STUDY OF THYROPROTEIN THERAPY ON RAM SEMEN

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ABSTRACT

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Five suspected infertile rams from privately owned sheep breeding farms, including one from the Michigan State University Sheep Breeding Farm, were used in the study. The two control, known fertile rams, were selected from the Michigan State University Sheep Breeding Farm. Volume, concentration and total number of sperm produced were evaluated. Respiration rate of the sperm was also measured on a multi-unit constant-pressure respirometer.

Starting on May 21, oral treatment of .2 gm of thyroprotein (Protamone) was given daily to the suspected infertile rams. No statistically significant difference was found between the fertile and infertile rams, in volume and total sperm ejaculated. Concentration was found to be statistically significantly higher in the fertile group than the infertile group.

The sperm from the ejaculates collected during the treatment period consumed more oxygen than from the ejaculates of the pretreatment period. The treatment appeared to have some favorable effect on the respiration rate of the sperm.

On July 11, the thyroprotein dosage was increased to .4 gm daily. Volume of the semen was not found to be statistically significantly different between the control and the treated group. Concentration and total number of sperm ejaculated during this period were highly significantly higher in the control rams than the treated rams.

No respiration studies could be made during the higher level of thyroprotein feeding.

Study on the ribonucleic acid (RNA) and desoxyribose nucleic acid (DNA) content of the sperm collected from the fertile and infertile rams was also started but the experiment had to be abandoned before any relevant data could be collected.

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INTRODUCTION

In the past quarter of a century many studies have been done on the effect of high temperatures on the semen production of rams and other male farm animals. The effect of both high atmospheric temperature and artificial insulation on the scrotum on different traits of the semen has been studied.

The importance of the thyroid gland in the normal production of semen in rams has long been established. Thyroxine has also been tried as a "cure" for the so-called "summer sterility" of rams, a phenomenon in which the thyroid gland reduces its secretion of thyroxine because of the increased atmospheric temperatures resulting in part, in the inferior quality of semen produced during these hot months.

Originally this research was designed to study three things:

I. To compare the volume, concentration and the total number of sperm produced in known fertile and suspected infertile rams.

II. To study the effect of thyroprotein-feeding through the summer months on the semen production of the suspected infertile rams.

III. To compare the respiration rate of the sperm in the semen of known fertile and suspected infertile rams and to see the effect of feeding thyroprotein on it.

However, for the reasons explained further in these pages experiment III had to be terminated before all the data could be collected relative to number III.

Comparison of ribonucleic acid (RNA) and desoxyribose nucleic acid (DNA) contents in the sperm of the fertile and infertile rams was also started but the experiment had to be discontinued before any relevant data could be collected, because very frequently all of the infertile rams did not ejaculate half a billion sperm.+

+Personal communication from Dr. Manuel Gordon, formerly of the Department of Dairy, Michigan State University, East Lansing.

REVIEW OF LITERATURE

Smelser (1934) compared thyroidectomized rats with control littermate rats and found that the thyroidectomized rats produced fewer sperm than the controls. On autopsy it was found that the treated rats had smaller accessory reproductory organs than the controls.

Phillips and McKenzie (1934) showed that the scrotum of the ram functions very much as a thermostat and maintains the temperature of the testes constant and considerably lower than that of the body cavity. That an artificial insulation on the scrotum has a detrimental effect on the semen production, was also noted. Low temperatures (lowest 6-8°C.) did not have any harmful effects on the testes of rams.

An inferior quality of semen was produced by rams during late winter when McKenzie and Berliner (1937) kept them in a room artificially kept at high temperature.

Berliner and Warbritton (1937) studied seasonal variation in rams and found summer as the lowest season. Semen production fell in thyroidectomized rams but injections of 4 mg. of thyroxine improved the semen toward normal. Green (1940) found that the general quality of semen decreases slightly but steadily from January to May, becoming quite inferior in June and July, presenting an increased number of the abnormal sperm. But from July to October a very rapid readjustment towards higher quality is underway. After October all sperm types become relatively stationary in their proportional relationship.

At Cornell, Phillips et al. (1943) during their study of seasonal variation in the semen of sheep and goats, found relatively little difference in volume between the seasons. Strangely enough, the concentration was found lowest in the fall instead of summer and highest in winter.

No deleterious effect on ram semen was found by Accame et al. (1944) when the rams were subjected to reduced barometric pressure.

The effect of synthetic thyroprotein on sterility in bulls was studied by Reineke (1946). A definite improvement in the libido of bulls with some indication of improved fertility was observed. Number of services per conception was also reduced.

Bogart and Mayer (1946) administered thyroxine to rams subcutaneously and later orally. They found that it did not prevent the gradual decline in the motility and did not have any effect on the volume. The concentration and/or total sperm ejaculated were, however, stabilized at a higher level than that during the pretreatment period. Increase in the percentage of abnormal sperms produced was also prevented.

Shaffner (1948) found that thyroprotein fed to mature cocks reduced their fertility. Volume and concentration, however, were not affected.

Eaton et al. (1948) made the comparison of feeding two different levels of thyroprotein on the quality of semen produced in rams. Results did not indicate that feeding thyroprotein was beneficial to rams since semen concentration, percent normal sperm and motility was higher in the untreated groups than either of the treated groups. Volume was, however, higher in the higher level (2 gm.) of thyroprotein than the lower level (1 gm.).

Huston and Wheeler (1949) fed the synthetic thyroprotein to mature cocks. They found that the volume of semen from the treated birds was significantly lower than from the untreated. The concentration and total number of sperm ejaculated were higher in the treated ones, although not statistically significant.

Black et al. (1950) did not find any increase in the number of total sperm produced by feeding thyroprotein to rams. Volume was increased but it was not significantly different. Concentration was affected to a very small extent.

Casady et al. (1953) tested the effect of high ambient temperature on spermatozoa of dairy bulls kept in mechanically controlled chambers. They concluded that under chamber conditions spermatogenesis in young dairy bulls may be impaired when the animals are kept continuously exposed to temperature exceeding 85° F. for 5 weeks.

Wilwerth et al. (1954) fed thiouracil to male chickens. The treated birds exhibited a trend toward decreasing volume. When these animals were fed thyroprotein it increased the volume. A higher level of thyroprotein increased the concentration also without effecting the volume. A very high level significantly decreased the concentration and volume. Henneman, Reineke and Griffin (1955) found that in sheep the thyroid secretion rate was lowest during July.

Dutt and Hamm (1955) exposed rams to artificially maintained high temperatures of 90° C. during winter. They found that the semen quality for unsheared rams in the hot room was inferior but there was very little effect on the sheared rams in the same room. Eight weeks after the test period, there was no significant difference between the treated and untreated groups.

El-sheikh and Casida (1955) working with rabbits concluded that increased ambient temperature may effect the male fertility without bringing about any apparent depression of sperm motility. The ratio of

fertilized to unfertilized ova in test females was lower for the treated animals.

Dutt and Bush (1955) and Dutt and Simpson (1957) found that keeping the rams in a cooled room the temperature of which was artificially maintained between 45°-48° F., improved their semen characteristics in motility and percent abnormal cells significantly but volume and concentration were not significantly effected.

Foote et al. (1957) concluded that in high temperatures, shearing is more effective in preventing a rise in testes temperature when humidity is low than when humidity is high.

Simpson et al. (1959) found that rams which had free access to an air-conditioned room had better semen characteristics in volume, concentration, percent motile sperm and percent abnormal sperm than those which did not. Recently Brooks and Ross (1962) in a series of experiments found the same results as Dutt and Bush (1955) and Simpson et al. (1959) except that in their experiment volume was higher in the heated room than in the cooled room.

Wilson et al. (1959) concluded that rams kept in a chamber in which both temperature and light conditions were simulated to October conditions had a higher fertility rate than others, including those in which only temperature conditions were simulated to October conditions.

Whiteman and Brown (1959) cooled rams during the day and found that cooled rams made more matings and produced more lambs per mating than the controls, although it was found not statistically significant.

Brooks, Ross and Pipes (1960) found that the thyroid secretion rate decreases with increasing temperatures. The correlation coefficient was found to be -.363.

Brooks and Ross (1962) found that thyroxine given at the rate of .4 mg/100 lb. bodyweight was detrimental to semen quality at high environmental temperatures. In a cooled room the thyroxine therapy had little effect except that sperm concentration was higher in the treated rams. The .3 mg/100 lb. bodyweight treatment did not have any appreciable effect on the quality of semen in the heated room but in the cooled room the treated rams had somewhat superior semen than the untreated, in motility, percent normal sperm and sperm concentration. When thyroxine therapy was reduced .2 mg/100 lb. bodyweight in the heated room there were no significant differences but the treated rams had higher sperm concentration and higher percent of normal sperm while the untreated rams had a higher volume, higher motility and a higher percent of live sperm. In the cooled room, the semen from untreated rams was of a higher quality in all indices. It was suggested that there is a lowering of semen quality associated with thyroxine therapy at higher environmental temperatures.

Gray (1928) working with the sea urchin spermatozoa, found that the oxygen consumption per spermatozoa is inversely related to the number of sperm present. The same results have also been observed on ram and boar spermatozoa by Winchester (1941) and on unaltered human semen by Ross, Miller and Kurzrok (1941). Bishop et al. (1954) diluted the whole bull semen four times with egg yolk phosphate and still obtained the same results.

Henle and Zittle (1942), however, concluded that with epididymal bull spermatozoa in Ringer's solution, maximal oxygen uptake was at the approximate concentration of 5×10^8 spermatozoa/ml.

Bishop (1942) measured the oxygen consumption of fox sperm and concluded that the oxygen uptake is directly related to the number of sperm cells.

Walton and Edwards (1938) reported on the use of respiration rate of the spermatozoa as a criterion of quality of bull semen. They found that bulls whose sperm exhibited higher respiration rates required fewer services per conception.

Windstosser (1935) found that the respiration rate of semen diluted 1:200 with saline solution, was higher than in whole semen, but in both the series the spermatozoa were immotile throughout the experiment.

Sergin (1939) found that the respiration of spermatozoa is lower than most of the other tissues of the body. He also found that the higher the concentration of the semen the higher was the oxygen consumption. Thus ram semen which is more concentrated than the semen of the boar or stallion absorbs more oxygen than semen from the other species.

Ely, Herman and Winchester (1942) thought as a result of their experiments that the spermatozoa exhibiting a greater activity may live longer than those of lower activity.

Bishop and Salisbury (1955) measured the oxygen uptake of both undiluted and seminal plasma diluted bull semen. The oxygen uptake per cell was found to be inversely related to the concentration of the sample subjected to the experiment.

Mohri and Horiuchi (1961) found that the presence or absence of carbon dioxide influences the respiration rate of sea-urchin spermatozoa.

EXPERIMENTAL PROCEDURE

Seven rams were used in the experiment (2 known fertile and 5 suspected infertile). The 2 known fertile rams were selected from the Michigan State University Sheep Breeding Farm, on the basis of their previous performance, in the number of ewes settled by them. The infertile rams were also randomly selected from the farms of breeders who were running private sheep breeding enterprises. Only one ram was taken from each farm, the ram settling the lowest number of ewes being selected. The infertile rams were not all available for the same length of time, thus the data presented here and used in the analysis is from the date all seven rams were available for the experiment, to the date the first ram was taken away.

All the rams were kept in the indoor pens and hand fed. The hay and grain given was of the same quality for each ram. Semen samples were collected twice every week at 3-5 day intervals with an electro-ejaculator. If the volume was not enough in the first ejaculate, another collection was tried immediately and the ejaculate collected in the same tube. Graduated tubes were used throughout the experiment. The collection tube was kept in a water jacket at a temperature of approximately 43° C. Immediately after the collection was completed the tube was securely corked, marked and transferred to a thermos flask in which frozen water in a can had been stored for about 15-20 minutes. The artificial vagina was washed and dried properly between the collections from different rams. After all seven collections were made the samples were taken to the laboratory immediately. Volume was recorded directly from the tube and concentration of sperm was counted on a haemocytometer slide. The staining fluid used had the following composition:

50 ml of H_2O
2 ml of 2% Eosin
1 ml of 3% Sodium Chloride

Unfortunately, at the beginning of the experiment only three traits of the semen, namely, volume, sperm concentration and total sperm ejaculate were considered. Later motility ratings in approximate percentages were also given to the samples.

Respiration studies were made on ejaculations taken prior to July 5. After July 5 no determinations could be made for respiration rate because of one of these three reasons:

a. either the samples did not contain any sperm,

b. the concentration per cc. was so low that .2 cc. of that sample would have had very few sperm, or

c. no semen sample was given by the animal.

The respiration study was made on the multiple-unit constant-pressure micro-respirometer devised by Reineke (1961), using .2 cc. samples of semen.

While analyzing the data, if no sample was obtained a zero was entered for that animal in all the three traits, namely volume, concentration and total sperm ejaculated, because it was thought that the animal did not have anything to offer. Whenever a urine sample was given it was omitted from calculation because it was difficult to know whether the reason was that he did not have anything to give or that this was due to the poor technique on the part of the collector. In either case, there usually was an erection of the penis. When a semen sample was given but no sperm were found in it, the volume was recorded and counted in the calculations. A zero was entered in the other two traits.

RESULTS AND DISCUSSION

The averages of the pretreatment period of the three semen traits studied in this experiment are tabulated in table 1, the analysis of variance for those traits is shown in table 2. No significant difference ($P<.05$) in volume was found between the fertile and infertile rams, although a significant difference ($P<.05$) was found between the rams within the groups.

The mean square for between rams/groups was used in all cases to test the significance of the mean square between groups. This was done because of the assumption that these rams were just samples of rams from two populations, fertile and infertile, and what we are really interested in testing is the difference between these two populations.

No significant difference ($P<.05$) was found in the sperm concentration of the fertile and infertile rams. The sperm concentration between the rams within the groups was also not statistically significant (table 2).

In the total number of sperm produced per ejaculate, again, no significant difference ($P<.05$) was found between the groups but there was a significant difference ($P<.05$) between the rams within the groups (table 2).

Since no significant difference was found in any of the three semen traits considered, between the fertile and the infertile rams, it can safely be said that the reason why these rams were suspected to be infertile is something other than these. Since these rams were selected on their previous fertility performance records there is a possibility that the semen of these rams is inferior in some traits other than these three. It is unfortunate that the motility, percent normal sperm

and percent live sperm were not taken into consideration from the very beginning of the experiment. The poor fertility record could also be because of lack of libido in some of the rams. Since there was no way of determining the libido in these infertile rams, it cannot be ruled out. On the other hand, the two control rams were selected from the Michigan State University Sheep Breeding Farm on their previous record and were known to be fertile.

From table 1 it can be seen that the total number of sperm produced by either of the two fertile rams was considerably higher than 4 of the infertile rams. Ram No. 7 was higher than the two fertile rams. But there was no significant difference between the two groups.

Table 1. AVERAGES OF VOLUME, CONCENTRATION AND TOTAL NUMBER OF SPERM PER EJACULATE PRODUCED DURING THE PERIOD OF APRIL 9 TO MAY 17.

Trait	Fertile		Infertile				
	Ram 1	Ram 2	Ram 3	Ram 4	Ram 5	Ram 6	Ram 7
Number[a]	10	11	9	10	10	7	10
Volume (cc.)	1.39	0.67	0.54	0.66	0.47	0.57	1.17
Concentration (billion/cc.)	1.16	1.71	1.25	1.15	1.13	0.69	1.30
Total number of sperm ejaculated (billion)	1.56	1.18	0.71	0.73	0.54	0.39	1.69

[a]Number of observations.

Table 2. ANALYSIS OF VARIANCE FOR VOLUME (A), CONCENTRATION (B) AND TOTAL NUMBER OF SPERM (C) PER EJACULATE PRODUCED DURING THE PERIOD OF APRIL 9 TO MAY 17.[a]

Source	Mean squares for traits			
	df	A	B	C
Between groups	1	1.48	1.46	3.88
Between rams/groups	5	1.16*	0.69	2.12*
Between ejaculates/rams	60	0.15	0.34	0.56

*P<.05.

From May 24 to July 9, .2 gm of Protamone was given orally in a gelatin capsule to the infertile rams. The averages of the three traits for this period are tabulated in table 3. The analysis of variance for the three traits is reported in table 4.

No significant difference in volume (P<.05) was found between the two groups. The rams within the groups were, however, significantly different (P<.05). Table 4.

Total number of sperm produced by the two groups was also found not to be statistically significant (P<.05). The rams within the groups were also not significantly different (P<.05). Table 4.

In the analysis of the pretreatment data no significant difference was found to exist between the two groups of fertile and infertile rams in volume, concentration or total sperm produced. However, all the infertile rams were kept in the "suspected infertile" group, because of their previous poor fertility performances. By the time this experiment was started, moderately high temperatures had already set in, in this area, which must have effected the semen production. Since all the rams, fertile and infertile, were fed the same ration and housed together

there were no known environmental differences between the two groups. In other words the deterioration in the semen quality because of the high temperatures should have been the same for both the groups. Still no significant difference was found in the volume, although average volume produced by 3 of the infertile rams was higher than their volume of the pretreatment time and the other 2 were almost the same. Both the fertile rams also produced a little more volume, on an average, in this period than in the pretreatment period, which as far as the knowledge of the author goes, is unaccountable even on the basis of any previous work. Since no significant difference was found between the groups it will be safe to say that .2 gm. of thyroprotein did not have any great effect on the semen volume of the infertile rams. This agrees with the results of Bogart and Mayer (1946), Shaffner (1948), Wilwerth et al. (1954) and Brooks and Ross (1962) but does not seem to be in line with the results that Eaton et al. (1948), Warwick et al. (1948) and Black et al. (1950) obtained in their experiments. But no detrimental effect was apparent on the volume as was found by Huston and Wheeler (1949) working with cock semen.

The sperm concentration was statistically different between the fertile and infertile groups. On the average all the seven rams produced semen of lower concentration than their respective concentrations of the pretreatment period. Since in the pretreatment period there was no statistical difference between the two groups, the only conclusion that can be drawn from the available data is that the thyroprotein did not have any effect on the concentration of the sperm.

Table 3. AVERAGES OF VOLUME, CONCENTRATION AND TOTAL NUMBER OF SPERM PER EJACULATE PRODUCED DURING THE PERIOD OF MAY 24 TO JULY 9.

Trait	Fertile		Infertile[a]				
	Ram 1	Ram 2	Ram 3	Ram 4	Ram 5	Ram 6	Ram 7
Number[b]	14	14	14	14	12	14	14
Volume (cc.)	1.54	0.71	0.90	0.53	0.60	0.89	1.00
Concentration (billion/cc.)	1.14	1.40	0.93	0.81	0.46	0.54	1.03
Total number of sperm ejaculated (billion)	2.01	1.13	0.94	0.58	0.35	0.55	1.31

[a]Rams received .2 gm. protamone daily.
[b]Number of observations.

Table 4. ANALYSIS OF VARIANCE FOR VOLUME (A), CONCENTRATION (B), AND TOTAL NUMBER OF SPERM (C) PER EJACULATE PRODUCED DURING THE PERIOD OF MAY 24 TO JULY 9.[a]

Source	Mean squares for traits			
	df	A	B	C
Between groups	1	2.16	5.10*	12.89
Between rams/groups	5	1.40*	0.74	2.62
Between ejaculates/rams	87	0.34	0.36	1.48

[a]Infertile rams given .2 gm. of protamone daily.
*$P<.05$.

Thyroprotein did not have any effect on the volume of the semen of the treated rams. Bogart and Mayer (1946) had concluded on the same kind of results in their experiment that this was due to the fact that

thyroprotein did not effect the interstitial activity of the testes. However, their conclusion that thyroprotein affected the spermatogenic activity was not substantiated in this experiment because of the fact that sperm concentration and total number of sperm were not affected by thyroprotein feeding. But one difference that very definitely exists between the two experiments is that their experiments were on the known fertile or supposedly fertile rams, whereas, this study was on suspected infertile rams.

On July 11, the thyroprotein dose was increased to .4 gm. daily. In the analysis of variance and in the averages only six rams are used because on July 20, Ram No. 7 a Michigan State University Sheep Breeding Farm ram was removed from the experiment and was put on pasture. After about 3 weeks, a sample was collected from him by the same method but he did not ejaculate any sperm. From the day he was put on pasture the treatment was also discontinued. Since the ram was taken away in the middle of the experiment, it was decided to omit him from all analyses.

The averages of the three traits of the semen for this period are tabulated in table 5 and the analysis of variance in table 6.

There was no statistically significant difference ($P<.05$) in volume between the two groups (table 6). It seems that although the difference in the volumes was not significant, this higher level of thyroprotein had some undesirable effect on the rams. When we compare table 5 with table 3, it appears that the treatment did not have a desirable effect on the rams. This conclusion was made by comparing the performance of the infertile rams for this period with their performance of previous

periods. The fertile (control) rams had also shown some decline in their semen characteristics but since the same rams had been kept as controls for all the periods, a relative comparison between the performance of the infertile rams for different periods was made. The relative decline in the semen of the fertile rams was also kept in view, although there was one infertile ram (No. 4) whose average volume for this period was higher than the pretreatment and the period during which .2 gm. of thyroprotein was given. Ram No. 3 produced a higher volume than in the pretreatment period but was lower than during the lower level period. All the other rams produced their lowest volume of the experiment in this period.

Sperm concentration was highly significantly ($P<.01$) higher in the fertile rams than in the infertile rams (table 6). The total number of sperm produced in the control group was also highly significant ($P<.01$) over the treated group (table 6). There was no difference within the groups in both the traits. As can be seen from table 5, this higher level of thyroprotein was harmful on the spermatogenic activity of the testes. These results are in accordance with the results of Warwick et al. (1948) who had fed a lower level but of a highly potent thyroprotein; Wilwerth et al. (1954) who found that .08% and .16% of protamone were detrimental to cock semen in both volume and concentration; and Brook and Ross (1962) who concluded that there appeared to be a lowering of semen quality associated with thyroxine therapy at higher environmental temperatures.

The averages of the oxygen consumption/billion sperm/hour of the pretreatment period and the period in which .2 gm. of thyroprotein was

Table 5. AVERAGES OF VOLUME, CONCENTRATION AND TOTAL NUMBER OF SPERM PER EJACULATE PRODUCED DURING THE PERIOD OF JULY 12 TO AUGUST 2.

Trait	Fertile		Infertile[a]			
	Ram 1	Ram 2	Ram 3	Ram 4	Ram 5	Ram 6
Number[b]	7	7	7	6	7	7
Volume (cc.)	0.76	0.41	0.61	1.12	0.24	0.33
Concentration (billion/cc.)	0.044	0.047	0.021	0.017	0.001	0.006
Total number of sperm ejaculated (billion)	0.029	0.031	0.013	0.013	0.001	0.003

[a]Rams received .4 gm. of protamone daily.
[b]Number of observations.

Table 6. ANALYSIS OF VARIANCE FOR VOLUME (A), CONCENTRATION (B) AND TOTAL NUMBER OF SPERM (C) PER EJACULATE PRODUCED DURING THE PERIOD OF JULY 12 TO AUGUST 2.[a]

Source	Mean squares for traits			
	df	A	B	C
Between groups	1	0	0.0109**	0.00483**
Between rams/groups	4	0.84	0.0005	0.00021
Between ejaculates/ram	35	0.45	0.0006	0.00044

[a]Infertile rams given .4 gm. of protamone daily.
**P<.01.

given orally are tabulated in table 7. The analysis of variance is tabulated in table 8. The between ejaculates/rams mean square was used to test the between group mean square for the pretreatment period in table 8 since the only reason that the between rams/groups can be smaller is sampling variation.

As can be seen in the pretreatment period table 7, the average oxygen consumption of both the known fertile rams was higher than six of the suspected infertile rams. One infertile ram had a higher average than the fertile ones. The range in the oxygen consumption for any one ejaculate in the fertile rams was from 35.90 µl/billion/hour to 191.81 µl/billion/hour. For the infertile rams it was 29.11µl/billion/hour to 124.00 µl/billion/hour.

The analysis of the available data showed that there was no significant difference between the fertile and infertile rams, though the 'F' value approached significance ($P<.05$).

In the treatment period although the average oxygen consumption of the infertile rams was higher, or almost equal, to that of the fertile rams, still there was no statistically significant difference between them. This may be because of a bigger range in both the groups. The range for the fertile rams was 40.33 µl/billion/hour to 210.00 µl/billion/hour and for the infertile rams 20.71 µl/billion/hour to 506.00 µl/billion/hour.

On an average the sperm in the semen of all the infertile rams consumed more oxygen in the treatment period than they did in the pretreatment period. By looking at table 7 we can see that the semen of all the

treated rams consumed relatively more oxygen than the semen from the fertile rams. Semen from Ram No. 1 consumed less oxygen in this period than during the pretreatment period, whereas the semen from Ram No. 2 consumed a little more oxygen than it did in the control period. By making a relative comparison between the performances of the fertile and the infertile rams it appears that the thyroprotein did have an effect on the respiration rate of the spermatozoa, although it was not statistically significant as demonstrated by table 8.

A statistical comparison was also made on the difference in the average respiration rate between the pretreatment and the treatment periods (table 9). Although there seems to be a difference in the average change in the respiration rate of the fertile and the infertile rams, after feeding the thyroprotein to the infertile rams, it was not found to be statistically significantly different ($P<.05$). This may be because of the few degrees of freedom and also because of a large variation between the individual rams from the pretreatment to the treatment period.

From tables 1 and 3 it was seen that the concentration for the same period (May 24 to July 9) was significantly higher in the untreated rams. In other words the concentration of sperms was higher in the pretreatment period in the infertile rams than it was during the treatment period. On the contrary the oxygen consumption was higher during the treatment period than the pretreatment period. This agrees with the result of Gray (1928), Rothschild (1950), Winchester (1941), Ross, Miller and Kurzrok (1941) and Windstosser (1935) although it does not agree with some of the other workers' results, that higher the concentration, lower the oxygen consumption.

Table 7. AVERAGE μl. OF OXYGEN CONSUMED/BILLION SPERM/HOUR FOR THE PRETREATMENT PERIOD (PP) AND TREATMENT PERIOD (TP).[a]

Period	Fertile		Infertile				
	Ram 1	Ram 2	Ram 3	Ram 4	Ram 5	Ram 6	Ram 7
PP	(3)[b] 83.29	(4) 86.80	(5) 46.00	(5) 47.46	(3) 51.47	(3) 88.41	(5) 49.06
TP	(6) 80.88	(4) 106.52	(7) 150.31	(2) 105.37	(4) 215.30	(5) 112.34	(7) 72.91
TP-PP	-2.41	19.72	104.31	57.91	163.83	23.93	23.85

[a]Infertile rams given .2 gm. protamone daily.

[b]Figures in parentheses show the number of observations.

Table 8. ANALYSIS OF VARIANCE FOR OXYGEN CONSUMPTION/BILLION SPERM/HOUR OF THE PRETREATMENT PERIOD (PP) AND THE TREATMENT PERIOD (TP).[a]

Source	df		Mean squares	
	PP	TP	PP	TP
Between groups	1	1	5169	9628
Between rams/groups	5	5	783	11808
Between ejaculates/rams	21	28	1257	10480

[a]Infertile rams given .2 gm. of protamone daily.

Table 9. ANALYSIS OF VARIANCE OF THE CHANGE IN THE RESPIRATION RATE OF SPERM (µl. of oxygen/billion sperm/hour) DUE TO .2 GM. OF PROTAMONE FED DAILY TO THE INFERTILE RAMS.

Source	df	Mean squares
Between groups	1	6243.80
Between rams/groups	5	2902.19

SUMMARY AND CONCLUSIONS

Five suspected infertile rams were selected from the nearby privately owned sheep farms, including one from the Michigan State University Sheep Breeding Farm. Two known fertile rams were selected randomly from the University flock. All the rams were kept in indoor pens, in the same barn and hand-fed the same ration.

Samples were collected, by electro-ejaculator in an artificial vagina, twice a week at an interval of 3-5 days. Volume, concentration and total sperm ejaculated were evaluated.

Starting on May 21, oral treatment of .2 gm of thyroprotein (Protamone) was given daily in a gelatin capsule. No statistically significant difference was found to exist between the fertile and infertile groups in volume and total sperm ejaculated. Concentration was found to be statistically significantly higher in the fertile ones over the infertile during this period.

On July 11, the thyroprotein dosage was increased to .4 gm daily. Volume was not found to be statistically different but concentration and total number of sperm produced were highly significantly higher ($P<.01$) in the control rams as compared to the treated rams.

Respiration rate of the sperm was also measured on the multiple-unit constant-pressure respirometer devised by Reineke (1961), using .2 ml of the sample, before and after the oral treatment with .2 gm of protamone. In both the groups the sperm from the ejaculates collected during the treatment period consumed more oxygen than the ejaculates of the pretreatment period. The treatment appeared to have a favorable effect on the respiration rate of the sperm, although it was not statistically significantly different.

The most important semen characteristic of the three, studied in this study, is the total number of sperm ejaculated, because it takes into consideration both the other traits which were studied, namely volume and concentration. Judging from this point of view, out of the 5 infertile rams, there were only two rams, Ram No. 3 and Ram No. 6 which at the .2 gm level ejaculated more sperm than their pretreatment performance. They also produced a relatively larger volume during this period as compared to their pretreatment period. The higher number of sperm ejaculated could have been because of this larger volume rather than as an effect of the thyroxine therapy. Ram No. 5 also produced a

little higher volume but the concentration was lower than during the pretreatment period. The highest number of sperm in the period, in the infertile group, were ejaculated by Ram No. 7, although it was lower than the pretreatment performance. The only thing that can be concluded from the available data is that at least three of the five infertile rams, Ram No. 4, 5 and 7 were not infertile because of any thyroxine deficiency. They could be infertile because of some other factor or a combination of factors. Ram No. 7 could be suspected for being one of those in which one of the factors could be thyroxine deficiency because this was the only ram in which the decline in the semen quality was not as acute as in others during the summer months. The thyroxine therapy could counteract only that factor but the semen quality still declined because of the other factor or factors involved.

Ram No. 3 and 6 could be suspected of suffering from thyroxine deficiency. They did show some improvement as far as the total number of sperm is concerned on feeding the thyroprotein at the .2 gm level.

It would also be safe to conclude that the .4 gm. level did not have any desirable effect on these three semen characteristics.

On an average, the samples from the infertile ram consumed more oxygen in the treatment period (not statistically significant) but it is hard to say whether this was because of the lower concentration of sperm present in the samples or because of the effect of thyroxine therapy.

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APPENDIX TABLE A

KNOWN FERTILE

Ram #1 - MSU 0029 - Columbia (2 years) Sheared April 26 and September 7

Date	Volume (c.c.)	Concentration per c. c. (billion)	Total No. of sperm in ejaculate (billion)	Motility (percent)	Atmospheric temperature degrees Fahrenheit (average)
January 8	0.7	1.50	1.05		20.0
" 17	1.0	1.80	1.80		15.0
February 17	0.9	1.50	1.35		25.5
March 25	0.7	1.60	1.12		38.5
April 9	0.8	1.40	1.12		45.0
" 13	2.5	1.19	2.975		40.5
" 18	1.2	1.20	1.44		45.5
" 23		No sample (urine)			
" 27	2.1	1.01	2.121		71.5
" 30	1.2	1.5	1.8		65.5
May 2	1.7	0.56	0.952		51.0
" 5	1.6	1.20	1.92		62.5
" 8	1.5	1.27	1.905		47.5
" 12	1.0	0.98	0.98		54.5
" 17	0.3	1.24	0.372		75.0
" 24	2.0	1.86	3.72		68.0
" 27	3.1	1.01	3.131		53.5
" 31	1.0	1.12	1.12		74.0
June 4	0.8	1.08	0.864		67.5
" 8	1.2	1.80	2.16		65.0
" 11	1.3	1.09	1.417		72.0
" 14	1.5	0.32	0.48		66.0
" 18	1.2	0.97	1.164		76.0
" 21	1.2	1.87	2.244		65.5
" 25	3.0	2.18	6.54	50	70.5
" 28	2.7	1.42	3.834	70	70.0
July 2	1.5	0.69	1.035	55	67.5
" 5	0.8	0.52	0.416	50	71.5
" 9		No sperms			
" 12	1.8	0.04	0.072	30	74.0
" 16	0.8	0.08	0.064	25	66.0
" 19	0.4	0.08	0.032	30	69.5
" 23	0.3	0.04	0.012	10	66.0
" 26	0.4	0.02	0.008	10	62.0
" 30	0.3	0.05	0.015	10	72.5
August 2	1.3	No sperms			60.5
" 7	0.8	No sperms			73.0
" 15	0.6	0.31	0.186	15	65.5
" 23	0.3	0.21	0.063	20	71.0
" 29	0.4	0.21	0.084	40	74.0
September 5	0.6	0.20	0.120	40	63.5
" 12	0.3	0.02	0.006	40	71.0
" 19	1.5	1.73	2.59	50	54.5
" 26	2.1	1.37	2.877	55	53.0
October 4	3.1	2.28	7.068	50	62.5
" 10	2.3	3.12	7.176	65	55.5
" 17	3.0	2.72	8.160	60	51.5

APPENDIX TABLE B

KNOWN FERTILE

Ram #2 - MSU 444 - Shropshire (2 years) Sheared April 26 and September 7

Date	Volume (c.c.)	Concentration per c.c. (billion)	Total No. of sperm in ejaculate (billion)	Motility (percent)	Atmospheric temperature degrees Fahrenheit (average)
April 9	0.7	1.19	0.833		45.0
" 13	1.0	2.03	2.03		40.5
" 18	0.8	0.98	0.784		45.5
" 23	1.0	2.83	2.83		50.5
" 27	0.4	1.02	0.408		71.5
" 30	0.5	1.11	0.555		65.5
May 2	0.4	3.38	1.352		51.0
" 5	0.6	2.02	1.212		62.5
" 8	0.8	1.90	1.52		47.5
" 12	0.6	1.81	1.086		54.5
" 17	0.6	0.57	0.342		75.0
" 24	1.1	2.98	3.278		68.0
" 27	1.0	1.82	1.82		53.5
" 31	0.4	2.40	0.96		74.5
June 4	0.5	2.01	1.005		67.5
" 8	0.3	1.98	0.594		65.0
" 11	1.1	1.11	1.221		72.0
" 14	1.1	1.57	1.727		66.0
" 18	1.4	1.21	1.694		76.0
" 21	0.7	1.05	0.735		65.5
" 25	1.0	1.98	1.98	60	70.5
" 28	0.8	0.98	0.784	70	70.0
July 2		No sample			67.5
" 5	0.1	0.44	0.044	50	71.5
" 9	0.5	0.08	0.04	35	69.5
" 12	0.1	0.01	0.001	20	74.0
" 16		No sample			66.0
" 19	0.3	0.04	0.012	20	69.5
" 23	0.2	No sperms			
" 26	0.9	0.10	0.090	25	62.0
" 30	0.4	0.11	0.044	30	72.5
August 2	1.0	0.07	0.070	20	60.5
" 7	0.6	0.09	0.054	45	73.0
" 15	0.3	0.06	0.018	40	65.5
" 23	0.2	0.12	0.024	45	71.0
" 29	0.2	0.18	0.036	40	74.0
September 5	0.4	0.07	0.028	50	63.5
" 12	0.3	0.21	0.070	60	71.0

Sold by MSU Sheep Farm

APPENDIX TABLE C

SUSPECTED INFERTILE

Ram #3 - Taylor 362 - Columbia (3 years) Sheared April 26

Date	Volume (c.c.)	Concentration per c. c. (billion)	Total No. of sperm in ejaculate (billion)	Motility (percent)	Atmospheric temperature degrees Fahrenheit (average)
January 8	0.7	1.30	0.91		20.0
" 17	0.4	1.20	0.48		15.0
February 17	0.6	1.00	0.60		25.5
March 25	0.4	1.40	0.56		38.5
April 9	1.1	1.61	1.771		45.0
" 13	0.5	0.90	0.45		40.5
" 18	0.4	1.72	0.688		45.5
" 23	0.6	1.62	0.972		50.5
" 27	0.4	1.50	0.60		71.5
" 30	0.8	1.02	0.816		65.5
May 2		No sample (urine)			51.0
" 5	0.4	0.98	0.392		62.5
" 8		No sample (urine)			47.5
" 12	0.3	1.01	0.303		54.5
" 17	0.4	0.91	0.364		75.0
May 21		Protamone started .2 gm			
" 24	2.7	1.58	4.266		68.0
" 27	1.1	1.10	1.21		53.5
" 31	0.6	0.72	0.432		74.0
June 4	0.3	1.02	0.306		67.5
" 8	0.8	1.53	1.224		65.0
" 11	1.2	0.96	1.152		72.0
" 14	1.3	0.80	1.04		66.0
" 18	0.9	0.62	0.558		76.0
" 21	0.5	1.75	0.875		65.5
" 25	0.5	1.23	0.615	40	70.5
" 28	0.5	0.32	0.16	55	70.0
July 2	1.0	0.86	0.86	50	67.5
" 5	0.9	0.49	0.441	45	71.5
" 9	0.3	0.01	0.003	25	69.5
July 11		Protamone .4 gm			
" 12	1.6	0.02	0.032	25	74.0
" 16	0.7	0.02	0.014	15	66.0
" 19	0.7	0.02	0.014	20	69.5
" 23	0.3	0.01	0.003	10	66.0
" 26	0.2	0.02	0.004	5	62.0
" 30	0.5	0.02	0.01	10	72.5
August 2	0.3	0.04	0.012	10	60.5
" 7	0.6	No sperms			73.0
" 15	0.4	0.07	0.028	20	65.5
" 23		No sample			71.0

Taken away by the owner.

APPENDIX TABLE D

SUSPECTED INFERTILE

Ram #4 - Frank 443 - Corriedale (5 years) Sheared April 26 and September 17

Date	Volume (c.c.)	Concentration per c. c. (billion)	Total No. of sperm in ejaculate (billion)	Motility (percent)	Atmospheric temperature degrees Fahrenheit (average)
January 8	0.3	1.00	0.300		20.0
" 17	0.4	1.30	0.520		15.0
February 17	0.8	1.10	0.880		25.5
March 25	0.5	1.30	0.650		38.5
April 9	0.8	1.01	0.808		45.0
" 13	2.0	1.13	2.26		40.5
" 18	0.6	No sperms			45.5
" 23	0.3	1.70	0.510		50.5
" 27	0.2	1.34	0.268		71.5
" 30	0.6	1.06	0.636		65.5
May 2	0.6	2.57	1.542		51.0
" 5	0.4	1.20	0.480		62.5
" 8	0.6	0.66	0.396		47.5
" 12		No sample (urine)			54.5
" 17	0.5	0.86	0.430		75.0
May 21		Protamone started .2 gm			
" 24	1.8	1.62	2.916		68.0
" 27	0.6	0.91	0.546		53.5
" 31	0.7	0.93	0.651		74.5
June 4	0.2	0.71	0.142		67.5
" 8	0.3	0.83	0.249		65.0
" 11	0.5	1.01	0.505		72.0
" 14	0.9	1.54	1.386		66.0
" 18	0.5	0.43	0.215		76.0
" 21		No sample (urine)			65.5
" 25	0.1	1.13	0.113	50	70.5
" 28	0.4	0.41	0.164	40	70.0
July 2		No sample (urine)			67.5
" 5	0.1	0.23	0.023	40	71.5
" 9	0.2	0.02	0.004	25	69.5
July 11		Protamone .4 gm			
" 12	1.1	No sperms			74.0
" 16	0.6	0.04	0.024	15	66.0
" 19	4.0	0.01	0.040	10	69.5
" 23		No sample (urine)			66.0
" 26	0.3	0.03	0.009	5	62.0
" 30	0.2	0.02	0.004	15	72.5
August 2	0.5	No sperms			60.5
" 7	0.1	No sperms			73.0
" 15		No sample			65.5
" 23	0.4	No sperms			71.0
" 29	0.3	0.11	0.033	15	74.0
September 5	1.0	0.11	0.11	10	63.5
" 12	0.1	No sperms			71.0
" 19	0.3	0.04	0.012	20	54.5

Taken away by the owner in the afternoon of September 19

APPENDIX TABLE F

SUSPECTED INFERTILE

Ram #6 - Bortel - Dorset (Westhoven) - (5 years) Sheared September 7

Date	Volume (c.c.)	Concentration per c. c. (billion)	Total No. of sperm in ejaculate (billion)	Motility (percent)	Atmospheric temperature degrees Fahrenheit (average)
April 9	0.6	0.98	0.588		45.0
" 13		No sample (urine)			40.5
" 18		No sample (urine)			45.5
" 23	0.4	0.72	0.288		50.5
" 27	0.5	0.64	0.320		71.5
" 30	0.6	0.56	0.336		65.5
May 2		No sample (urine)			51.0
" 5		No sample (urine)			62.5
" 8	0.7	0.71	0.497		47.5
" 12	0.4	0.69	0.276		54.5
" 17	0.8	0.51	0.408		75.0
May 21		Protamone started .2 gm			
" 24	2.0	1.31	2.620		68.0
" 27	1.2	1.10	1.320		53.5
" 31	0.7	0.82	0.574		74.0
June 4	0.3	0.67	0.201		67.5
" 8	0.4	0.72	0.288		65.0
" 11	1.2	0.22	0.264		72.0
" 14	0.6	0.59	0.354		66.0
" 18	0.6	0.70	0.420		76.0
" 21	1.7	0.26	0.442		65.5
" 25	1.0	0.31	0.310	10	70.5
" 28	0.6	0.35	0.210	15	70.0
July 2	1.5	0.44	0.66	10	67.5
" 5	0.3	0.06	0.018	5	71.5
" 9	0.4	No sperms			69.5
July 11		Protamone .4 gm			
" 12	0.5	No sperms			74.0
" 16	0.5	0.04	0.020	5	66.0
" 19	0.3	No sperms			69.5
" 23		No sample			66.0
" 26	0.4	No sperms			62.0
" 30		No sample			72.5
August 2	0.6	No sperms			60.5
" 7		No sample			73.0
" 15		No sample			65.5
" 23		No sample			71.0
" 29		No sample			74.0
September 5		No sample			63.5
" 12	0.5	No sperms			71.0
" 19		No sample			54.5
September 22		Last dose of protamone			
" 26	0.3	0.28	0.084	15	53.0
October 4	1.7	0.39	0.663	15	62.5
" 10	1.4	0.52	0.728	10	55.5
" 17	1.0	0.47	0.470	10	51.5

APPENDIX TABLE G

SUSPECTED INFERTILE

Ram #7 - MSU 237874 - Shropshire (3 years) Sheared April 26

Date	Volume (c.c.)	Concentration per c.c. (billion)	Total No. of sperm in ejaculate (billion)	Motility (percent)	Atmospheric temperature degrees Fahrenheit (average)
March 25	0.6	1.90	1.140		38.5
April 9	1.4	1.34	1.876		45.0
" 13	0.9	1.50	1.350		40.5
" 18	1.0	1.42	1.420		45.5
" 23	1.5	1.75	2.625		50.5
" 27	1.2	1.11	1.332		71.5
" 30	2.1	2.34	4.914		65.5
May 2		No sample (urine)			51.0
" 5	1.1	1.72	1.892		62.5
" 8	1.0	0.42	0.420		47.5
" 12	0.8	0.81	0.648		54.5
" 17	0.7	0.58	0.406		75.0
May 21		Protamone started .2 gm			
" 24	2.6	2.88	7.488		68.0
" 27	1.2	1.10	1.320		53.5
" 31	1.0	0.92	0.920		74.0
June 4	0.5	0.82	0.410		67.5
" 8	0.6	1.12	0.672		65.0
" 11	0.4	1.02	0.408		72.0
" 14	1.0	0.83	0.830		66.0
" 18	1.1	1.45	1.595		76.0
" 21	1.4	0.56	0.784		65.5
" 25	1.4	2.04	2.856	55	70.5
" 28	0.5	0.71	0.355	40	70.0
July 2	0.7	0.53	0.371	45	67.5
" 5	0.9	0.38	0.342	50	71.5
" 9	0.7	No sperms			69.5
July 11		Protamone .4 gm			
" 12	0.4	No sperms			74.0
" 16	0.5	0.02	0.01	10	66.0
" 19		No sample			69.5
" 23					
" 26		Taken away and put on pasture - treatment discontinued			
" 30					
August 2					
" 7	0.9	No sperms			73.0

Taken away again put on pasture.
Tried to collect a sample on a non-cyclic ewe but refused to mount.
Later on tried to collect a sample by an electro-ejaculator but did not give any sample.

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