85	3.02	2.71	Cooled and Thyroxine[1]
5786	.11	.01	.01	.01	.02		.25	1.76	3.37	2.15	5.71	5.00	Cooled and Thyroxine
5961		.02	.02	.01	.01	.03	.05	.94	3.02	3.04	5.97	7.39	Cooled and Thyroxine
5868	.10	.28	.39	.31	1.99	1.04	3.42	4.61	3.74	2.08	1.14	2.12	Heated
5969	4.13	3.92		3.95	4.32			3.08	4.87	4.40	4.59	4.33	Heated
6008	4.10	6.02	5.82	3.26	4.16	2.57	3.95	3.75	4.16				Heated
5952	2.43	2.89	1.63	1.22	2.20	.95	1.12	1.29	.70	.22	.05		Heated and Thyroxine
5864	3.18	2.84	4.00	4.11	5.56	4.40	7.08	5.81	3.21	4.56	6.03	6.14	Heated and Thyroxine
5810	2.43	5.99	5.86	2.68	3.03	2.79	4.79	2.38	4.20	4.17	5.15	5.69	Heated and Thyroxine
5937	5.28	5.17	5.83	5.21	5.93	4.47	5.73	5.66	6.34	6.81	6.37	6.49	Heated and Thyroxine

[1]L-thyroxine injected daily at level of 0.2 mg. per 100 pounds body weight.

D. Discussion

Undoubtedly, the most striking facet of this study was the influence of the higher environmental temperatures upon the apparent fertility of the rams used. This trend was established in the first trial and continued to hold true for the three which followed.

Reports by Gunn *et al.* (1942) and others indicated an effect of temperature on semen quality, but it was not realized that the results would be so strongly affected, especially since the average temperatures in the heated room were much lower than those used by Casady *et al.* (1953) who determined the effect of temperatures of 100° and 86° F. on semen quality of bulls, or Dutt and Bush (1955) who subjected rams to temperatures of 90° F. in studying effects on semen quality.

A possible cause of the increased sensitivity of rams to heat was proposed by Foote *et al.* (1957) who observed that increases in humidity tended to cause rises in rectal and testis temperature. In the present experiment, humidity was not controlled but could be recorded. Over a period during which trial temperatures averaged 82° F., the humidity in the heated room averaged 71 percent. That is well below the range (78-88 percent) which Foote *et al.* reported as sufficient, when temperature was also high, to bring about increased body and testis temperatures.

It would appear, therefore, that constant average environmental temperatures of approximately 80° F. such as were experienced in these trials constitute a critical temperature above which semen quality of rams is reduced.

Much individual variation in semen quality was observed. Rams 5964 and 5963 assigned to the heated room in trial one maintained good apparent fertility even though the semen quality of all others in that group, with the possible exception of ram 5786, was definitely lowered from pre-test levels. In trial two, the semen quality of rams 5969, 6008, and 5937 remained satisfactory under conditions of the heated room whereas that of 5810, 5864, and 5868 was adversely affected.

Such findings suggest differences in heat tolerance within the Hampshire breed of sheep and, further, that rams can be found which are capable of maintaining a good fertility status under relatively high ambient temperatures.

Motility and the percentage of live sperm were affected relatively more by the higher temperatures than were the other three criteria of semen quality. That they would be affected similarly is not surprising since they are normally closely allied.

The percentage of normal spermatozoa was not affected to the same extent by high temperatures as were motility and percentage of live sperm. Furthermore, although the overall percentages of normal sperm cells from rams in the heated room were greatly reduced, they did not generally reach such typically low levels as those reported by Dutt and Hamm (1955). Since it has been postulated by McKenzie and Berliner (1937), among others, that sperm abnormalities are associated with even a slight rise in body temperature, this result may

further indicate that the temperatures in the heated room were indeed quite close to a critical point above which both semen quality declines and body temperature rises.

Exposure to the heated room was followed shortly by distinct and significant decreases in concentration of sperm in the semen of the majority of rams tested. This finding was in accord with those of McKenzie and Berliner (1937) and Bogart and Mayer (1946). In cases where concentration did decline in the hot room, it was noted that the decline was usually progressive, possibly indicating disruption of the integrity of seminiferous tubules or, an effect on various stages of the spermatogenic process.

Since it has been shown (Ortavant, 1956) that spermatogenesis in the ram requires about 50 days, it was surprising that rams kept in the heated room in trial one and which had ended the trial with low sperm concentrations regained a normal sperm count rather quickly after being placed in the cooled room.

The sperm concentration of ram 5944 rose from a very low level on September 16, while he was in the heated room to a normal level by October 20. On that date, he had been in the cooled room for about 33 days. Sperm concentration in ram 5961 was normal after only about 28 days in the cooled room.

Such results indicate that spermatogonia were not damaged by conditions found in the heated room. In view of the very low sperm concentration found in some samples, it would seem that spermatozoa and spermatids were destroyed by heat and possibly even the spermatocytes were affected.

In late November, 1960, in the interval between the ending of trial three and the start of trial four, temperatures in the heated room were allowed to reach abnormally high levels. Over the three day period from November 25 to November 27, temperatures averaged 92° F. with a high point of 97° F.

Semen samples collected on December 2, from rams housed in the heated room were of very poor quality. Motility was quite low as were also the percentage of live sperm and, in general, sperm concentration. The rams were rotated to the cooled room on December 5. By January 13, 1961, the apparent fertility of ram number 5964 was much improved. It is interesting to note that he was one of the two least affected by heat in trial one. Satisfactory semen quality for rams 5944, 5787, and 5786 was not reached until the collection period of January 28, 1961.

An even more extreme response to heat was that of ram 5961. He did not produce a satisfactory semen sample until February 12, a period of just over two months. Meanwhile, ram number 5963, the second of the two whose semen was little affected by heat in trial one, was the only one of the group of six which continued to produce spermatozoa in quantity after removal from the heated room, but, while it was true that his sperm concentration appeared relatively normal throughout trial four, his semen motility rating and percentages of live and normal sperm remained at very reduced levels until the conclusion of the test on February 17, 1961.

After the rams were exposed to the very high temperatures the data indi-

cate that about eight weeks were required before good quality semen was again produced. It is evident that sufficiently high environmental temperatures will destroy cells in all different stages of spermatogenesis.

The relationship of volume of semen to temperature did not appear to follow a consistent pattern and, although it was higher for the heated rams in trial one, it was not repeated for similar groups in succeeding trials.

In none of the trials did thyroxine therapy alone significantly affect any semen quality criterion other than volume. Since untreated rams produced a greater volume of semen than those receiving the hormone, it is possible that androgen production was lowered in the treated animals.

An interaction was found between treatment and temperature in the data from trials two, three, and four. This was probably attributable to the fact that rams which were subjected to high temperature and received no thyroxine had considerably greater semen volumes than did rams under other treatment—temperature regimes.

Results of experiment four differed from the preceding three trials in that average semen quality did not decline sharply. There appeared, in fact, to be relatively little change in apparent fertility of rams in the heated room over the course of the test. An explanation for this is rather difficult to establish. The only known changes made which may have caused it to differ from the first three were that the thyroxine level was lowered as scheduled and a rotating fan was installed to replace the exhaust fan used in the previous experiments. The effect of the fan, although it seemed minor at the time, now has taken on special significance. The increased air circulation so provided may well have aided the rams to dissipate excess heat and thereby kept testicular temperatures below the previously mentioned critical temperature.

In all of the four trials there was no evident effect of thyroxine therapy on ram semen quality. Since the reports of Eaton *et al.* (1948), Warwick *et al.* (1948) and others indicate that hyperthyroidism is detrimental to the semen quality of rams it is probable that the levels of thyroxine (0.2, 0.3, and 0.4 milligrams per 100 pounds body weight) used in the present study did not induce hyperthyroidism. Furthermore, the injected levels fell within the normal thyroxine secretion rate range (0.2 to 0.7 milligrams per 100 pounds body weight) determined for these rams at average temperatures of 46° to 87° F.

EFFECT OF THYROID BLOCK OR THYROIDECTOMY ON SEMEN QUALITY OF RAMS

The objectives of this work were:

(1) to establish whether a complete thyroidal block or surgical thyroidectomy would result in changes in the semen quality of rams.

(2) to determine the effect of thyroidectomy upon libido.

A. Experimental Procedure

Six, one and two year old rams of varied breeding were purchased at a central market and used in the initial phase of the test. In addition, one ram was added on January 10, the 15th day of the experiment, and four others on March 1, the 66th day.

Quarters were provided in a partially open 14 ft. by 12 ft. by 10 ft. box stall. Feeding practices were identical to those described in Chapter III.

At least two pre-trial semen collections were made from each of the initial six rams. Either the artificial vagina or the electro-ejaculator was used in making collections. Semen was taken at approximately weekly intervals and each sample was evaluated as described previously.

After completion of the pre-trial period the rams were divided into a control group of two and a treated group of four. Within each group was one ram from which semen could be secured with the artificial vagina. The ram added on the 15th day and those added on the 66th day were assigned to the treated group.

Pilot studies had shown that 400 milligrams of tapazole per 100 pounds body weight was sufficient to essentially suppress the uptake of radioiodine by the thyroid of lambs, thus indicating inhibition of thyroid function. Because no similar figures were available for mature rams, an arbitrary level of 600 mg. per 100 pounds body weight was used for the first eight days of the test beginning December 27, 1960. It was given daily as an aqueous drench. Then, on January 4, 1961, the dosage was increased to 800 milligrams per 100 pounds of body weight. The change was made as a precautionary measure to insure that a thyroid blocking level was being administered. A five day radioiodine uptake measured on February 7, confirmed that such a dosage had been chosen.

Two semen collections were taken from the group of four rams added on the 66th day. One was taken just prior to their being placed on test. The second was made after they had been receiving 800 milligrams of tapazole daily per 100 pounds body weight for twenty-two days. On March 24 the dosage was decreased to 600 milligrams per 100 pounds body weight in an attempt to forestall apparent undesirable side effects of the drug. Administration of tapazole was discontinued on March 30. This terminated the first part of the test.

The second, major phase was initiated with fifteen rams purchased from a central market and one from the University flock. They were from one to five years old and of varied breeding. Prior to the start of the trial they were sheared, dipped and vaccinated for enterotoxemia and soremouth.

Two partially open 14 ft. by 12 ft. by 10 ft. box stalls provided housing. Feeding practices were similar to those described for the preceding group of rams with the exception that access to pasture was usually allowed for about one-half hour each day. This also provided some exercise.

At least one pre-trial semen collection was made from each ram. Either the artificial vagina or electro-ejaculator was used in obtaining samples. Previous work had shown that semen collected by the electro-ejaculation method varied

widely in volume, concentration and to a lesser extent, motility both within and between rams. Consequently, in the second part of this experiment only motility, percentage of live cells and percentage of normal spermatozoa were used as indices of semen quality of samples collected by electro-ejaculation. Those taken by the artificial vagina were evaluated according to the procedures already described. Samples were taken throughout the experiment approximately once weekly.

Following the pre-trial period four rams were selected to undergo surgical thyroidectomy. These included the only three rams (numbers two, six and 12) of the entire group from which semen was obtained with the artificial vagina during the pre-test period. Rams three and one were trained during the course of the trial to serve the artificial vagina. The purpose of thyroidectomizing these particular rams was to observe the effect of the operation on libido as well as semen quality. One thyroidectomy (ram two) was performed May 26, the others (rams three, six, and 12) on May 29.

Four rams of the 12 others were randomly chosen for controls, the remaining eight received tapazole. The drug was administered as an aqueous drench. A daily dosage of 400 milligrams per 100 pounds body weight was given over the period from May 27, 1961, to June 26, 1961.

On June 23, each ram was injected with 100 μc of radioiodine. On June 26, a 72 hour I^{131} uptake was measured, using the scintillation detector and count rate meter used in the thyroxine secretion rate studies. This procedure was carried out to indicate: (1) the degree of thyroid inhibition in tapazole treated rams, (2) whether thyroidectomized rams had remaining thyroidal tissue and (3) the percentage of I^{131} uptake in controls.

B. Results

The first phase of this experiment differed appreciably from the second and thus will be considered separately. Pre-trial collections showed semen quality to be very good in all of the six rams being used. On January 16, a ram from the treated group and from which semen was being collected with the artificial vagina, died unexpectedly. Unfortunately this animal was not autopsied immediately and only a probable diagnosis of enterotoxemia could be made. The last semen collection from this ram, made on January 12, indicated a very high apparent fertility.

On January 29, severe convulsions were observed in ram seven, the one which had been added to the test on January 10. The seizures were characterized by rapid breathing, frothing at the mouth, violent body tremors and grinding of the teeth, all occurring while the ram was prostrate. This animal was removed from the trial that day and put under the care of the University Veterinary Clinic where he made an uneventful recovery. His last semen sample, taken on January 28, was of good quality.

Convulsions similar to those noted in ram seven were seen in ram four on February 8. He had been losing weight for a short period prior to his attack and

was in relatively poor condition at that time. On February 11, he died, apparently as the result of hemorrhage from an abscessed lung. His last semen sample collected on February 10, by electro-ejaculation, was of very satisfactory quality in all respects. The testes were entirely normal both grossly and microscopically. However, as reported by the Veterinary Pathology Laboratory:

The thyroid was very hyperemic. No colloid was present in the acini. Most of the acini showed enfolding or papillary proliferation of the lining epithelium. The cells were uniform in size but somewhat large. The gland was showing definite evidence of goiter. The thyroid glands were 2½ inches long and about 1 inch wide. They were connected by a broad isthmus. They weighed 21.6 grams.

On February 26, ram 3 was noticeably depressed and would not eat. He was taken to the University Veterinary Clinic where he died on February 28. Post-mortem examination disclosed death was caused by peritonitis resulting from a thrombus in the left testicular vein. A description of the thyroids and testes prepared by the Veterinary Pathology Laboratory read as follows:

The thyroids weighed 45 grams. The two lobes were somewhat unequal in size. The larger lobe was three inches long and an inch and a half wide. The testicle and thyroid were examined histologically. Spermatogenesis was normal in the right testicle. The left testicle had undergone complete infarction. There was an absence of colloid in the thyroid. The acini were greatly enlarged and contained many papillary proliferations. The cells were swollen.

Again, semen quality was maintained at a high level in all collections made from this animal.

On March 2, ram six was observed in convulsions. He was the last surviving ram of the four which had begun receiving tapazole on December 27. Tapazole administration continued until his death on March 13. The complete necropsy report from the Pathology Laboratory follows:

The ram was relatively thin. The ingesta in the rumen had no odor. The small intestine was empty. The contents of the colon were too dry. There was edema in the wall of the gallbladder. The testicles appeared relatively small. The thyroids weighed 47.2 grams. There was diffuse hemorrhage and edema in the wall of the right ventricle of the heart. There was a 2 ml. subendothelial hematocyst in this area. The heart muscle over an area of 3 cm. in diameter was involved. The lungs were heavy and wet due to congestion and edema. Death was caused by congestive heart failure.

The heart, thyroid and testicles were examined microscopically. Spermatozoa were being produced but it appeared that they were being produced in decreased numbers. There was severe goiter in the thyroid. A number of vessels in the area of the damaged heart were occluded. There was an infiltration of neutrophils around the periphery of these vessels. The muscle fibers were still alive. The cause of this lesion was not obvious. The cause may be associated with the drug that is being given.

The necropsy report suggested a probable reduction in the rate of spermatogenesis at the time of death. However, at no time while on test did this ram produce a semen sample indicative of a low fertility status.

On March 27, convulsions were seen in rams eight and ten, this after they

had been receiving tapazole for twenty-five days. Ram eight received no tapazole after March 28. However, on March 29, he appeared depressed, refused feed and again suffered convulsions. He underwent a third seizure on March 30, a day on which rams nine and ten also had severe tremors, the latter both in the morning and afternoon. All tapazole administration was stopped after March 29.

By April 1, the animals, with one exception, were noticeably much more alert and lively. Ram nine had a final convulsion that day and refused to eat. His condition was complicated by a case of soremouth.

Semen quality data for this phase of the experiment are presented in Tables VIII, IX, X, XI and XII.

On June 14, during phase two of the experiment, ram number four was somewhat listless and lacking appetite. He was among the eight rams receiving 400 milligrams of tapazole daily per 100 pounds body weight. He remained on treatment and was given a final administration of tapazole on June 19. His condition did not improve and he died on June 21. The necropsy report submitted by the Veterinary Pathology Laboratory read in part as follows:

> Sixty mls. of blood tinged fluid were present in the abdominal cavity. A few suffusion hemorrhages were present in the stomach. There was a watery exudate in the intestinal tract. A number of nodular worms were present. The thoracic and cervical lymph nodes were hemorrhagic. Numerous ecchymotic hemorrhages were present on the surface of the heart. The anterior one-third of the lungs was consolidated. There was a catarrhal hemorrhagic tracheo-bronchitis. Pasteurella bacteria were isolated from the lungs. Death was caused by pneumonia.
>
> The thyroid gland weighed 13.2 gms. Histologically the colloid stained poorly. The epithelium in many of the acini was invaginated. A few of the acini were filled with cells. Histologically, there was an absence of spermatogenesis. The spermatic tubules were lined with a single layer of sustentacular cells. Very few spermatogonial cells were present. There was no mitotic activity.

The semen quality of ram 4 had declined from pre-test levels and his last semen sample, taken on June 14, indicated a very poor apparent fertility status. However, none of the remaining rams receiving tapazole appeared to be affected in the same way while on test. Semen quality remained generally satisfactory for the group. Semen data for all rams used in phase two of the experiment are shown in Tables XIII, XIV, XV, XVI, and XVII.

In previous tests, it was observed that data on concentration and volume of semen obtained from rams by electro-ejaculation were unreliable indices of apparent fertility. Thus, only data on motility and percentages of live and normal sperm were used as criteria for studying effect of tapazole on fertility of rams requiring the electro-ejaculator for collection. Since one ram of the group (ram number 1) did serve the artificial vagina, it was possible to obtain data on concentration and volume of sperm from at least one ram treated with tapazole. Ram 1 was apparently very fertile throughout the test. There appeared to be no lessening of libido.

Semen samples were collected with the artificial vagina throughout the test

from three of the four thyroidectomized rams. The fourth, ram number 3, was trained during the experiment to serve the artificial vagina and his last three weekly semen samples were taken in that manner. No decline in either semen quality or libido was noted among these four rams during the test. All remained healthy and vigorous and no gross signs of thyroid deficiency such as general lethargy, thickening of the skin or loss of appetite were noted.

The results of the I^{131} uptake test are shown in Table XVIII. The average I^{131} uptake of the control rams was considerably higher than the average for either the tapazole treated or thyroidectomized rams. One thyroidectomized ram, number six, had a much higher uptake than others of his group, an indication of probable remaining thyroid tissue. There was a good deal of variation among tapazole treated rams in radioiodine uptake, suggesting only partial thyroid blockage in some. However, it was interesting to note that the lowest uptake of I^{131} occurred in ram one.

Ram six, which had the highest radioiodine uptake among the thyroidectomized group, and 12, the ram with the lowest uptake, were sacrificed and examined on July 5 to determine whether all thyroid tissue had been excised at the time of operation. In number six, a mass of normal thyroid tissue weighing 1.9 gms. was found. Spermatogenesis was normal.

The report of post-mortem examination on number 12, as prepared by the Veterinary Pathology Laboratory contained the following information:

> This ram was in excellent physical condition. He was bright and alert. The wool was firmly attached. There was no evidence of subcutaneous edema. The digestive tract was well filled with feed. In short, we found no evidence of a cretin.
>
> There was considerable scar tissue in the area of the surgery. Several small abscesses were present adjacent to the proximal end of the trachea. No thyroid tissue was demonstrated either grossly or microscopically. However, this observation was not conclusive as small fragments of thyroid could easily have been lost in the scar tissue which was present. Spermatogenesis was occurring at a normal rate as determined by histological examination of the testicle.

C. Discussion

In no instance was there a change in apparent fertility which could be linked either to experimental treatment or the thyroid gland directly.

Since definite goiters were produced in phase I, it appears obvious that the dosage of tapazole administered was sufficient to induce hypothyroidism. Also, in each succeeding necropsy examination, larger amounts of thyroid tissue were found, a reflection of the effect of tapazole and length of time the rams had been on test.

The absence of colloid in the thyroids studied histologically was yet another strong indication that the rams were in a hypothyroid state. A lack of thyroid hormone induces a rise in thyrotropin level which in turn causes increased thyroxine secretion by increasing the secretory epithelium of thyroid follicles and by mobilizng the colloid contained therein. Hypertrophy and hyperplasia occur if

enough thyroxine containing colloid cannot be mobilized to meet body requirements for the hormone. Then a goiter is formed.

With respect to semen quality it might be argued that the rams were not hypothyroid for long enough to allow changes in semen quality, if mediated through the thyroid gland to become evident. However, Freinkel and Lewis (1957) have shown the half life of exogenous I^{131} labeled thyroxine to average 25 hours in shorn and 38 hours in unshorn sheep. Similar results have been obtained with ewes by Annison and Lewis (1959). In the dairy cow, Pipes *et al.* (1959) estimated the half-life of injected I^{131} labeled thyroxine to be only 2.47 days. They further postulated that "the rise in the body thyroxine pool is slow during periods of increasing thyroxine secretion, whereas the decline is rapid during periods of decreasing thyroxine secretion." In the light of such experimental findings it seems highly unlikely that the tapazole treated rams retained significant amounts of thyroxine after the first few days of the test. Thus, it is difficult to explain how treated rams maintained satisfactory semen quality throughout the test, in one instance (ram six) for over 60 days, if the thyroid gland is essential for high fertility.

The high degree of mortality associated with the treated group was very disturbing. Except for the first ram which died (number two) a similar syndrome was noticed in each of the other rams which died during the test. Such symptoms were not seen in the control rams. Ram seven was removed from the experiment after several seizures and no further attacks occurred. The obvious conclusion is that in addition to causing thyroid blockage, tapazole administration had unexpected and deleterious side effects. The mechanism for such an action is not readily apparent. In phase two of the trial no such effects were seen, possibly a reflection of the lower level of drug administered.

No decline in apparent fertility from pre-test levels was observed in the tapazole treated rams in phase two. Their response to tapazole administration was therefore similar to that noted for rams in phase one.

Among the thyroidectomized rams no changes in behavior, physical appearance or semen quality were noticed following the operation. Libido remained very high in all instances, a probable indication that androgen production was curtailed little, if at all, by an absence of circulating thyroxine.

No evidence was obtained, either through the I^{131} uptake study or by postmortem examination, that ram 12 retained any thyroid tissue after thyroidectomy. His semen quality data therefore are of a special significance. All semen collections made from him, including one taken prior to the date he was sacrificed, indicated a very good fertility status. His libido was unquestionably normal. Probably, the lack of thyroid tissue in no way curtailed his breeding capacity or spermatogenic activity.

SUMMARY

Studies were made over a three year period to determine the influence of the thyroid gland upon apparent fertility of mature rams. The research was conducted in three investigations. In the first, estimates of the effect of environmental temperature on the output of thyroxine were made. The second involved determinations of semen quality in Hampshire rams subjected to thyroxine therapy under two levels of ambient temperature. The last was designed to study the effect of goitrogenic immobilization of the thyroid gland and surgical thyroidectomy upon semen quality of rams.

The thyroxine secretion rate study involved twenty-one Hampshire rams subjected to environmental temperatures averaging from 46° to 87° F. Sixty-four individual secretion rates were estimated. These ranged from 0.2 to 0.7 milligrams of L-thyroxine per 100 pounds of body weight with an average of 0.36 milligrams. An inverse relationship was noted between thyroxine secretion rate and environmental temperature. Plotted as a regression the slope of the line was best described by the equation $Y = .620 + (-.0038)\ X$ with Y representing thyroxine secretion rate and X the temperature between 46° and 87° F. The regression coefficient of -.0038 was highly significant ($P < .01$), indicating a major influence of temperature on thyroxine secretion rate.

The second series of experiments dealt with the effect of a combination of environmental temperature and thyroxine therapy on the semen quality of Hampshire rams. In the four trials conducted using an average of 13 rams, it was determined that trial environmental temperatures of approximately 80° F. were sufficient to cause a decline in semen quality. About four weeks exposure to approximately 70° F. temperatures allowed semen quality to return to normal in rams whose apparent fertility had decreased under 80° F. ambient temperatures. Approximately eight weeks were required for recovery of apparent fertility in rams subjected to 92° F. temperatures for only three days.

Daily injected L-thyroxine levels of 0.2, 0.3, or 0.4 milligrams per 100 pounds body weight had no significant effect on semen quality. However, it was observed that thyroxine treated rams under higher environmental temperatures produced semen which was consistently of lower quality than that of untreated rams under similar conditions. Thus, there is a possibility that thyroxine administration at temperatures of 80° F. or above may even be detrimental to ram semen quality.

The final study was designed to establish whether inhibition of thyroid function by the goitrogen, tapazole, or surgical thyroidectomy mediated changes in semen quality. Evidence obtained through radioiodine uptake measurements and necropsy reports on treated animals indicated that severe hypothyroidism was induced by the daily administration of 800 milligrams of tapazole per 100 pounds of body weight. No detrimental effect on semen quality was observed which could be attributed to administration of the drug, but tapazole at the 800 milligram level was associated with a high degree of mortality among treated rams.

Thyroidectomies were performed upon four rams. Following the operation, semen samples were collected and evaluated over a five week period. There was no apparent effect on fertility of rams due to removal of the thyroid nor was there any change in libido.

Two of the four rams were sacrificed after I^{131} uptake measurements were taken over the thyroid region. In one ram a considerable degree of radioactivity was noted, indicating probable remaining thyroidal tissue. Confirmation of that was obtained on post-mortem examination. In the second ram a very low I^{131} uptake was recorded and no thyroid remnants were detected, a finding which lent special emphasis to the fact that he maintained excellent semen quality for five weeks after having been thyroidectomized.

CONCLUSIONS

Under the conditions of these experiments the following was concluded:

1—An inverse relationship exists between thyroxine secretion rate of rams and environmental temperature.

2—There is a marked variation in thyroid activity among rams.

3—Semen quality of rams declines at ambient temperatures of 80° F. or above.

4—The rapidity of fertility recovery after exposure to high temperature is dependent upon the degree of the temperatures.

5—Rams vary considerably in their response to heat as measured by apparent fertility status.

6—Injections of 0.2, 0.3, or 0.4 milligrams of L-thyroxine daily per 100 pounds body weight do not prevent semen quality decline in rams under high ambient temperatures.

7—Tapazole blockage of the thyroid gland does not lower the apparent fertility of rams.

8—Tapazole administration at the level of 800 milligrams daily per 100 pounds body weight is deleterious to the health of rams.

9—Over a five week period following thyroidectomy no effect in semen quality or libido can be observed.

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