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Effects of Radioactive Phosphorus In Vitro on Oxygen Consumption of Semen

Glenn D. Folmar
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Glenn D. Folmar entitled "Effects of Radioactive Phosphorus In Vitro on Oxygen Consumption of Semen." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Charles S. Hobbs, Major Professor

We have read this thesis and recommend its acceptance:

R.L. Murphree & J.G. Carlson

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 11, 1952

To the Graduate Council:

I am submitting herewith a thesis written by Glenn D. Folmar, Jr. entitled "Effects of Radioactive Phosphorus In Vitro on Oxygen Consumption of Semen." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Charles S. Hobbs
Major Professor

We have read this thesis
and recommend its acceptance:

R. L. Murphree

J. H. Carlson

Accepted for the Council:

E. A. Yates
Dean of the Graduate School

EFFECTS OF RADIOACTIVE PHOSPHORUS *IN VITRO* ON OXYGEN CONSUMPTION OF SEMEN

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the Degree of
Master of Science

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by
Glenn D. Folmar, Jr.

August 1952

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INTRODUCTION

The increasing use of radioactive isotopes and radiation in industry, chemistry, biochemistry, biophysics, biology and medicine has stimulated interest in the possible effects of radiation on the reproductive functions of both the male and female. It has been demonstrated that whole-body X-irradiation of the male increases the numbers of abnormal sperm (Boche, 1946) and that either X-irradiation of the scrotal region or whole-body X-irradiation increases the pre-natal mortality rate of the off-spring (Strandskov, 1932; Henson, 1942; and Murphree et al., 1952). Likewise, it has been shown that X-irradiation of sperm in vitro increases the pre-natal mortality rate, although no gross morphological changes were observed in sperm even at levels of radiation which completely inhibited the fertilizing capacity (Kesin, 1944 and Amoroso and Parkes, 1947).

It would appear, therefore, that the reduction in the functional capacity of sperm may occur before gross alterations in morphology are observed. It is possible that these results may be due to disturbances in cellular function. It has long been recognized that the more common methods of evaluating semen, such as motility, percentage alive, survival in storage, and morphology of sperm do not afford a completely satisfactory method of predicting potential fertility of normal sperm. In view of the fact that no gross changes in morphology or motility due to radiation damage have been observed (Kesin, 1944 and Amoroso and Parkes, 1947) it is apparent that a more sensitive test of radiation damage to sperm is needed. This study was initiated as an effort to find such a test.

The rate of oxygen consumption was chosen as a possible indication of radiation damage because it represents a more quantitative measure than do the more subjective evaluations of sperm viability previously listed. It was also considered that the sensitivity of the manometric technique might allow observations of smaller differences between irradiated and non-irradiated semen specimens.

Radioactive phosphorus (P^{32})[1] was chosen as the source of radiation because it could be easily incorporated into the medium without chemical damage to sperm while the oxygen consumption values were being obtained. In addition, P^{32} is readily available and has certain desirable radiation characteristics. Its beta energy of 1.71 million electron volts is strong enough to ensure uniform distribution of ionizing collisions, but is not strong enough to permit the major portion of beta particles to escape from the one to two milliliter semen samples used in this study. The confinement of radiation to the sample is also desirable from the standpoint of reduced hazard to personnel. Radioactive phosphorus has a half-life of 14.3 days which is long enough so that decay corrections do not have to be made on data collected over a period of a few hours.

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[1] The term P^{32} will hereafter be used to indicate radioactive phosphorus.

EXPERIMENTAL PROCEDURE

Source and Evaluation of Semen

Boar Semen. The semen used in this study was obtained from two Hampshire boars and was collected in an artificial vagina. Complete ejaculates were collected from Boar 31, while with one exception, incomplete ejaculates were collected from Boar LPR. These incomplete ejaculates were partitioned collections in which only the intermediate or highest-sperm-containing fraction was retained for subsequent study. This procedure was followed in an effort to obtain a comparison of the oxygen consumption values of semen samples of widely different sperm concentrations. Immediately following collection, the semen was brought to the laboratory where the following observations were made: volume, estimation of initial motility, and sperm concentration. Volume was measured in a 250 milliliter graduated cylinder. Following microscopic examination, an estimation of motility was recorded on the basis of percentage of motile sperm and on the degree of motility (with 0 indicating no motility and 5 maximum motility). Sperm counts were made with a hemacytometer and expressed as numbers of sperm per cubic millimeter of semen.

The semen characteristics are shown in Table I. The average sperm concentration of incomplete ejaculates from Boar LPR was approximately two and one-half times the average concentration of complete ejaculates from Boar 31. While the higher motility of semen from Boar LPR may have been due largely to individual variation between boars, it also may have been partially due to the almost unavoidable tendency to

TABLE I

BOAR SEMEN CHARACTERISTICS

Source of Semen	Ejaculate	Volume in Milliliters	Initial Motility[a]	Concentration per Cubic Millimeter
Boar 31	A	-[b]	60%-3.0	202,500
	B	230	80%-3.0	187,500
	C	170	60%-3.0	120,000
	D	240	70%-3.0	165,000
	E	230	60%-3.0	180,000
	F	240	70%-4.0	240,000
	G	115	50%-3.0	220,000
	H	260	60%-3.0	200,000
Average	-	212	64%-3.1	189,375
Boar LPR	I	270	80%-4.0	240,000
	J	120	90%-4.0	530,000
	K	-	70%-3.5	540,000
	L	115	95%-5.0	430,000
	M	-	90%-4.0	540,000
Average	-	-	85%-4.1	510,000[c]

[a] The first figure represents the percentage of sperm showing any movement and the second figure represents degree of motility.

[b] The volumes of Ejaculates A, K, and M were inadvertently not recorded.

[c] Average of Ejaculates J, K, L, and M, since Ejaculate I was a complete ejaculate.

rate the motility of a highly concentrated specimen higher than a less concentrated sample when examining semen microscopically. The differences in the semen characteristics of the two boars are attributed largely to the fact that the intermediate fractions of ejaculates, with the exception of Ejaculate I, were collected from Boar LPR. McKenzie et al. (1938) demonstrated that the intermediate fraction of an ejaculate of boar semen has a higher sperm concentration than the initial and terminal fractions. Winchester and McKenzie (1941) reported that differences in concentration and motility of semen from two boars were not greater than day to day variations in the semen of either animal.

On the basis of a rank correlation test (Snedecor, 1946) there was no apparent association of initial motility or concentration and subsequent oxygen consumption of the semen of either boar, even though the initial motility and concentration of semen from Boar LPR were consistently higher than the initial motility and concentration of semen from Boar 31. Since no significant difference between boars was demonstrated in the oxygen consumption of semen, the data were pooled for presentation and discussion.

Bull semen. The bull semen used in this study was obtained from two bulls at the University of Tennessee dairy bull stud at Knoxville and from two bulls maintained by the UT-AEC Agricultural Research Program at Oak Ridge. Semen from the bulls at Knoxville was brought to Oak Ridge immediately following collection. In addition to motility estimates and concentration counts, percentage of living sperm in the bull semen was determined using the eosin-fast

green FCF staining technique (Mayer et al., 1947).

Bull semen characteristics are shown in Table II. There was considerable variation in the quality of semen from the four bulls which may be due largely to the individual variation that is known to occur between bulls (Herman and Swanson, 1941).

Preparation of Semen Samples

Boar semen. Before sampling the semen was stirred gently to ensure a uniform sperm concentration. Two milliliters of undiluted semen was added to each of a series of Warburg flasks with a pipette. The number of replicates for each ejaculate varied from two to eight. Initially, it was planned to use only two replicates from each semen sample. However, since it was noted early in the study that there was considerable variation between replicates, the maximum number of replicates, as determined by either the volume of semen or the capacity of the Warburg apparatus, were used in subsequent trials.

The different shipments of P^{32}, as received in the laboratory, were in solutions of 0.03 to 0.05 normal hydrochloric acid. These solutions were neutralized to pH 7.0 with sodium hydroxide prior to addition to treated semen to prevent possible harmful effects of the acid on the sperm. A micropipette was used to add P^{32} to semen samples. The amount of P^{32} which was added to replicates of the different ejaculates was varied in order to give an estimation of the effect of varying levels of radiation on oxygen consumption of semen. The quantities of P^{32} which were added to replicates from each ejaculate are shown in Table III. Aliquots of six different ejaculates from

TABLE II

BULL SEMEN CHARACTERISTICS

Animal	Semen Sample	Volume in Milliliters	Initial Percentage Alive	Initial Motility[a]	Concentration per Cubic Millimeter
Two UT Bulls	1	5.0[b]	88	80%-4.0	1,420,000
Bull UT-01	2	4.0	40	50%-3.0	390,000
UT Bull	3	5.0	67	50%-2.0	2,000,000
Bull UT-00	4	2.8	81	70%-3.0	720,000
Two UT Bulls	5	5.5[b]	75	70%-4.0	1,150,000
Average	-	4.5	70	64%-3.2	1,136,000

[a] The first figure represents the percentage of sperm showing any movement and the second figure represents degree of motility.

[b] Two pooled ejaculates.

TABLE III

DOSE PER REPLICATE OF BOAR SEMEN IN MILLICURIES OF P^{32}

Animal	Ejaculate	Millicuries of P^{32}
Boar 31	A	1.00
	B	1.00
	C	1.00
	D	1.00
	E	1.00
	F	1.50
	G	2.30
	H	1.00
Boar LPR	I	0.50
	J	0.25
	K	-[a]
	L	0.25
	M	0.12

[a] No P^{32} was added to samples from Ejaculate K.

Boar 31 received 1.0 millicurie of P^{32} per replicate in 0.150 to 0.200 milliliters of neutralized solution, while replicates from two additional ejaculates from the same boar received 1.5 and 2.3 millicuries each in a similar volume. Semen samples from two of the five ejaculates from Boar LPR received 0.25 millicuries of P^{32} per replicate while samples from two additional ejaculates received 0.12 and 0.50 millicuries per sample. No radioactive phosphorus was added to samples from one other ejaculate, but the oxygen consumption of those samples was included in the data on non-treated samples. Using methods described by Marinelli *et al.* (1948), it was calculated that each sample of semen received approximately 8,320 r per hour for each millicurie of P^{32}. A comparable volume of a control solution of neutralized hydrochloric acid was added to each non-treated semen sample. In the later discussion, semen to which P^{32} was added will be referred to as treated samples, while semen which received no radioactivity will be referred to as non-treated. The data for non-treated and treated semen for the first hour of measurement were obtained on ninety replicates representing thirteen different ejaculates and seventy-nine replicates representing twelve different ejaculates, respectively. The number of replicates decreased slightly during the later intervals (second to fifth hours, inclusive). The failure to obtain values for replicates during the later intervals was due to technical difficulties in the operation of the Warburg apparatus.

Bull semen. The procedure for treatment of bull semen was

essentially the same as for bear semen except that the semen was diluted 1:1 with M/8 phosphate buffer at pH 6.9 prior to sampling and 1.0 milliliter aliquots of diluted semen were taken for measurement of oxygen consumption. In two cases, single ejaculates from two bulls were pooled to obtain sufficient volume of semen (Table II). In the other three instances, a single ejaculate was used in each trial. Replicates from two semen samples received 0.1 millicurie of P^{32} while replicates from a third semen sample received 1.0 millicurie. The data for non-treated and treated semen for the first hour of measurement were obtained on five replicates representing three different semen samples and six replicates representing three different semen samples, respectively.

Seminal fluid. Sperm-free seminal fluid was obtained by centrifuging whole semen at 2,000 r.p.m. for ten minutes and then decanting the fluid.

Manometric Technique

The instrument used to measure oxygen consumption was the Aminco-Lardy Rotary Manometric Apparatus equipped with Warburg manometers. The general principles and procedures described by Umbreit et al. (1945) were employed in the manometric determinations. Flask constants, needed to determine the amount of gas consumed from observed pressure changes, were calculated from a nomogram (Dixon, 1945). The temperature of the water bath was adjusted to $37.5^{\circ} \pm 0.02^{\circ}$ Centigrade by means of a mercury thermoregulator. A shaking speed of seventy oscillations per minute was used with a three centimeter amplitude of

oscillation. All manometer readings received thermobarometer corrections. Two-tenths of a milliliter of 20 per cent potassium hydroxide was added to a coil of filter paper in the center well of the Warburg flask to absorb carbon dioxide given off by the semen preparation. To prevent creeping of KOH into the sample, the top surface of the center well was given a light coating of petroleum jelly.

Following addition of the semen to the Warburg flasks and attachment of the manometers to the water bath, a ten-minute shaking period was allowed, for the temperature of the preparation to reach equilibrum, before the manometer system was closed. During this period all plugs and connections were adjusted and tightened to ensure against leaks in the system due to softening of the petroleum jelly used to seal the connections. At the end of the equilibration period, fluid in the closed side of the manometer was adjusted to the initial reading point (150 Millimeters) with the stopcock open to atmospheric pressure. The system was then closed and the initial reading taken.

The time between collection and the initial manometer reading for boar and bull semen was one to one and one-half hours and one and one-half to four hours, respectively. Consequently, the oxygen consumption values for the first hour represent oxygen consumption for the first hour of observation. Oxygen consumption was recorded at thirty minute intervals. The analysis of variance, rank correlation test and standard deviations of the data on boar semen were calculated according to the methods of Snedecor (1946). The averages in the data on boar semen are weighted averages, while the averages in the data on bull semen are unweighted.

RESULTS AND DISCUSSION

Boar Semen

Oxygen consumption of boar semen. The average oxygen consumption of non-treated and treated semen during the first hour of measurement was 201.5 and 187.4 microliters of oxygen per billion sperm, respectively (Table IV and Figure 1). The results for the individual hourly intervals (first to fifth hours, inclusive) are included in Tables XIII through XXII in the Appendix. The differences in the oxygen consumption between non-treated and treated semen are not statistically significant (Table XXIII). These results are in agreement with the work of Kosin (1944) who observed that irradiation of cock sperm *in vitro* with 10,000 r from an X-ray source did not affect the rate of oxygen consumption. While there was considerable variation in the oxygen consumption of replicates from a given ejaculate, this variation was small in comparison to the differences in oxygen consumption between ejaculates from a given boar (Tables XIII through XXII). Within the levels of radiation administered in this study, there was no appreciable effect upon the rate of oxygen consumption of the irradiated sperm.

As previously stated, the average oxygen consumption of non-treated semen during the first hour of measurement was 201.5 microliters of oxygen per billion sperm, with a range of 84.6 to 295.8. Since semen samples of two milliliters were used in this experiment, the oxygen consumption per sample was recalculated (Table V) for the purpose of comparison with the results of Winchester and McKenzie (1941). These workers reported fifteen observations on what appears to be nine ejaculates. Their values

TABLE IV

AVERAGE OXYGEN CONSUMPTION OF BOAR SEMEN

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm per Hourly Interval[a] 0 - 1	1 - 2	2 - 3	3 - 4	4 - 5
Boar 31	A	2	137.5 (182.3)	115.9 (155.9)	52.9 (7.2)	92.6 (17.8)	64.3 (19.5)
	B	3	237.1 (227.6)	89.7 (82.7)	20.1 (24.5)	31.0 (9.8)	29.6 (21.9)
	C	4	105.4 (115.5)	34.9 (59.2)	27.1 (32.5)	-[b] (-)	- (-)
	D	7[c]	129.1 (87.1)	54.2 (36.7)	75.5 (74.2)	53.0 (46.6)	17.6 (16.8)
	E	8	284.4 (236.8)	254.3 (174.4)	87.7 (80.1)	104.4 (56.3)	80.1 (36.7)
	F	8	140.9 (136.6)	-60.6 (-104.7)[d]	1.6 (17.5)	27.2 (50.7)	- (-)
	G	8	194.8 (175.6)	164.6 (131.5)	60.9 (38.1)	41.7 (41.8)	6.6 (-6.7)
	H	8	240.5 (233.1)	64.4 (60.7)	-40.4 (-41.0)	-29.0 (-24.9)	-18.1 (-15.5)
Average	-	-	191.6 (172.7)	91.7 (65.0)	35.0 (32.0)	41.0 (31.7)	24.7 (9.6)
Boar LPR	I	8	168.7 (159.7)	29.7 (33.2)	-22.6 (-21.0)	-46.5 (-61.3)	-47.7 (-27.6)
	J	8	230.0 (212.9)	144.3 (152.6)	82.7 (134.9)	20.5 (90.8)	8.3 (43.6)
	K	8	194.4 (-)[e]	81.6 (-)	14.3 (-)	1.2 (-)	- (-)
	L	8	253.6 (242.8)	122.6 (120.2)	27.9 (29.3)	-4.6 (-4.9)	-5.4 (-8.4)
	M	10[f]	216.9 (236.7)	139.5 (143.1)	29.7 (43.5)	7.4 (7.1)	-8.0 (-8.7)
Average	-	-	212.9 (211.4)	105.3 (110.2)	26.5 (46.9)	4.2 (8.5)	-12.9 (0.3)
Average (Both Boars)	-	-	201.5 (187.4)	98.0 (82.2)	31.1 (37.7)	23.0 (22.4)	6.4 (5.4)

[a] The first figures are the values for non-treated semen, while the figures in parentheses are the values for treated semen.

[b] The failure to obtain values during the later intervals was due to technical difficulties in the operation of the Warburg apparatus.

[c] There were seven replicates of non-treated semen and eight replicates of treated semen from Ejaculate D.

[d] A minus sign indicates the evolution of an unknown gas or gases, other than carbon dioxide, instead of consumption of oxygen.

[e] No P^{32} was added to replicates from Ejaculate K.

[f] There were ten replicates of non-treated semen and six replicates of treated semen from Ejaculate M.

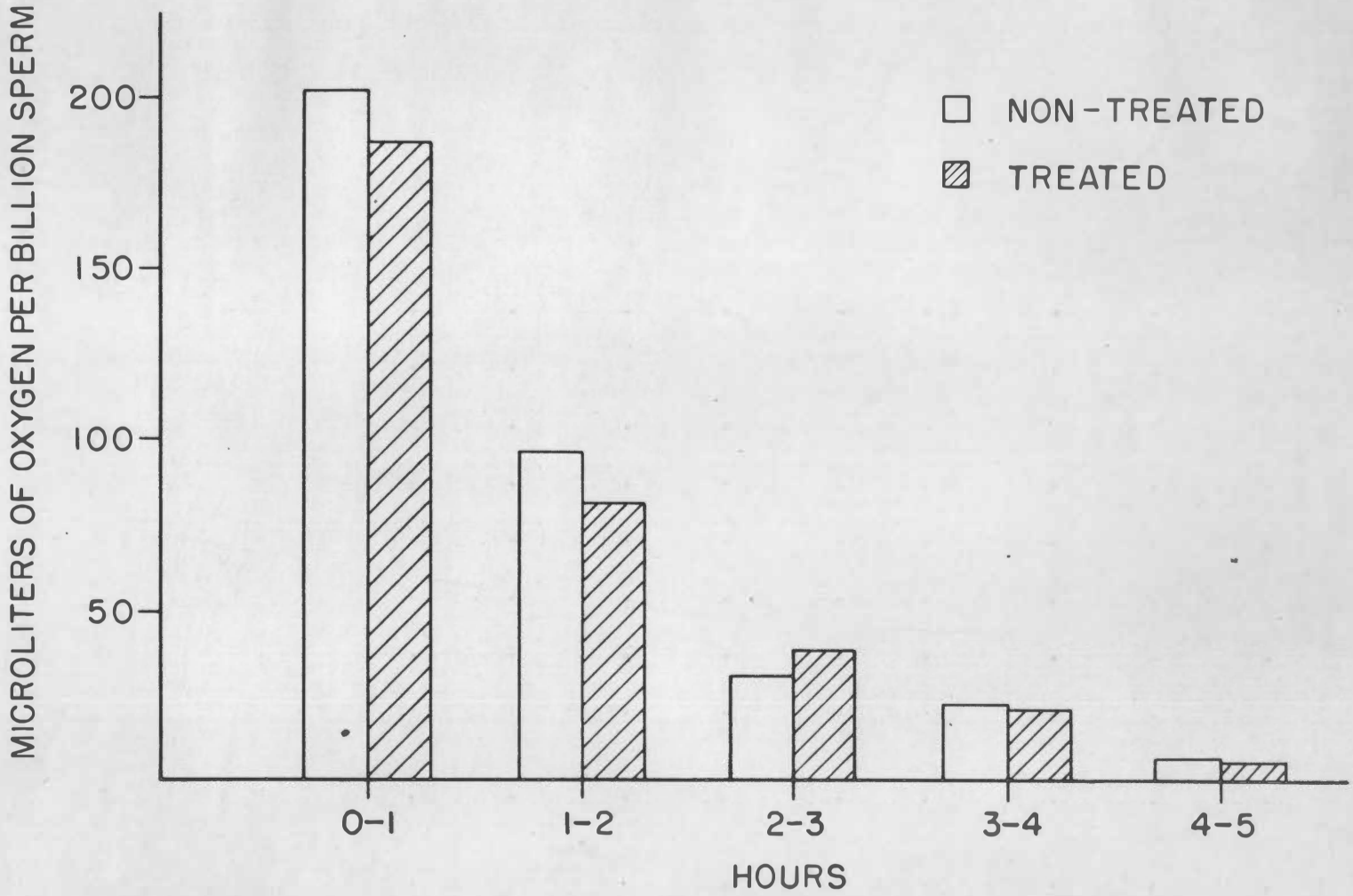

FIGURE I. AVERAGE OXYGEN CONSUMPTION OF BOAR SPERM

TABLE V

OXYGEN CONSUMPTION (PER SAMPLE) OF NON-TREATED BOAR SEMEN DURING THE FIRST HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Two Milliliters of Semen			
			Range			Standard
			Low	High	Mean	Deviation
Boar 31	A	2	43.3	55.7	49.5	8.8
	B	3	79.6	83.0	80.9	1.9
	C	4	18.5	28.1	23.0	5.0
	D	7	34.7	46.7	40.9	3.9
	E	8	90.1	96.8	93.1	2.4
	F	8	57.0	66.3	60.1	2.8
	G	8	61.6	76.4	68.6	4.9
	H	8	70.7	90.0	77.0	6.0
	All	48	18.5	96.8	64.8	21.1
Boar LPR	I	8	61.0	67.2	64.8	1.9
	J	8	183.3	199.7	194.8	5.6
	K	8	206.3	216.2	209.9	3.6
	L	8	186.0	199.0	193.4	4.2
	M	10	212.8	254.8	230.7	14.4
	All	42	183.3[a]	254.8[a]	208.6[a]	17.9[a]
Both Boars	All	90	18.5	254.8	119.1	72.6

[a] Ejaculate I, which was a complete ejaculate, was not included in the calculation of these results.

covered a range of 30.5 to 144.3 microliters of oxygen per milliliter of semen per hour. In this study, the average oxygen consumption of semen from Boar 31 was 32.4 microliters of oxygen per milliliter of semen during the first hour of measurement with a range of 9.3 to 48.4. The average oxygen consumption of the more concentrated samples of incomplete ejaculates from Boar LPR was 104.3 microliters of oxygen per milliliter of semen during the first hour of measurement, with a range of 91.7 to 127.4. The average oxygen consumption per milliliter of semen from Boar 31, which was 32.4, is approximately the same as the lowest value reported by Winchester and McKenzie, while the lowest value for semen from this boar, which was 9.3, is only approximately one-third of the lowest value reported by those workers. The highest value recorded for oxygen consumption per milliliter of semen from Boar LPR was 127.4 which is lower than the highest value reported by Winchester and McKenzie.

Oxygen consumption of boar seminal fluid. The average oxygen consumption of seminal fluid during the first hour of measurement was 0.04 microliters of oxygen per milliliter of seminal fluid (Table VI). Seminal fluid from only two ejaculates consumed oxygen, whereas, the seminal fluid from three additional ejaculates evolved a small amount of an unknown gas or gases, other than carbon dioxide, instead of consuming oxygen. The oxygen consumption of boar seminal fluid observed in this study is not in agreement with the findings of Winchester and McKenzie (1941) that boar seminal fluid consumed oxygen in amounts of 5 to 22 per cent of that of whole semen. In view of the low level of

TABLE VI

OXYGEN CONSUMPTION OF BOAR SEMINAL FLUID DURING THE FIRST HOUR OF MEASUREMENT

Animal	Ejaculate[a]	Number of Replicates	Microliters of Oxygen per Two Milliliters			
			Range			Standard
			Low	High	Mean	Deviation
Bear 31	-	4	-7.6[b]	-3.7	-5.7	1.9
	-	1	-	-	-1.2	-
	-	1	-	-	-6.1	-
	A	4	1.2	16.0	5.9	7.3
	B	3	-1.3	6.7	3.5	4.7
	C	4	-2.3	1.1	-0.6	1.7
	All	17	-7.6	16.0	0.08	4.8

[a] The data on the first three ejaculates listed in this Table were obtained during preliminary work, hence these ejaculates are not identified by a letter.

[b] A minus sign indicates the evolution of an unknown gas or gases, other than carbon dioxide, instead of consumption of oxygen.

oxygen consumption of seminal fluid observed in this study, no corrections for seminal fluid were taken into consideration when the oxygen consumption of whole semen was calculated.

Survival of boar sperm during incubation. Motility estimates at hourly intervals showed a gradual decline in motility during incubation of semen samples in the Warburg apparatus (Table VII). However, the rate of oxygen consumption (Figure 1) decreased more rapidly than did motility.

Bull Semen

Oxygen consumption of bull semen. The average oxygen consumption of non-treated and treated semen during the first hour of measurement was 95.6 and 86.1 microliters of oxygen per billion sperm, respectively (Table VIII). The average oxygen consumption per hourly interval is shown in Table IX. The differences between non-treated and treated semen are not significant (Dean and Dixon, 1951). These results are in agreement with the work of Kosin (1944).

The average oxygen consumption of twelve replicates of non-treated bull semen representing five semen samples was 79.4 microliters of oxygen per billion sperm, with a range of 29.4 to 172.1, during the first hour of measurement (Table VIII). However, the rate of oxygen consumption for semen samples from three of the four bulls used in this study ranged from 29.4 to 87.6 microliters of oxygen per billion sperm during the first hour of measurement. Ely et al. (1942) reported that semen from ten dairy bulls consumed oxygen within a range of 26.7 to 308.6 microliters of oxygen per billion sperm for one hour. They stated that wide variations

TABLE VII

MOTILITY OF NON-TREATED BOAR SPERM FOLLOWING INCUBATION AT 37.5° CENTIGRADE

Animal	Ejaculate[a]	Motility at Hourly Intervals[b]					
		Initial	1	2	3	4	5
Boar 31	-	80%-3	-	60%-3	-	-	-
	-	80%-4	-	60%-3	40%-2	5%-1	0%-0
	A	60%-3	60%-3	60%-3	50%-3	50%-2	40%-2
	B	80%-3	80%-3	50%-2	50%-2	40%-2	30%-2
	C	60%-3	50%-2	-	-	-	-
	D	70%-3	-	60%-3	-	-	-

[a] The data on the first two ejaculates listed in this Table were obtained during preliminary work, hence these ejaculates are not identified by a letter.

[b] The reason that motility was not recorded in every instance is that it was not desirable to interrupt the oscillation of the samples in the Warburg apparatus.

TABLE VIII

OXYGEN CONSUMPTION OF BULL SEMEN DURING THE FIRST HOUR OF MEASUREMENT

Animal	Semen Sample	Number of Replicates	Microliters of Oxygen per Billion Sperm[a] Range Low	High	Mean	Standard Deviation
Two UT Bulls	1[b]	4	72.2 (-)[c]	77.1 (-)	74.9 (-)	2.4 (-)
Bull UT-01	2	3	33.4 (-)	39.8 (-)	35.7 (-)	3.8 (-)
UT Bull	3	2	29.4 (28.2)	31.0 (30.6)	30.2 (29.4)	1.4 (2.1)
Bull UT-00	4	1[d]	172.1 (147.9)	172.1 (157.9)	172.1 (152.9)	- (8.9)
Two UT Bulls	5[b]	2	81.2 (71.8)	87.6 (80.0)	84.4 (75.9)	5.7 (7.3)
	-[e]	5	29.4 (28.2)	172.1 (157.9)	95.6 (86.1)	71.6 (62.4)
	All[f]	12	29.4	172.1	79.4	56.9

[a] The first figures are the values for non-treated semen, while the figures in parentheses are the values for treated semen.

[b] Two pooled ejaculates.

[c] No P^{32} was added to replicates from Semen Samples 1 and 2.

[d] There was one sample of non-treated semen and two replicates of treated semen from Semen Sample 4.

[e] These data are for Semen Samples 3, 4 and 5 only.

[f] These data are for all Semen Samples.

TABLE IX

AVERAGE OXYGEN CONSUMPTION OF BULL SEMEN

Animal	Semen Sample	Number of Replicates	Microliters of Oxygen per Billion Sperm per Hourly Interval[a] 0 - 1	1 - 2	2 - 3	3 - 4	4 - 5
Two UT Bulls	1[b]	4	74.9 (-)[c]	58.7 (-)	-[d] (-)	- (-)	- (-)
Bull UT-01	2	3	35.7 (-)	44.5 (-)	32.2 (-)	33.9 (-)	- (-)
UT Bull	3	2	30.2 (29.4)	44.5 (37.3)	58.6 (37.8)	- (-)	- (-)
Bull UT-00	4	1[e]	172.1 (152.9)	86.5 (64.0)	36.4 (51.4)	29.1 (28.0)	25.5 (19.3)
Two UT Bulls	5[b]	2	84.4 (75.9)	50.4 (72.0)	42.4 (31.5)	31.2 (20.6)	- (-)
Average[f]	-	-	95.6 (86.1)	60.5 (57.7)	45.8 (40.2)	30.2 (24.3)	25.5 (19.3)
Average[g]	-	-	79.4	56.9	37.0	31.4	25.5

[a] The first figures are the values for non-treated semen, while the figures in parentheses are the values for treated semen.

[b] Two pooled ejaculates.

[c] No P^{32} was added to replicates from Semen Samples 1 and 2.

[d] The failure to obtain values for replicates during the later intervals was due to technical difficulties in the operation of the Warburg apparatus.

[e] There was one sample of non-treated semen and two replicates of treated semen from Semen Sample 4.

[f] Average of Semen Samples 3, 4 and 5 only.

[g] Average based on all Semen Samples.

occurred in the oxygen consumption of semen from different ejaculates of the same bull and are similar to variations occurring in other characteristics of different ejaculates from a given bull. That there are wide variations in the oxygen consumption of semen from different bulls is demonstrated by the work of Ghosh et al. (1949). They observed that in sixty-five semen samples from eight dairy bulls, the great majority showed oxygen consumption values between 3 and 10 microliters of oxygen per hundred million sperm per hour, while the highest value recorded was 12.5. They state that they believe those oxygen consumption values to be low for some unknown reason since semen from bulls at another breeding station during the same time had oxygen consumption values of 17 to 25 microliters of oxygen per hundred million sperm. The bulls in question also showed a "normal" rate of oxygen consumption a year previously. Lardy and Phillips (1943) observed that the oxygen consumption of nineteen samples of bull semen varied from 16.1 to 29.8 microliters of oxygen per hundred million sperm. Since Lardy and Phillips (1941) found that the rate of oxygen consumption remains constant for sperm concentrations of one hundred million to one billion sperm, it is felt that results calculated on a basis of either one hundred million or one billion sperm can be used in comparisons when results calculated on a basis of one hundred million sperm are multiplied by a factor of ten.

The minimum rate of oxygen consumption of bull semen in this study is within the range reported by Ely et al. (1942), but the maximum value recorded in this study does not approach the highest value

reported by Ely and his co-workers. The average oxygen consumption of semen in this study is below what Ghosh et al.(1949) believed to be "normal", but it is within the ranges which they report for two different groups of bulls. The highest value for oxygen consumption of bull semen in this study is approximately the same as the lowest value reported by Lardy and Phillips (1943). In view of the wide variation reported in oxygen consumption of semen from different bulls, the comparatively lower rates of oxygen consumption of bull semen in this study are probably characteristic of the bulls from which the semen was collected.

Oxygen consumption of bull seminal fluid. The rate of oxygen consumption of bull seminal fluid is shown in Table X. Seminal fluid from only one semen sample consumed oxygen, whereas, eleven replicates from four semen samples evolved an average of 15.4 microliters of an unknown gas or gases per milliliter of seminal fluid during the first hour of measurement. The absence of oxygen consumption of seminal fluid in four instances in this study is not in agreement with results reported by Ely et al. (1942). They found that seminal fluid consumed oxygen at rates varying from 3.3 to 24.0 per cent of normal semen.

Since seminal fluid in this study was demonstrated to evolve appreciable quantities of an unknown gas or gases, oxygen consumption of bull semen was corrected by adding the quantity of gas evolved by seminal fluid to the oxygen consumption of semen.

Survival of bull sperm during incubation. Motility of non-treated bull sperm did not decrease appreciably during the first hour of incubation in the Warburg apparatus (Table XI). Percentage of living sperm (non-

TABLE X

OXYGEN CONSUMPTION OF BULL SEMINAL FLUID DURING THE FIRST HOUR OF MEASUREMENT[a]

Animal	Semen Sample	Number of Replicates	Microliters of Oxygen per Milliliter			
			Range			
			Low	High	Mean	Standard Deviation
Two UT Bulls	1[b]	2	-12.9[c]	-14.9	-13.9	1.8
Bull UT-01	2	3	-17.6	-18.6	-18.3	0.6
UT Bull	3	4	-17.0	-21.3	-10.8	2.1
Bull UT-00	4	2	-17.6	-19.7	-18.7	1.9
Two UT Bulls	5[b]	2	10.6	14.4	12.5	3.4
	All	13	-17.6	14.4	-9.8	12.9
	-[d]	11	-17.6	-14.9	-15.4	3.8

[a] Bull seminal fluid samples were diluted 1:1 with M/8 phosphate buffer.

[b] Two pooled ejaculates.

[c] A minus sign indicates the evolution of an unknown gas or gases, other than carbon dioxide, instead of consumption of oxygen.

[d] These data are for Semen Samples 1, 2, 3, and 4 only.

TABLE XI

MOTILITY OF NON-TREATED BULL SPERM FOLLOWING INCUBATION AT 37.5° CENTIGRADE

Animal	Semen Sample	Motility at Hourly Intervals[a]					
		Initial	1	2	3	4	5
Two UT Bulls	1[b]	80%-4	80%-4	-	-	60%-3	10%-2
Bull UT-01	2	50%-3	40%-2	-	5%-1	0%-0	-
UT Bull	3	50%-2	50%-2	-	30%-2	-	-
Bull UT-00	4	70%-3	-	-	-	-	-
Two UT Bulls	5[b]	70%-4	-	-	-	-	-

[a] The reason that motility was not recorded in every instance is that it was not desirable to interrupt the oscillation of the samples in the Warburg apparatus.

[b] Two pooled ejaculates.

treated) at hourly intervals are shown in Table XII.

TABLE XII

PERCENTAGE OF LIVING BULL SPERM (NON-TREATED) FOLLOWING INCUBATION AT 37.5° CENTIGRADE

Animal	Semen Sample	Percentage Alive at Hourly Intervals[a]					
		Initial	1	2	3	4	5
Two UT Bulls	1[b]	88	88	-	-	89	73
Bull UT-01	2	40	33	-	39	30	33
UT Bull	3	67	67	-	53	55	46
Bull UT-00	4	81	-	67	63	54	56
Two UT Bulls	5[b]	75	-	-	-	-	-

[a] The reason that percentage alive was not recorded in every instance is that it was not desirable to interrupt the oscillation of the samples in the Warburg apparatus.

[b] Two pooled ejaculates.

SUMMARY

Under the conditions of this study, the levels of radiation used did not significantly alter the oxygen consumption of boar and bull sperm.

The average oxygen consumption of ninety replicates of non-treated boar semen representing thirteen different ejaculates from two boars was 201.5 microliters of oxygen per billion sperm during the first hour of measurement, with a range of 84.6 to 295.8. The average oxygen consumption of seventy-nine replicates of treated boar semen representing twelve different ejaculates from the same two boars was 187.4 microliters of oxygen per billion sperm during the first hour of measurement, with a range of 70.2 to 264.6.

The average oxygen consumption of seventeen replicates of boar seminal fluid representing six different ejaculates was 0.04 microliters of oxygen per milliliter of seminal fluid during the first hour of measurement. Seminal fluid from only three of the ejaculates consumed oxygen, while the seminal fluid from the other three ejaculates evolved an unknown gas or gases, other than carbon dioxide, instead of consuming oxygen.

The average oxygen consumption of twelve replicates of non-treated bull semen representing five different semen samples from four bulls was 79.4 microliters of oxygen per billion sperm during the first hour of measurement, with a range of 29.4 to 172.1. The average oxygen consumption of five replicates of non-treated bull semen representing three different semen samples from three bulls was 95.6 microliters of oxygen per billion sperm during the first hour of measurement, with a range of 29.4 to 172.1.

The average oxygen consumption of six replicates of treated bull semen representing the same three semen samples from the same three bulls was 86.1 microliters of oxygen per billion sperm during the first hour of measurement, with a range of 28.2 to 157.9.

Bull seminal fluid from only one out of five semen samples consumed oxygen, while eleven replicates representing four semen samples evolved an average of 15.4 microliters of an unknown gas or gases, other than carbon dioxide, per milliliter of seminal fluid during the first hour of measurement.

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APPENDIX

TABLE XIII

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE FIRST HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard
			Low	High	Mean	Deviation
Boar 31	A	2	120.3	154.7	137.5	30.6
	B	3	233.4	243.3	237.1	5.8
	C	4	84.6	128.6	105.4	21.6
	D	7	109.6	147.6	129.1	14.1
	E	8	275.2	295.8	284.4	7.2
	F	8	133.6	155.4	140.9	7.6
	G	8	174.9	216.9	194.8	14.7
	H	8	220.9	281.3	240.5	21.1
	All	48	84.6	295.8	191.6	62.8

TABLE XIII

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE FIRST HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			
			Low	High	Mean	Standard Deviation
Boar LPR	I	8	158.7	175.0	168.7	5.7
	J	8	216.3	235.6	230.0	6.8
	K	8	191.1	200.2	194.4	3.2
	L	8	243.7	260.6	253.6	5.9
	M	10	200.0	239.5	216.9	13.0
	All	42	158.7	260.6	212.9	29.9
Both Boars	All	90	84.6	295.8	201.5	51.1

TABLE XIV

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE FIRST HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard
			Low	High	Mean	Deviation
Boar 31	A	2	161.4	203.2	182.3	37.2
	B	3	219.5	237.8	227.6	10.8
	C	4	94.3	134.1	115.5	19.5
	D	8	70.2	100.1	87.1	10.5
	E	8	173.5	264.6	236.8	31.9
	F	8	126.2	143.4	136.6	6.0
	G	8	165.0	185.1	175.6	7.0
	H	8	212.1	249.4	233.1	13.1
	All	49	70.2	264.6	172.7	59.4

TABLE XIV

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE FIRST HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			
			Low	High	Mean	Standard Deviation
Boar LPR	I	8	152.3	166.2	159.7	4.9
	J	8	205.5	218.1	212.9	4.4
	K	-	-	-	-	-
	L	8	228.2	248.4	242.8	7.1
	M	6	230.9	245.4	236.7	5.8
	All	30	152.3	248.4	211.4	34.2
Both Boars	All	79	70.2	264.6	187.4	54.0

TABLE XV

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE SECOND HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard
			Low	High	Mean	Deviation
Boar 31	A	2	107.3	124.5	115.9	12.2
	B	3	85.8	93.0	89.7	5.1
	C	4	-5.6[a]	52.0	34.9	27.3
	D	7	45.1	61.7	54.2	6.1
	E	8	240.2	266.2	254.3	10.9
	F	8	-79.2	-46.5	-60.6	11.2
	G	8	148.8	175.3	164.6	9.4
	H	8	49.1	97.5	64.4	16.7
	All	48	-79.2	266.2	91.7	101.3

TABLE XV

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE SECOND HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard Deviation
			Low	High	Mean	
Boar LPR	I	8	20.3	44.1	29.7	7.5
	J	8	125.8	151.3	144.3	8.5
	K	8	65.9	94.0	81.6	10.1
	L	8	103.9	134.1	122.6	10.5
	M	10	125.9	149.0	139.5	8.5
	All	42	20.3	151.3	105.3	44.0
Both Boars	All	90	-79.2	266.2	98.0	79.3

[a] A minus sign indicates the evolution of a gas or gases rather than consumption of oxygen.

TABLE XVI

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE SECOND HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			
			Low	High	Mean	Standard Deviation
Boar 31	A	2	150.6	161.1	155.9	7.5
	B	3	64.3	110.0	82.7	24.1
	C	4	55.5	64.4	59.2	3.7
	D	8	29.4	45.8	36.7	6.7
	E	8	86.7	201.3	174.4	42.5
	F	8	-112.4[a]	-91.6	-104.7	7.3
	G	8	124.6	143.2	131.5	6.9
	H	8	44.3	75.6	60.7	11.4
	All	49	-112.4	201.3	65.0	91.4

TABLE XVI

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE SECOND HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard Deviation
			Low	High	Mean	
Boar LPR	I	8	13.4	44.5	33.2	9.8
	J	8	144.2	159.7	152.6	5.5
	K	-	-	-	-	-
	L	8	110.6	124.1	120.2	4.7
	M	6	132.7	157.2	143.1	9.3
	All	30	13.4	159.7	110.2	49.4
Both Boars	All	79	-112.4	201.3	82.2	80.9

[a] A minus sign indicates the evolution of a gas or gases rather than consumption of oxygen.

TABLE XVII

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE THIRD HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard Deviation
			Low	High	Mean	
Boar 31	A	2	50.5	55.3	52.9	3.4
	B	3	10.7	32.5	20.1	11.2
	C	4	17.3	44.7	27.1	12.7
	D	7	69.3	82.8	75.5	5.3
	E	8	59.7	151.1	87.7	28.0
	F	8	-9.2[a]	8.1	1.6	5.6
	G	8	40.9	71.6	60.9	9.6
	H	8	-54.3	-18.8	-40.4	12.0
	All	48	-54.3	151.1	35.0	46.9

TABLE XVII

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE THIRD HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			
			Low	High	Mean	Standard Deviation
Boar LPR	I	8	-25.2	-19.1	-22.6	2.1
	J	8	62.9	109.8	82.7	15.6
	K	8	9.0	18.1	14.3	3.9
	L	8	13.6	36.2	27.9	7.5
	M	10	19.7	38.6	29.7	6.7
	All	42	-25.2	109.8	26.5	34.4
	All	90	54.3	151.1	31.1	41.5

[a] A minus sign indicates the evolution of a gas or gases rather than consumption of oxygen.

TABLE XVIII

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE THIRD HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			
			Low	High	Mean	Standard Deviation
Boar 31	A	2	0.0	14.3	7.2	10.1
	B	3	14.8	30.3	24.5	8.5
	C	4	22.2	37.5	32.5	6.9
	D	8	69.3	77.6	74.2	3.0
	E	8	16.3	115.6	80.1	41.8
	F	8	11.8	24.2	17.5	4.8
	G	8	20.8	48.1	38.1	9.8
	H	8	-64.5[a]	-26.7	-41.0	13.4
	All	49	-64.5	115.6	32.0	44.1

TABLE XVIII

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE THIRD HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			
			Low	High	Mean	Standard Deviation
Boar LPR	I	8	-37.8	-10.4	-21.0	8.5
	J	8	122.9	145.1	134.9	7.9
	K	-	-	-	-	-
	L	8	21.5	37.1	29.3	5.4
	M	6	37.4	52.1	43.5	5.3
	All	30	-37.8	145.1	46.9	59.5
Both Boars	All	79	-64.5	145.1	37.7	50.6

[a] A minus sign indicates the evolution of a gas or gases rather than consumption of oxygen.

TABLE XIX

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE FOURTH HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard Deviation
			Low	High	Mean	
Boar 31	A	2	90.8	94.3	92.6	1.7
	B	3	10.3	46.9	31.0	18.8
	C	-	-	-	-	-
	D	7	34.7	90.3	53.0	19.7
	E	8	38.7	153.6	104.4	32.8
	F	8	21.3	32.0	27.2	3.6
	G	8	22.3	51.1	41.7	9.5
	H	8	-36.6[a]	-22.7	-29.0	5.3
	All	44	-36.6	153.6	41.0	46.0

TABLE XIX

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE FOURTH HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard Deviation
			Low	High	Mean	
Boar LPR	I	8	-51.2	-41.9	-46.5	3.5
	J	8	8.6	47.9	20.5	12.0
	K	8	-1.2	2.4	1.2	1.3
	L	8	-5.2	-3.3	-4.6	0.6
	M	10	2.4	9.1	7.4	2.0
	All	42	-51.2	47.9	4.2	19.1
Both Boars	All	86	-51.2	153.6	23.0	40.8

[a] A minus sign indicates the evolution of a gas or gases rather than consumption of oxygen.

TABLE XX

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE FOURTH HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard Deviation
			Low	High	Mean	
Boar 31	A	2	14.0	21.5	17.8	5.3
	B	3	7.6	11.1	9.8	1.9
	C	-	-	-	-	-
	D	8	36.7	55.4	46.6	6.1
	E	8	-43.4[a]	112.8	56.3	61.0
	F	8	42.5	59.1	50.7	4.7
	G	8	28.6	87.3	41.8	19.1
	H	8	-37.4	-15.8	-24.9	7.4
	All	45	-43.4	112.8	31.7	39.1

TABLE XX

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE FOURTH HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			
			Low	High	Mean	Standard Deviation
Boar LPR	I	8	-79.7	-41.0	-61.3	12.8
	J	8	79.8	103.5	90.8	8.6
	K	-	-	-	-	-
	L	8	-6.5	-1.7	-4.9	1.7
	M	6	6.6	12.5	7.1	2.2
	All	30	-79.7	103.5	8.5	51.5
Both Bears	All	75	-79.7	112.8	22.4	48.3

[a] A minus sign indicates the evolution of a gas or gases rather than consumption of oxygen.

TABLE XXI

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE FIFTH HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard Deviation
			Low	High	Mean	
Boar 31	A	2	53.8	74.8	64.3	14.9
	B	3	25.1	36.1	29.6	5.8
	C	-	-	-	-	-
	D	7	7.6	36.0	17.6	12.5
	E	8	64.1	95.4	80.1	12.4
	F	-	-	-	-	-
	G	8	-7.4[a]	20.5	6.6	10.2
	H	8	-22.9	-12.2	-18.1	3.4
	All	36	-22.9	95.4	24.7	36.9

TABLE XXI

OXYGEN CONSUMPTION OF NON-TREATED BOAR SEMEN DURING THE FIFTH HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard Deviation
			Low	High	Mean	
Boar LPR	I	8	-53.1	-41.6	-47.7	4.0
	J	8	4.6	10.8	8.3	1.8
	K	-	-	-	-	-
	L	8	-6.8	-4.5	-5.4	0.9
	M	10	-11.9	-5.4	-8.0	2.4
	All	34	-53.1	10.8	-12.9	20.7
Both Boars	All	70	-53.1	95.4	6.4	35.6

[a] A minus sign indicates the evolution of a gas or gases rather than consumption of oxygen.

TABLE XXII

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE FIFTH HOUR OF MEASUREMENT

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			Standard Deviation
			Low	High	Mean	
Boar 31	A	2	17.9	21.0	19.5	2.2
	B	3	11.1	31.9	21.9	10.4
	C	-	-	-	-	-
	D	8	8.0	27.9	16.8	7.2
	E	8	-4.1[a]	67.7	36.7	32.3
	F	-	-	-	-	-
	G	8	-11.0	7.0	-6.7	6.1
	H	8	-23.6	-7.6	-15.5	5.8
	All	37	-23.6	67.7	9.6	25.0

TABLE XXII

OXYGEN CONSUMPTION OF TREATED BOAR SEMEN DURING THE FIFTH HOUR OF MEASUREMENT (Continued)

Animal	Ejaculate	Number of Replicates	Microliters of Oxygen per Billion Sperm			
			Range			
			Low	High	Mean	Standard Deviation
Boar LPR	I	8	-33.6	-24.2	-27.6	3.3
	J	8	40.0	46.4	43.6	2.4
	K	-	-	-	-	-
	L	8	-9.9	-6.3	-8.4	1.0
	M	6	-10.4	-6.1	-8.7	1.5
	All	30	-33.6	46.4	0.3	27.8
Both Boars	All	67	-33.6	67.7	5.4	26.5

[a] A minus sign indicates the evolution of a gas or gases rather than consumption of oxygen.

TABLE XXIII

ANALYSIS OF VARIANCE: OXYGEN CONSUMPTION OF BOAR SEMEN

Interval	Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F Value
First Hour	Between Treatments	1	8,308	8,308	0.240
	Between Boars	1	37,677	37,677	1.080
	Among Ejaculates Within Boars	11	383,953	34,905	
	Within Ejaculates	155	38,024	245	
	Total	168	467,962		
Second Hour	Between Treatments	1	10,577	10,577	0.120
	Between Boars	1	35,064	35,064	0.400
	Among Ejaculates Within Boars	11	976,075	88,734	
	Within Ejaculates	155	58,804	379	
	Total	168	1,080,520		

TABLE XXIII

ANALYSIS OF VARIANCE: OXYGEN CONSUMPTION OF BOAR SEMEN (Continued)

Interval	Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F Value
Third Hour	Between Treatments	1	1,833	1,833	0.060
	Between Boars	1	96	96	0.003
	Among Ejaculates Within Boars	11	311,198	28,291	
	Within Ejaculates	155	42,282	273	
	Total	168	355,409		
Fourth Hour	Between Treatments	1	523	523	0.030
	Between Boars	1	36,641	36,641	1.870
	Among Ejaculates Within Boars	10	195,923	19,592	
	Within Ejaculates	148	82,000	554	
	Total	160	315,087		

TABLE XXIII

ANALYSIS OF VARIANCE: OXYGEN CONSUMPTION OF BOAR SEMEN (Continued)

Interval	Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F Value
Fifth Hour	Between Treatments	1	34	34	0.003
	Between Boars	1	19,268	19,268	1.800
	Among Ejaculates Within Boars	8	85,607	10,700	
	Within Ejaculates	126	28,761	228	
	Total	136	133,670		