Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 22, Number 2, April, 1949.

Printed in the Union of South Africa by the Government Printer, Pretoria.

The Sperm Picture of Rams of Different Breeds as an Indication of their Fertility. II.—The Rate of Sperm Travel in the Genital Tract of the Ewe.

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CONTENTS.

Received for publication on 20th August, 1946.—Editor.

1. Introduction.

The increasing incidence of infertility in domestic animals in South Africa has shown the necessity for research in this field. Quinlan (1929) studied the causes of infertility in cattle and came to the conclusion that genital infection was the chief aetiological factor in female infertility. At the same time he pointed out that there were many cases unexplained by genital examination. These were classed as functional infertility, either nutritional or hormonal. Quinlan and co-workers (1931, 1932, 1933, 1936, 1941) felt that their pathological investigations needed amplification by a study of the sex physiology of the male and female domestic animals under the environmental conditions prevailing in South Africa. There were many cases of infertility which could not be explained by pathological lesions in the genital tract. Consequently, it was felt that a study of the semen of animals was needed. Eighteen rams were put at the author's disposal for this investigation.

This opportunity was welcomed because during an extended period in the field service the author undertook many investigations into the causes of breeding failures of stock, and realised that such examinations were unsatisfactory without a thorough understanding of the different semen characteristics and their relation to good, indifferent, and poor fertility. The limitations of a clinical examination alone of the male genitalia were repeatedly demonstrated in practice since pathological conditions in infertile animals were frequently not revealed clinically. In fact, Quinlan, Mare, and Roux (1932) showed that when clinically normal rams were mated to clinically normal ewes, the breeding results differed considerably. The percentages of ewes fertilized by thirteen such rams varied from 26·1 to 100. Nor could such desirable characteristics as keen mating desire and vigour of service be regarded as evidence of sexual soundness, because totally infertile animals have been noticed to exhibit normal libido.

It has been shown by workers in other countries and also in South Africa, that change in environment may produce a marked decrease in fertility of rams. Consequently, this suggested a study of the semen of rams under the seasonal environmental conditions prevailing at Onderstepoort, and the experiment was planned so that observations on spermatogenic activity could be made during the different seasons. The necessity for such observations is evident when it is realised that climatic conditions vary considerably in different parts of South Africa.

Concurrently with this study the environmental temperature was studied. The body temperature, respirations and pulse counts, as well as the skin, scrotal and intratesticular temperatures were recorded. These latter observations will be the subject of a separate paper, which will be published at a later date.

PART I.

II. Materials and Methods.

Eighteen rams were available for this work. They comprised four Merinos, five Ronderib Afrikaners, two Romney Marshes, two Dorset Horns, one Blackhead Persian, one Karakul, two Welsh Mountain-Ronderib Afrikaners, and one Southdown-Blackhead Persian. The Merinos were kept in a paddock 75 feet by 45 feet, in which was a building that could be entered and left as they chose. The remainder were in a paddock of approximately the same size. This paddock was surrounded by poplar trees which provided good shade in summer and very little protection in winter when their leaves had dropped. The rams were invariably to be seen in the shade during the hot weather.

The grain ration per ram per day was as follows:—

	Protein.
½ lb. crushed yellow maize	0·08
¼ lb. crushed oats	0·04
¼ lb. crushed palm kernal	0·10
	0·22

In addition to the grain mixture they received lucerne hay, green feed when available and veld hay. Water was available *ad lib.*

Preliminary semen collections were made by means of the artificial vagina, but the Ronderib Afrikaners were so nervous and unmanageable that they could not be induced to mount a ewe in the presence of a human being. Electrical stimulation was therefore resorted to for all the rams, and proved very successful. Merino ewes were used for the breeding tests. They were tested for oestrus by means of vasectomised teasers. The Ronderib Afrikaners, except No. 62418, did not respond to careful and patient handling and refused to serve. They were ejaculated electrically and their semen artificially inseminated into the ewes.

The rams were divided into four groups for convenience, two groups with four each and two with five. Semen was collected from each group at fortnightly intervals. Graduated test-tubes were found to be very convenient for the collections. The vermiform appendix and part of the

glans being inserted into the opening of the tube before ejaculation. The semen volume could thus be read off direct. When sufficient experience had been gained, it was possible to ejaculate four rams in approximately twenty-four minutes.

The colour of the semen was noted and each sample was examined for motility without delay; usually within thirty minutes of collection. The sperm concentration (number per cubic millimetre) was determined by making counts over a Bürker counting chamber. Semen smears were prepared and stained for morphological study of the spermatozoa, which were classified according to the particular abnormal type.

Portions of ejaculates were stored at approximately 2° C. in order to determine how long the spermatozoa would remain viable. This part of the experiment could not be carried to conclusion, however, because of repeated failure of the electric current.

III. Collection of Semen.

Semen can be collected from the female genitalia after natural copulation or by artificial means. Some of the older methods are obsolete and have been replaced by better ones. Lambert and McKenzie (1940) have described and discussed most of the methods that have been used in the past as well as those that are now used for the different species of animals.

In the sheep three methods are possible, viz., recovery of semen from the vagina of the ewe after natural copulation, from ejaculation into an artificial vagina, and by means of electrical stimulation.

With trained rams ejaculation into the artificial vagina probably approaches the natural process very closely, and the various semen characteristics can be regarded as representative of the semen of the particular ram. For reasons already explained, this method could not be used for all the rams, although it was used for preliminary collections from the Merinos. Semen recovered from the vagina is mixed with secretions from that organ, with the result that sperm counts would have been difficult to make and the results would have been extremely inaccurate. Moreover, a different ewe would have had to be used for each ram.

It was therefore decided to produce seminal ejaculation artificially by electrical stimulation as devised by Gunn (1936). This method proved very satisfactory, giving clean, uncontaminated semen suitable for all the examinations required. The source of the electrical current was the 50 to 60 cycle 250 voltage alternating current of Pretoria. The current was reduced in voltage by a transformer and passed through a voltmeter and a milliammeter before being applied to the animal (Fig. 1.). The electrode for the rectal pole was a brass rod 9 inches long and 9 millimetres in diameter at its free end. The rest of the rod was turned down to about 7 millimetres and insulated with rubber tubing. A wooden handle carrying one lead was screwed into the brass rod (Fig. 2A). For the anterior electrode, Gunn used a stout needle soldered to the other insulated wire lead (Fig. 2C). Such a needle was used in the beginning of this work and gave good results, but it was soon replaced by an electrode consisting of a zinc disc about $\frac{3}{4}$ inch in diameter and covered with calico (Fig. 2B).

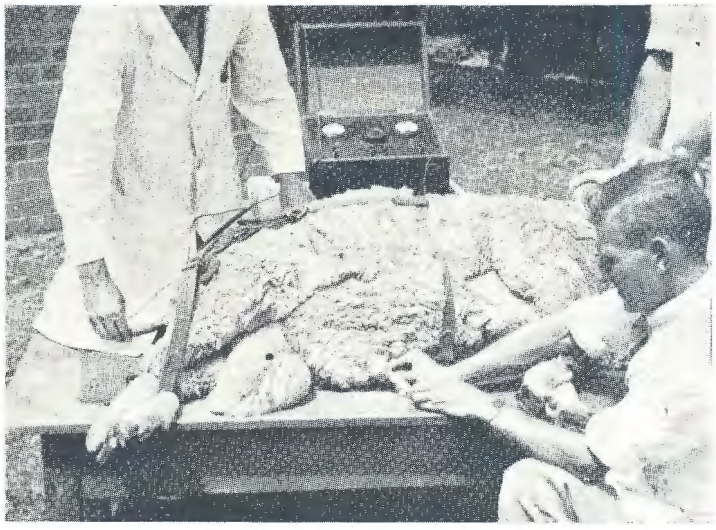

FIG. 1.—Collection of semen by means of electrical ejaculation.

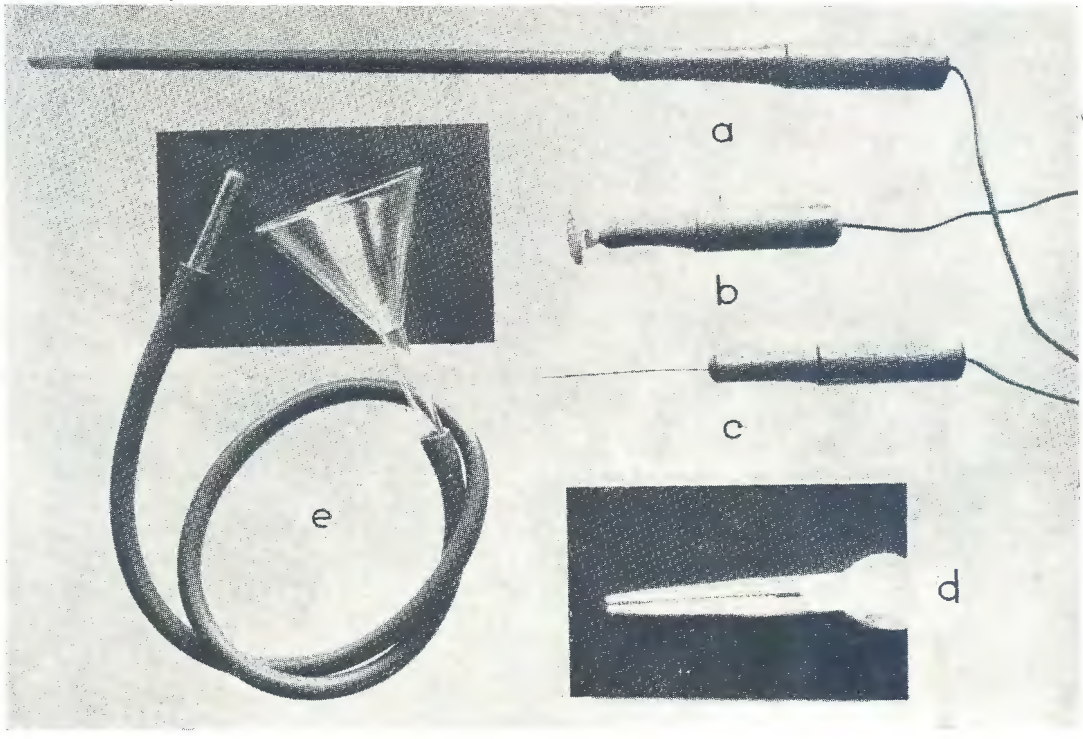

FIG. 2.—(*a*) Posterior electrode (rectal pole).
(*b*) Anterior electrode—zinc disc type, (anterior pole).
(*c*) Anterior electrode—needle type (anterior pole).
(*d*) Graduated test-tube.
(*e*) Funnel and rubber tubing for giving enema.

1. *Restraint.*

A low table, 55 by 27 by 22 inches, is convenient to work on. Upon this table the ram was placed on its left side and secured in the extended position as recommended by Gunn (1936), because the general body reaction is towards extension. Straps bolted to the table secure the neck, legs, thorax, and hindquarters (Fig. 1). Injury to the lumbar region, especially in heavy rams, may be caused if the hindquarters are forcibly raised during the process of lifting the ram onto the table.

2. *Technique.*

The rams to be ejaculated were tethered in the shed. A saline enema, approximately 150 c.c., was given through a funnel and rubber tubing. The enema facilitated evacuation of the rectum and ensured better contact of the rectal pole. A small area on the right side in the region of the third and fourth lumbar vertebrae, and midway between the spine and free border of the lumbar transverse processes was clipped short, and cleaned if necessary. Foreign matter adherent to the hair and wool around the opening of the sheath was clipped away and the opening swabbed with moist cotton wool. To protrude the penis, it was grasped well back with the left hand and pushed forward at the same time as the sheath was pushed back with the right hand. The protruding penis was secured by wrapping a strip of broad tape round it behind the glans.

When the needle was used as the anterior pole it was inserted into the longissimus dorsi muscle opposite the fourth lumbar vertebra.

In making the collection the vermiform process and part of the glans were introduced into the opening of the graduated test tube in order to ensure collection of the whole ejaculate.

The rectal pole was now inserted carefully into the rectum for about eight inches. The anterior pole was pressed firmly over the longissimus dorsi muscle at the site previously clipped opposite about the fourth lumbar vertebra. The current was switched on and slowly adjusted to 30 volts. This stimulation was maintained for 10 to 15 seconds and the anterior pole disengaged. The rectal pole was withdrawn slightly and the current adjusted to 20 volts. The anterior pole was applied suddenly, but this time for about two seconds only. Ejaculation sometimes occurred after the first stimulation, but usually took place immediately the second short stimulus was applied; if not, then the anterior pole was removed for a few seconds and again applied. After the fairly long initial stimulation, ejaculation could often be induced by merely tapping the site over the fourth lumbar vertebra with the tips of the fingers. At the moment of emission the tip of the vermiform appendix curled round onto its base and then whipped round spraying semen a considerable distance with great force.

A weak electric current due to leakage or faulty contact might give a thin watery semen. A disadvantage of the metal disc used for the anterior pole is that it must be renewed from time to time because of corrosion. Retardation of ejaculation due to central inhibition as experienced by Gunn (1936) was also met with. This was mainly overcome by applying the stimulus unexpectedly. Some rams occasionally showed slight inability to

extend their hind legs immediately on release, but this soon passed off. The eighteen rams were ejaculated for fourteen months and none suffered any ill effects.

IV. Preparation of Smears.

Well and carefully prepared semen smears with the spermatozoa distributed as evenly as possible, save time in examination and enable the worker, when he has gained sufficient experience, to obtain remarkably uniform counts of abnormal spermatozoa. It is much easier to make good smears from clean semen as obtained, for example, in the case of the ram, by electrical ejaculation or with the artificial vagina, than when semen is taken from the vagina after natural copulation. Many different methods of making smears were tried, including drawing semen over the surface of a slide with the edge of another, blowing it over the slide, pouring undiluted semen and semen diluted with physiological saline, onto the slide and allowing it to run off; but finally the following method was adopted: A drop of semen was placed on a slide, the slide grasped at one end between the thumb and forefinger of the left hand and the second slide laid on the drop at right angles to the first. The drop was allowed to spread and without delay the top slide was drawn along the bottom one smoothly and without any added pressure. The top slide was used except when the semen was very thin.

When the sperm concentration approached three million per cubic millimetre and over, it was better to dilute the semen. In order to obtain a uniform mixture of semen and diluent, the test-tube was rolled between the palms of the hands. The smears were air-dried. Salisbury, Willett and Seligman (1942) statistically analysed the influence of different methods of making semen smears on the proportion of abnormal spermatozoa in an ejaculate of the bull. They showed that the method used in making semen smears had a significant effect upon certain of their classifications of abnormal spermatozoon types. Thus they found that when 0·01 c.c. of semen was added to 2·0 c.c. of dilutor [Milovanov's (1933) S.G. C_2 dilutor] in a small test-tube and poured gently on to a slide and allowed to dry, the counts of the "true abnormals" (types possessing any abnormality with respect to the shape and size of the head, spermatozoa with enlarged abaxial, beaded or filiform middle pieces and those with thickened or double tails) were significantly higher. They suggest that this fact may be due to mechanical removal from the slide of these abnormal types by the other methods they employed. Again, when the semen smear was made by gently spreading a small drop of diluted semen over the slide with a dry, fine camel's hair brush, an unduly high proportion of tailless heads and a significantly lower proportion of coiled tails were produced. These results, the authors suggest, are probably due to mechanical effects of the brush. They conclude that it would appear that the most satisfactory method to be used in the preparation of semen smears, is that in which a drop of diluted semen is spread over a clean slide by simply placing another clean slide face down on it, and pulling the two apart lengthways.

Gunn, Sanders and Granger (1942) came to the conclusion that the histological finding was not affected to any marked extent when smears were prepared by running semen diluted with physiological normal saline over the slide, blowing undiluted semen over the slide or even by smearing as for a blood smear (Williams and Savage, 1926), that is, with the edge of a slide held at an angle of 45°.

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In the case of good quality semen the experienced worker should have little difficulty in distinguishing between pathologically abnormal spermatozoa and those which have been mechanically damaged in the process of making the smear. In poor quality semen it is more difficult because spermatozoa which are undergoing a process of degeneration are very easily damaged in spite of due care.

V. Staining Semen Smears.

For morphological study of spermatozoa staining is necessary and a staining technique which gives a clear, sharp delineation of the outline of the spermatozoa together with good differentiation of the different parts of the cell should be used. After experimenting with various stains that have been recommended, the technique introduced by W. W. Williams (1920) was used throughout the work. It is not so simple as some other staining methods; in fact, a good deal of practice is necessary to obtain good results. It is a double stain and is made up as follows:—

Stain 1.

Saturated alcoholic solution of eosin (bluish) 1 part.

Ziehl-Nielsen carbol-fuchsin, 2 parts.

Alcohol 95 per cent., 1 part.

Mix the eosin and carbol-fuchsin, filter and add the alcohol to the filtrate. The stain is rather unstable and loses its efficiency after 2 or 3 days.

Stain 2.

Loeffler's methylene blue, 1 part.

Distilled water, 4 parts.

Procedure.

Immerse the smear in 0·5 per cent. solution of chlorazene for 5 to 7 minutes to dissolve any mucus, wash gently with water and then dip into 95 per cent. alcohol.

Stain 1 is applied for $3\frac{1}{2}$ to 4 minutes. To prevent precipitation during the process add more stain from time to time. Wash in water and counterstain with Stain 2 for 3 seconds. Wash and dry.

This staining method is endorsed by Webster (1932), and by Lagerlof (1934). Lambert and McKenzie (1940) recommend either of the following methods: (1) Dried smears are stained for 3 minutes with 0·5 per cent. alcohol solution of gentian violet; (2) air-dried slides are put in a saturated solution of chlorazene for 5 to 10 minutes, rinsed in distilled water, fixed in 10 per cent. formalin for 3 to 5 minutes rinsed in distilled water, stained with Ziehl's carbol-fuchsin for 2 minutes, washed gently in running tap water and dried.

Swanson and Herman (1941) stained bull semen smears for 5 to 10 minutes with 3 per cent. aqueous Rose Bengal solution. Gunn, Sanders and Granger (1942) had good results with the stain introduced by Williams (1920), but found that the method required great care to prevent precipitation of the stain and that it may cause distortion of the sperm necks and

tails. For rapid results in the field they used a mixed stain consisting of 7 parts of stock solution of carbol-fuchsin and 1 part saturated alcoholic solution of eosin. They had uniformly satisfactory results with a modification of the method of Cary and Hotchkiss (1935). By this method smears are fixed with methyl alcohol for 2 minutes, washed in water, stained with an acidified solution of Mayer's haemalum for 6 minutes, washed in water for 3 minutes, and then counterstained with an acidified saturated alcoholic solution of eosin for 2 minutes and washed in water.

Salisbury, Willett and Seligman (1942) cleared the smears by immersion in a 1·0 per cent. solution of chlorazene for 5 minutes, rinsed in tap water and dried rapidly under an electric fan. The smears were then stained for 45 seconds in Ziehl's carbol-fuchsin, washed thoroughly in tap water, dried under the fan, counterstained for 45 seconds in aniline gentian violet, washed in tap water and dried. They claim that compared with other staining methods and stains, this procedure is the easiest as well as giving the best delineation of cellular outline.

To demonstrate middle piece heads, smears must be carefully prepared and gently handled during the staining process to avoid damage (Figs. 11 and 12). They stand out clearly in negative preparations made with India ink (Fig. 13). The semen smear was prepared in the same way as for ordinary staining, air-dried and then diluted India ink was poured over it. The ink was poured off after a few seconds and the slide stood on its end to drain and dry. The background is brownish black and the spermatozoa show up as negative figures. Lagerlöf (1934) used opal blue (Bresslau) to demonstrate these bodies with excellent results.

With the object of determining the relative number of live and dead spermatozoa in semen specimens, Lasley, Easley, and McKenzie (1942) developed a new staining technique. They had observed that several stains would enter certain spermatozoa and that others remained unstained. The sperm in which the stain entered were non-motile before and after staining. The following mixture gave the best results:—

Stain A.

2 per cent. water solution eosin in M/8 phosphate buffer (pH 7·3).

Stain B.

1 part opal blue (undiluted)
1 part H/8 phosphate buffer (pH 7·4) } pH 5·7

[Phosphate buffer (pH 7·4): 80·4 c.c. M/8 Na_2HPO_4 and 19·6 c.c. M/8 KH_2PO_4].

For the staining mixture one part of A and one part of B were used. Slides were made by placing a drop of stain on a clean glass slide and mixing a little semen with it by means of a glass rod, and then drawing the smear with the flat surface of another slide. The posterior portion of the sperm head stains almost red to dark purple and the anterior part a light pink or not at all. The unstained cells show up in clear outline against a light blue background.

In order to eliminate the effects of changes in environment, the authors recommend that both the stain and the semen should be brought to a definite temperature between 20-30° C. and that the smears should be prepared at

this temperature. It is claimed " that this new staining technique stains dead sperm or sperm which have reached a state of irreversible inactivation and fails to stain live (active or potentially active) sperm ".

VI. Semen Characteristics.

1. *The Ejaculum.*

According to Gunn (1936) the typical ejaculum produced by electrical stimulation consists of a small amount of clear watery material which is believed to be essentially prostatic secretion; then 1 to 2 c.c. of thick material, creamy in colour and consistency containing the majority of spermatozoa; thereafter a certain amount of watery secretion from the accessory sex glands. He noticed that usually a greater total quantity is ejaculated during hot days and hot seasons than on cold days and during cold seasons. Gunn states further that from the data obtained from a number of adult rams submitted to stimulation daily or every two days for long periods, and from the examination of about one hundred stained specimens of the ejacula, no evidence has been found of any changes in the quantity of the ejaculum, the number of spermatozoa ejaculated, or in the motility, morphology or staining reactions of the spermatozoa. He concludes that even very large numbers of services by rams are unlikely to lead directly to sterility.

Normal ram semen is creamy or greyish white in colour with a creamy consistency. With low concentrations the appearance is milky to watery. In the case of the ram No. 62542, the semen was always flocculated; motility was invariably poor and on microscopical examination the spermatozoa were " clumped ". Neutrophiles in large numbers were present in every ejaculum. Lemon-yellow coloured semen, due to admixture of urine, was encountered on occasions. The initial motility of such discoloured samples was usually only fairly good to poor. A faint pink colour resulting from the presence of fresh blood did not affect the semen, but when it was a dark brown the motility was often adversely affected and the number of abnormal spermatozoa was increased. The presence of blood in the semen was due to the fact that some of the rams were at the same time in another experiment in which intratesticular temperatures were taken every fourteen days. A sterile needle containing a thermo-couple was inserted into the parenchyma of the testicle. Intratesticular haemorrhage was occasionally produced, but no abscessation was experienced. Although the rams suffered no other ill effects from the use of the thermo-couple, it was perhaps unfortunate that they had to be used in the concurrent experiment on bio-climatology.

Walton (1933) ascribes a pinkish or reddish colour in semen to an admixture of fresh blood derived mostly from injuries to the vaginal mucosa of the female from which the collection was made; and a brown or reddish-brown colour to products of degeneration of blood and tissues, indicating a degenerative process in the genital organs of the sire.

Greenish or distinctly yellowish coloured semen which indicates the pressure of pus was not met with in this work.

2. *Semen Volume.*

The semen volumes were read off directly on graduated test-tubes into which the ejaculations were made. The data show that in general, the volume per ejaculate of individual rams obtained by electrical stimulation varies remarkably little. A significance test revealed that on the average no significance could be attributed to the differences. Individual rams appear to have their own volume threshold.

The lowest individual average was 0·8 c.c. emitted by four rams among which was the Blackhead Persian, which gave the highest average concentration. The highest average volume per ejaculate was obtained from the two exogenous rams, the Dorset Horn 56956 and the Romney Marsh 56947, with 1·2 c.c. each (Dorset Horn ram 62548 and the Romney Marsh 56939 died before the completion of the experiment and are not included in the averages).

The Merinos as a group average 0·9 c.c. and the Ronderib Afrikaners 1·0 c.c. The average semen volume for all the rams, except the two that died, was 0·9 c.c. Gunn (1936) obtained an average volume of 1·5 c.c., from an average application of 18 stimuli per occasion. Lambert and McKenzie (1940) record a volume of 0·8 c.c. as the most common for the ram.

Swanson and Herman (1941) found a close relationship between the variation in average volume of the ejaculates among the different bulls and the relative size of the bulls within the breed. In boars, McKenzie, Miller, and Bauguess (1938) did not find a direct relationship between liveweight and volume of semen per ejaculate. Over respective collection periods the smallest boar produced the greatest volume on the hardest collection schedule, and the two largest boars the smallest volumes. Fewer collections per unit of time were made from the two largest boars than from the smallest one.

The largest ram in the experiment, Dorset Horn 56956, shared the highest average volume with the Romney Marsh 56947, but the lighest ram, Karakul 62544, and the smallest, Southdown-Blackhead Persian 62545, were well up to the average of the other rams (Table 1). There was no definite correlation between the volume of semen emitted at different ejaculations and the sperm concentration. This was so for all the rams. McKenzie and Berliner (1937) state that a decrease in volume was not always accompanied by a decrease in concentration, and that ejaculations with the smallest volume did not always contain the smallest number of spermatozoa.

3. *Sperm Concentration.*

The number of spermatozoa per cubic millimeter of semen was determined by means of the Bürker haemocytometer. A dilution of one thousand times in a 2 per cent. KOH solution was used except when the semen was very thin; then it was diluted one hundred times.

The dilution was made as follows:—

1. From a burette graduated to 0·1 c.c., run 9·9 c.c. KOH solution into a small flask.

TABLE 1.

Semen Volumes (c.c.).

Ram.	Oct.	Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Average.
MERINO RAMS.															
45106	0·6	1·4	0·6	1·0	0·9	1·0	0·7	0·7	0·7	0·8	0·8	0·7	0·8	0·9	0·8
45307	0·8	0·7	0·6	1·0	1·4	1·1	0·5	0·9	0·9	1·0	0·9	1·3	1·0	1·0	0·9
50549	1·3	0·7	1·0	0·9	0·8	1·0	0·7	0·7	0·7	0·8	0·9	0·7	1·0	0·6	0·8
50735	1·5	1·4	1·3	1·0	1·0	1·2	1·0	0·9	1·0	1·2	0·8	1·0	0·7	1·0	1·1
Average	1·1	1·1	0·9	1·0	1·0	1·1	0·7	0·8	0·8	1·0	0·9	0·9	0·8	0·8	0·9
ROMNEY MARSH RAMS.															
*56939	0·6	0·6	0·6	1·1	0·7	1·1	0·8	0·9	0·8	0·7	0·3	†	—	—	—
56947	1·1	1·5	1·6	0·9	1·4	1·3	1·0	1·1	1·4	0·9	1·0	1·2	0·9	1·2	1·2
BLACKHEAD PERSIAN RAM.															
62030	0·9	1·0	0·8	1·1	0·8	1·0	0·7	0·8	0·6	1·0	0·8	0·9	0·8	0·6	0·8
DORSET HORN RAMS.															
56956	1·4	0·8	1·1	1·7	1·2	1·2	0·3	1·0	1·1	1·4	1·4	1·9	0·9	1·0	1·2
*62548	1·1	0·6	1·0	1·5	1·4	1·0	0·7	1·0	1·1	†	—	—	—	—	—
RONDERIB AFRIKANER RAMS.															
62418	0·4	0·7	1·4	0·6	1·3	1·3	0·8	1·3	1·0	0·8	0·9	0·9	0·8	0·8	0·9
62546	1·7	0·7	1·8	1·0	1·2	1·2	1·3	0·9	0·9	1·1	1·1	1·0	0·9	0·8	1·1
62419	1·3	1·0	0·6	1·3	1·2	0·9	0·8	1·1	1·4	0·7	1·5	0·9	0·9	1·2	1·1
62549	0·8	0·8	1·4	1·0	1·5	1·1	0·8	1·0	1·3	1·1	0·8	0·8	0·8	0·6	1·0
62543	0·6	0·7	0·9	0·8	1·1	0·8	1·1	0·7	0·8	0·8	0·9	0·9	0·6	0·6	0·8
Average	1·0	0·8	1·2	0·9	1·3	1·1	1·0	1·0	1·1	0·9	1·0	0·9	0·8	0·8	1·0
WELSH MOUNTAIN—RONDERIB AFRIKANER RAMS.															
62541	0·7	0·8	1·0	0·9	1·0	1·1	0·8	1·0	1·0	1·2	0·6	0·7	0·5	0·6	0·9
62542	1·0	0·9	0·9	1·2	1·0	1·2	0·9	1·2	1·1	1·2	0·9	1·8	0·9	1·0	1·1
Average	0·9	0·9	1·0	1·1	1·0	1·2	0·9	1·1	1·1	1·2	0·8	1·3	0·7	0·8	1·0
SOUTHDOWN—BLACKHEAD PERSIAN RAM.															
62545	0·7	0·8	0·9	0·9	0·7	1·2	1·0	0·8	1·0	1·9	0·8	0·9	0·8	0·9	1·0
KARAKUL RAM.															
62544	—	1·0	0·5	0·8	0·9	0·9	0·9	0·7	0·9	0·9	0·8	0·9	0·8	1·4	0·9
Grand Average	1·0	0·9	0·7	1·0	1·1	1·1	0·8	0·9	1·0	1·1	0·9	1·0	0·8	0·9	0·9

* Not included in averages. † Died.

2. To this 9·9 c.c. KOH solution add 0·1 c.c. semen. A 1 c.c. pipette graduated to 0·01 c.c. was used.

3. This gives a dilution of 100 times.

To 9 c.c. KOH solution in another flask add 1 c.c. of the 1/100 semen solution.

5. This gives a dilution of one thousand times.

Before making the dilution the semen in the test-tube was well stirred up by rolling the test-tube between the palms of the hands. If the semen is shaken in the ordinary way air bubbles may become included in it and drawn up into the pipette. A different pipette was used for each dilution and for each ram. After use the pipettes were cleaned with a potassium bichromate sulphuric acid cleaning solution.

(a) The Counting.

The diluted semen was well mixed and a small drop from a finely drawn pipette was run between the coverslip and the counting slide. The drop should not be so large that it will flow over into the grooves round the counting slide. The desired size of drop can be gauged with a little practice. To ensure an even spread of fluid the counting slide and the coverslip must be absolutely clean and free from foreign particles. Eight to ten minutes should be allowed for the spermatozoa to settle before counting is commenced. Counting was done under a magnification of 200 times.

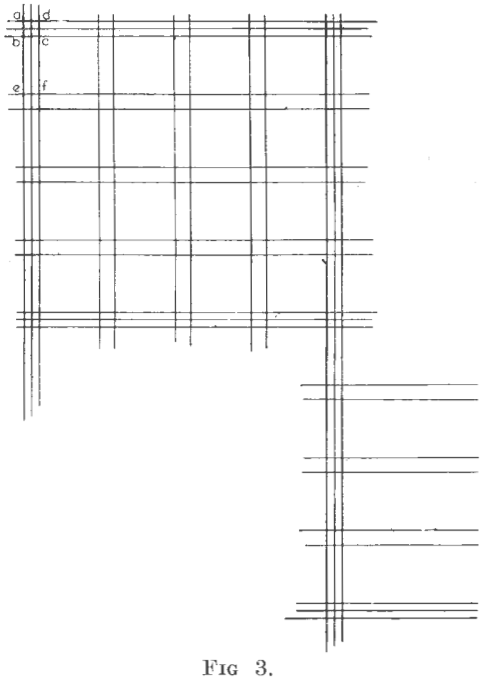


FIG 3.

Section of Bürker counting chamber, showing the ruling.

(*b*) *Calculation.*

Depth of counting chamber = 1/10 mm.
Square a,b,c,d (Fig. 3) = 1/20 × 1/20 = 1/400 sq. mm.
Volume of square a,b,c,d = 1/400 × 1/10 = 1/4000 cub. mm.

The rectangle a,e,f,d, was used as a unit for counting and represents 5 squares, of 5 × 1/4000 = 1/800 cub. mm.

Starting at the top left hand corner (Fig 3), four rectangles were counted downwards, then four to the right, again four down and so on to the right hand bottom corner of the counting chamber. Twenty-four rectangles were counted. Two separate counts were made of each sample and averaged.

Spermatozoa lying inside the rectangle a,e,f,d, and those touching the side d,a,e, were counted, but not those touching the side d,f,e even if inside the rectangle.

Number of spermatozoa in 24 rectangles = S
i.e., number of spermatozoa in 24 × 1/800 cub. mm. = S

$$\text{Number of spermatozoa per cub. mm.} = S \times \frac{800}{24} \times \text{dilution}$$

Example: Number of spermatozoa counted = 60

Dilution is 1,000 times.

$$\text{Number of spermatozoa per cub. mm.} = 60 \times \frac{800}{24} \times 1000$$
$$= 60 \times \frac{100{,}000}{3}$$
$$= 2{,}000{,}000.$$

Making sperm counts is a laborious procedure, and finding that it interfered with their other observations on the semen, Gunn, Sanders, and Granger (1942) evolved a simpler method of estimating the approximate number of spermatozoa in a semen sample, and adopted the following scale:—

Type of Semen.	*Approximate number of Spermatozoa in hundreds of millions per c.c.*
Very thick creamy	30
Thick creamy	25
Creamy	20
Thin creamy	15
Thick milky	10
Milky	5
Cloudy	1
Less than cloudy	insignificant.

These authors consider that, provided the ejaculations do not take place at less than twenty-four hour intervals, approximately the same number of spermatozoa are ejaculated by any given ram in good health on successive occasions. Russian workers (Kusnezov, 1934, Moskovits, 1934) quoted by

Gunn, Sanders and Granger (1942), found that for high fertility in the ram in artificial insemination a minimum of 50 million spermatozoa are required. Macomber and Sanders (1929) working with human semen concluded that "the total number in an ejaculation is of less significance in relation to fertility than the concentration". They found that there was markedly more variation in head lengths in sperms of low concentration.

Although rams of good fertility tend to produce semen with a satisfactory concentration there may, nevertheless, be a considerable difference in concentration in different ejacula of individual rams. Ram 62545, which gave a very high breeding test (95 per cent. pregnancies) had a range of 0·9 to 5·8 million spermatozoa per cubic millimetre with an average of 2·7; and the Ronderib Afrikaner ram 62543 whose highest abnormality count was 42 per 1,000, ranged from 0·5 to 6·9 million spermatozoa per cubic millimetre.

Gunn (1936) points out that in healthy, normal rams the ejaculation, by the electrical method, of a semen sample containing only a few spermatozoa, may be due to faulty technique. This may have been the reason why the semen of the Merino ram 45106 contained only 13,000 spermatozoa per cubic millimetre on 27.2.42, and 83,000 on 12.6.42. On both these dates, however, the motility was poor and the abnormality count correspondingly high with coiled tails almost entirely responsible for this high count. The coiling involved mainly the ends of the tails only. Phillips (1935) quotes Milovanow (1934) for the statement that the secretions of the accessary sex glands tend to reduce the span of life of spermatozoa of the boar and stallion.

A perusal of the protocols shows that a very low sperm concentration was invariably associated with poor initial motility, and a rather high abnormality count; but it is also clear that poor motility and many abnormal spermatozoa are by no means encountered only in semen of low concentration. The sperm concentration is but one of the factors involved in the quality of an ejaculate.

Investigating bovine sterility in New Zealand, Webster (1932) found counts of 250,000 and under to be associated with poor motility and a high abnormality count. Such low counts were not met in bulls of high fertility. Thirteen bulls with counts of 100,000 per cubic millimetre and less were completely stirile. Normal counts varied from 500,000 to 700,000 per cubic millimetre, but might exceed 1,000,000.

Statistical comparisons can, unfortunately, not be made of the different breeds used in this work because in some cases only one ram of a particular breed was available, but it can be said that the five Ronderib Afrikaner rams gave semen throughout of a more uniform concentration and a higher monthly average than the four Merinos. The Blackhead Persian had the highest average (3·3). Ten of the rams averaged over two million per cubic millimetre and the lowest average concentration was 1·1 million, given by the Merino ram 45307.

Lambert and McKenzie (1940) give the range of sperm concentration (per cubic millimetre) for the ram as 500,000 to 6,000,000 with 1,000,000 as the most common figure.

TABLE 2.

Sperm Concentrations (millions per cub. mm.).

Ram.	Oct.	Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Average.
MERINO RAMS.															
45106	2·6	3·5	3·5	2·5	1·6	2·9	2·5	1·9	2·2	3·4	1·2	3·3	1·6	5·3	2·7
45307	2·4	2·2	0·3	1·1	0·6	0·4	1·4	1·4	1·3	0·9	0·7	0·8	0·8	0·7	1·1
50549	3·9	1·3	2·6	1·2	0·8	1·9	1·5	2·4	5·5	3·5	6·1	0·8	0·02	0·08	2·3
50735	2·3	3·5	1·3	0·3	0·6	1·8	0·4	1·4	2·6	5·0	1·5	2·6	1·8	0·4	1·8
Average	2·8	2·6	1·9	1·3	0·9	1·8	1·5	1·8	2·9	3·2	2·4	1·8	1·1	1·6	2·0
ROMNEY MARSH RAMS.															
*56939	0·4	1·4	1·2	0·4	0·4	1·2	1·1	3·7	1·7	0·4	—	—	—	—	—
56947	1·4	1·7	2·7	0·7	0·6	1·0	1·8	2·4	2·5	1·0	1·9	3·3	2·1	0·4	1·7
BLACKHEAD PERSIAN RAM.															
62030	4·3	3·0	2·4	3·1	1·8	3·2	3·1	3·1	4·5	4·2	4·7	4·2	2·2	2·3	3·3
DORSET HORN RAMS.															
56956	1·0	1·0	0·8	1·0	1·8	1·3	—	0·8	1·8	0·7	3·2	2·6	1·0	0·9	1·3
*62548	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
RONDERIB AFRIKANER RAMS.															
62418	5·5	3·1	3·9	1·1	0·8	2·4	2·9	2·4	2·4	3·1	2·6	3·5	4·9	2·1	2·9
62546	4·0	2·0	4·7	3·3	1·8	2·7	2·0	1·4	2·2	3·3	2·5	2·9	3·9	1·8	2·8
62419	3·9	2·9	2·8	2·9	2·2	1·3	1·5	3·5	4·1	1·5	4·7	2·8	2·3	1·0	2·7
62549	5·1	2·1	3·0	3·0	2·1	1·0	2·1	2·0	2·2	2·9	1·2	3·6	4·3	1·5	2·6
62543	4·6	4·6	4·5	3·0	1·7	1·6	3·2	3·5	3·9	2·1	3·0	5·0	2·0	2·4	3·2
Average	4·6	2·9	3·8	2·7	1·7	1·8	2·3	2·6	3·0	2·6	2·8	3·6	3·5	1·7	2·8
WELSH MOUNTAIN—RONDERIB AFRIKANER RAMS.															
62541	2·3	1·0	4·4	2·1	0·8	1·8	1·4	2·6	1·4	2·7	2·2	3·5	4·3	3·0	2·4
62542	1·2	1·0	1·4	1·2	1·3	0·7	0·8	1·3	1·8	1·6	1·1	1·4	1·5	2·1	1·3
Average	1·8	1·0	2·9	1·7	1·1	1·3	1·1	1·9	1·6	2·2	1·7	2·5	2·9	2·6	1·9
SOUTHDOWN—BLACKHEAD PERSIAN RAM.															
62545	2·0	2·6	1·5	1·8	1·3	0·9	3·9	2·3	5·5	3·3	3·8	4·0	2·6	2·1	2·7
KARAKUL RAM.															
62544	—	1·1	3·3	2·8	1·3	1·4	1·8	5·0	3·4	0·4	0·8	0·1	1·5	0·2	1·8
Grand Average	2·9	2·3	2·7	1·9	1·3	1·6	1·9	2·3	3·0	2·5	2·6	2·8	2·3	1·6	2·3

* Not included in averages.

4. *Total Number of Spermatozoa.*

The total number of spermatozoa in an ejaculate was calculated from the semen volume and the concentration (number per cubic millimetre), and since both these semen properties are subject to variation it could be expected that the total number would show wide variations between semen samples. The highest total number of spermatozoa in an individual ejaculation was 10,810 million obtained from Ronderib Afrikaner ram 62546 the first time he was ejaculated (not shown in the records). During the experiment a few months later the same ram gave a total of 9,900 million in an ejaculation. His lowest total was 800 million.

Russian workers found that 50 million spermatozoa were necessary for an insemination, but a much larger number would be required for natural copulation where the semen is deposited in the vagina. Lasley and Bogart (1943) showed that the number of spermatozoa per insemination was correlated with fertility in the bull. Fertility was below average when there were fewer than 800 million total spermatozoa, or 275 million live spermatozoa, or 175 million resistant spermatozoa. Lasley and Bogart record a high correlation per ejaculate between total spermatozoa and semen volume, sperm concentration and motility rating. McKenzie, Miller and Bauguess (1938) could find no direct relation between the total number of spermatozoa and semen volume of the boar.

It has been indicated before in this work that semen volume and sperm concentration were not correlated. Neither could a definite relation be seen between the total spermatozoa per ejaculate and the volume. There was, however, a close relationship between the total sperm count and the concentration. The following example from four successive ejaculations at fortnightly intervals from Karakul ram 62544 shows how these properties are related.

Date.	Semen Volume (c.c.)	Concentration (Millions per mm^3).	Total Number of Spermatozoa. (Millions).
2/1/42.....	0·8	2·0	1,600
16/1/42.....	0·8	4·9	3,900
30/1/42.....	0·8	1·6	1,280
13/2/42.....	0·8	0·5	400

In most of the rams the total number of spermatozoa dropped to a low figure on some occasions, but in the case of the rams with low fertility a low total count was the rule rather than the exception. The Ronderib Afrikaners all had a higher average of total spermatozoa than the Merinos. It will be seen from Table 3 that there were big differences among the individual rams.

5. *Motility of Spermatozoa.*

In order to reach the ovum a spermatozoon must exhibit motility. But there are degrees of motility and it is obvious that all other factors being equal, the better and more vigorous it is the greater are the chances that the spermatozoa will reach the ovum descending the fallopian tube. It is

TABLE 3.

Total Number of Spermatozoa (millions).

Ram.	Oct.	Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Average.
MERINO RAMS.															
45106	1·4	5·4	2·1	2·4	1·9	2·9	1·6	1·2	1·4	2·6	1·0	2·4	1·5	4·8	2·3
45307	1·9	1·5	0·2	1·0	0·9	0·5	0·7	1·3	1·2	0·9	0·6	1·1	0·8	0·7	1·0
50549	3·5	0·9	2·9	1·0	0·7	1·9	1·1	1·6	4·0	3·0	5·2	0·6	0·03	0·05	1·9
50735	3·4	4·6	2·5	0·3	0·6	2·3	0·4	1·3	2·6	6·2	1·2	3·0	1·3	0·4	2·2
Average	2·6	3·1	1·9	1·2	1·0	1·9	1·0	1·4	2·3	3·2	2·0	1·8	0·9	1·5	1·8
ROMNEY MARSH RAMS.															
*56939	0·2	0·8	0·7	0·4	0·3	1·5	0·9	3·3	1·8	0·3	—	—	—	—	—
56947	1·6	2·6	4·3	0·6	1·0	1·4	1·8	2·6	3·6	1·0	2·2	4·4	1·9	0·5	2·1
BLACKHEAD PERSIAN RAM.															
62030	3·9	3·0	2·2	3·7	1·1	3·4	2·2	2·5	2·5	2·8	3·8	3·9	1·8	1·4	2·7
DORSET HORN RAMS.															
56956	1·5	0·8	0·9	2·5	2·0	1·8	0	0·2	2·0	1·5	4·5	8·5	0·9	0·9	2·0
*62548	0·9	0·5	1·1	0·6	0·3	0·5	0·1	1·6	0·9	—	—	—	—	—	—
RONDERIB AFRIKANER RAMS.															
62418	2·2	2·0	5·7	0·8	1·2	3·3	2·3	3·3	2·6	2·5	2·3	3·2	3·8	1·7	2·6
62546	6·3	1·4	8·5	3·4	2·2	3·4	2·6	1·1	2·0	4·1	2·7	2·7	3·7	1·4	3·3
62419	4·4	2·8	1·7	3·8	2·7	1·2	1·2	4·0	5·7	1·1	7·1	2·6	1·9	1·2	3·0
62549	3·9	1·2	4·0	2·9	3·2	1·1	1·5	2·1	2·3	3·8	1·0	2·8	3·2	0·9	2·4
62543	2·8	3·4	4·5	2·7	1·6	1·2	4·3	2·5	2·9	1·4	2·6	4·5	2·1	1·4	2·7
Average	3·9	2·2	4·9	2·7	2·2	2·0	2·4	2·6	3·1	2·6	3·1	3·2	2·9	1·3	2·8
WELSH MOUNTAIN—RONDERIB AFRIKANER RAMS.															
62541	1·9	0·8	4·1	1·5	0·7	1·7	1·1	2·4	1·3	3·5	1·3	2·6	2·0	1·8	1·9
62542	1·2	0·8	1·1	1·5	1·4	0·9	0·7	1·5	2·0	1·9	1·0	2·6	1·3	2·1	1·4
Average	1·6	0·8	2·6	1·5	1·1	1·3	0·9	2·0	1·7	2·7	1·2	2·6	1·7	2·0	1·7
SOUTHDOWN—BLACKHEAD PERSIAN RAM.															
62545	1·4	2·1	1·3	1·5	0·7	1·1	3·7	1·7	5·2	4·3	3·2	3·5	2·4	1·9	2·4
KARAKUL RAM.															
62544	—	1·1	1·7	2·3	1·3	1·2	1·8	3·3	3·0	0·4	0·6	0·1	1·3	0·3	1·4
Grand Average	2·8	2·[illegible]	3·0	2·0	1·4	1·8	1·7	2·0	2·8	2·6	2·5	3·0	1·9	1·3	2·2

* Not included in averages.

important that the site of fertilization should be reached in a reasonable time, for according to Phillips (1935) " the ability to fertilise the ovum persists for a much shorter time than the power to show motility ". If the fallopian tube has been reached before ovulation has occurred the spermatozoon may not find an ovum for a considerable period and during this delay its ability to fertilize will decline. Again, the life of the ovum after leaving the follicle is short. In the rabbit Hammond and Marshall (1925) state that the ovum is capable of fertilisation for only 2 to 4 hours after ovulation, and Day (1942) found that mares inseminated 2 to 24 hours after ovulation were all sterile.

It is accepted unquestionably that spermatozoa must be motile in order that fertilisation may result, but opinions differ as to the value that can be attached to the degree of motility shown by semen samples during examinations. Williams and Savage (1927) were unable to attach much clinical importance to this phenomenon in the case of bulls especially when considered alone. They point out that the movement of spermatozoa from highly fertile sires may be affected adversely by many trivial factors, and that abnormal spermatozoa from bulls with poor fertility were often found to be highly motile. Factors which may affect the motility rating are dilution of semen with extraneous fluid, admixture of vaginal blood and tenacious mucus, the time between collection and examination and the temperature at which the sample is kept and examined. Quinlan, Steyn and De Vos (1941) found that the optimum temperature for storage of ram semen was between 2° C. and 4° C. Semen was stored in narrow tubes and covered with sterile medicinal liquid paraffin. By this method live sperm were seen after a storage period of 100 days. Pregnancies were, however, not obtained with semen stored for longer than 72 days. Douham and Simms (1930) studying the genitalia obtained from the abattoir found that the secretions of the seminal vesicles of bulls either retarded or inhibited motility of spermatozoa.

Webster (1932) investigating bovine sterility in New Zealand considered that normal semen samples should remain motile at room temperature for 36 to 48 hours, although he occasionally found motility up to five days. In normal samples he found an active initial motility of 80 per cent., which dropped steadily as the age of the sample increased. Very frequently, the motility in the case of bulls that were sterile or of low fertility was extremely poor and of short duration. The percentage of motile spermatozoa and the degree of activity were both affected. Webster concluded that " from the results obtained it may be stated that in most cases the general motility of a sample of semen affords a reliable estimate of its probable fertility ". He says, however, that the first ejaculate of bulls after an extended period of sexual rest, usually shows poor motility and contained many degenerate sperms, whereas in a second ejaculation of a few minutes later the motility was normal. Lagerlöf (1934) agrees with this last observation and, like Williams and Savage (1927), also draws attention to extraneous factors that may influence the movement of spermatozoa, and which should be taken into account when an examination of semen is made. In most cases of highly fertile bulls Lagerlöf found sperm motility to be very good or good, while in bulls with reduced fertility, and in those that were sterile it was variable.

Gunn (1936) was unable to confirm the view that the percentage of motile spermatozoa, or the degree or duration of motility was reduced in

the first ejacula after a prolonged rest period. He obtained pregnancies in ewes by insemination with semen that showed less than the maximum degree of motility, but he goes on to say that " the percentages of motile spermatozoa and the degree of their motility were, in our experiments, the main means of comparing and evaluating semen samples ". Swanson and Herman (1941) came to the conclusion that the initial motility of the semen of the different bulls used in their work was not correlated with their fertility, except in samples with very poor initial motility, which were of poor fertility. In separate ejaculates of the same bull, however, there was a rough relationship between initial motility and the time of survival with good motility. The time of survival of vigorous motility under storage conditions at 40° F. was considered to give the closest correlation with fertility.

Among the many factors which may affect the vitality of spermatozoa outside the body, temperpature is one of the most important. To study this effect Hammond (1930) and Walton (1930) in collaboration conducted experiments with rabbit semen collected, respectively, from the vagina of the doe after mating and direct from the epididymis of the buck rabbit. At body temperature (40° C.) the maximal survival was about 13 hours, while at 45° C. the spermatozoa were almost instantly destroyed. At temperatures below that of the body, survival was increasingly prolonged until a maximum of about seven days at 15° C. was reached. Motility was reduced at lowered temperatures, but in a fresh suspension complete inhibition did not take place until about +5° C. (Walton). The results obtained by the two methods were approximately the same. The percentage of spermatozoa motile, when examined at room temperature, was considered by Hammond to be a fairly good indication of the percentage fertility.

Examinations for motility were made in all cases at room temperature within approximately thirty minutes after ejaculation. In cold weather an electric heater was switched on in the room. The hanging-drop technique with a magnification of 200 diameters was used for the examination.

No uniform method has as yet been adopted for the classification of the various degrees of motility encountered in semen samples. From a practical point of view what seems most desirable is a method which is simple, and at the same time enables one to indicate the degrees of motility which appear to be of importance. The classification adopted in this work is more or less similar to that of the Russian workers, quoted by Walton (1933). If an ejaculation is collected directly into a test-tube and held up to the light, a sort of vibration due to the movement of the spermatozoa can be seen in semen of good quality.

The percentage of spermatozoa viable and the vigour of their movements were both considered in assessing the motility rating. The highest mark, 5, was given when 95 to 100 per cent. of the spermatozoa showed vigorous progressive movement. Such samples give the impression of a seething mass with waves moving across the field with great rapidity. In the centre of the drop individual spermatozoa cannot be observed, but towards the edges they can be seen to move very actively. In the next grade, 4, 75-95 per cent. exhibited strong progressive movement. A few immotile spermatozoa could be seen and waves still traversed the mass rapidly. When 55 to 75 per cent. of the spermatozoa showed good progressive movement the motility was described as " 3 ". Waves were also observed, but they were

fewer and slower; movements of individual healthy cells being easily followed. Many spermatozoa showed oscillatory motion only. In "2" motility, 33 to 55 per cent. were motile. No waves were present, many motionless cells drifted about, forward movements were slow and about half of the motile spermatozoa showed oscillatory motion or moved in small circles. Abnormalities, when present could be studied. The mark 1, indicates that very few sperms were viable and the majority showed only oscillatory movement. The motility was described as 0, when no motile spermatozoa could be seen. This classification would be rather crude for detailed studies on sperm motility, but it was found to be satisfactory for clinical purposes.

Because motility is so readily influenced by extraneous factors an indifferent initial motility *per se*, of a single ejaculate cannot be regarded as a true index of the viability of the semen or of the sperm picture as a whole. There is, however, a direct correlation between the initial motility of successive ejaculates, especially when considered over an extended period, and the semen picture and, roughly, the fertility of the ram. The records show that the initial motility of the semen of good rams was generally good and that of the rams of poor fertility generally poor. When the motility reading was mostly 3 or below, the semen picture was, as a rule, poor or bad and the rams were of low grade fertility. Rams in this class are the Romney Marsh 56939, the Dorset Horn 56956 and the Welsh Mountain-Ronderib Afrikaner 62542. Their breeding records were respectively 21·7 per cent., 42 per cent. and 61·5 per cent. An exception is the Blackhead Persian ram 62030, which always had an excellent semen picture with good motility but whose breeding record was only 59 per cent. This ram was very keen, but he rather often dismounted too soon with the result that the semen was deposited round the vulva.

6. *Sperm Morphology.*

Stained semen smears were used for the study of sperm morphology. Numerous wet preparations were closely observed in order to determine whether the various abnormalities found in stained smears could be detected in the unfixed preparations and to what extent, if any, the processes of fixing and staining changed the picture. It can be said that all the aberrant forms listed, with the exception of those spermatozoa which showed poor staining affinity were observed in the wet smears. It was found that a fairly good picture of the ultimate finding could be forecast from a study of the spermatozoa in wet preparations.

It was possible to increase the proportion of tailless spermatozoa by careless preparation of smears. Heads and middle pieces were torn by foreign matter on the glass slides. Mechanical aberrations could be detected after sufficient experience had been gained. The head of the healthy spermatozoon does not separate from the body very easily. To facilitate differential counts of abnormal cells, semen should be spread over the slides as evenly as possible.

All spermatozoa which did not conform to what was regarded as normal (Fig. 4) were classified as abnormal under the different headings. Five hundred spermatozoa were counted in each sample from the different rams and classified. Any individual cell was recorded under one heading only. For example, a tailless head was classified as such only, irrespective of

any other aberration it might have shown as well; a pyriform head which also had a coiled tail was recorded as the former only. In general, abnormalities of the head were given preference over those of other parts of the spermatozoa, and deformities of the middle piece over those of the tail, provided the particular abnormality was definite enough.

By recording carefully all the different aberrant forms which were observed it was hoped that it might be possible to form an opinion of the significance of the various abnormal types to fertility. This question will be discussed later.

Williams (1920) believed that the noxious influence which caused death and disintegration of only a few cells also devitalised the remainder of the spermatozoa. Simeone and Young (1931) showed that spermatozoa attained full maturity in the epididymis and that those that were not discharged underwent regressive changes resulting in death and liquefaction in the epididymis and vas deferens. Variations in sperm morphology in the semen of different bulls was shown by Lagerlöf (1934) to be due to degenerative changes in the testicles. This does not explain the finding of sudden marked increases in the abnormality counts of particular ejaculates from healthy rams and just as sudden a return to normal.

(*a*) *Abnormalities of the Tail.*

Tailless Spermatozoa.—The separation of the head from the tail invariably occurs at the neck so that the separate tail includes the middle piece. A break anywhere else in the tail has been seen only as a result of mechanical tearing, as occurs when a smear was roughly prepared or when foreign matter had come between the slides.

As a rule the free heads only were found in the smear; very occasionally were the free tails seen. Loose heads were seen in wet preparations immediately after ejaculation and must have lost their tails previously. Williams and Savage (1925) observed free heads in the seminal tubules in one case. Williams (1920) considered the presence of tailless spermatozoa to indicate lowered vitality of all the cells present. Trophic disturbances in the later stages of spermatogenesis were thought to be responsible for these abnormal forms or that the secretions of the accessary glands played a limited role. In the latter case Williams says marked morphological changes in the nuclear portion of the head would not be expected. He found tailless spermatozoa to be associated with constricted nuclei.

In good sperm pictures and in the semen of rams of good fertility the number of tailless spermatozoa was usually small. It was observed that when free heads were a significant factor in the abnormal count the majority showed defective staining of the anterior head cap. The anterior cap stained fainter than normal and the outline was inclined to appear blurred. For example, on 28.8.42 (see Table 14 in Appendix), the Ronderib Afrikaner ram 62418 had a marked increase of free heads constituting 52 per cent. of the total abnormal count of 188 per thousand. All these free heads exhibited defective staining of the anterior head cap.

Few tailless spermatozoa were generally found in the ejacula of the good rams. Averages for individual rams in this category ranged from four to twelve per thousand and the group average was seven per thousand.

In the rams of poor fertility the individual averages were between thirty-five and sixty-two per thousand with an average of fifty thousand for the group.

Coiled Tails.—Various types and degrees of coiling have been observed, but were not differentiated in the classification of " coiled tails " (Figs. 5 and 6). In the extreme type the tail enveloped the head closely; in others it was coiled at the base of the head and more frequently the tail was looped back on itself near the end of the middle piece. In the other extreme the lower part or the end of the tail only was involved (Fig. 21).

The closer to the head the coiling or the looping was, the more adversely were the movements of the spermatozoon affected. Spermatozoa with tails coiled round the head were either non-motile or showed oscillatory movements only; and those with looped tails involving the middle piece were capable of rotary motion with no progression. When the coiling was near the end of the tail a fair degree of forward movement could be observed. Spermatozoa with the less severe types of coiled tails usually had normal-looking heads. Williams and Savage (1925) considered such cells to indicate merely a state of immaturity, but they point out that such a condition may mark the beginning of more serious abnormalities.

That in most cases coiled tails in themselves signify an immature state, and not a gross disturbance of spermatogenesis, seems to be evident from the fact that not infrequently during this work the percentage of abnormal spermatozoa was suddenly and markedly increased and fell just as suddenly. Coiled tails, either of the looped variety or with the coiling near the ends, were almost entirely responsible for the increased abnormality count. The following examples show the number of coiled tails and the total number of abnormal spermatozoa per thousand in ejaculations at the time of a sudden increase and, as a comparison, the figures for the ejacula immediately before and after, Collections were made fortnightly.

Ram.	Before.		At sudden increase.		After.	
	Coiled Tails.	Total Abnormals.	Coiled Tails.	Total Abnormals.	Coiled Tails.	Total Abnormals.
45106.....	8	24	92	144	6	20
45307.....	14	38	306	412	54	128
50549.....	4	86	118	140	6	68
62546.....	0	2	182	276	4	12
62541.....	58	74	200	220	62	74

The loops and coils were often filled with cytoplasmic material (Figs. 6 and 13), which appeared to hold the tails in those abnormal positions.

Of all the aberrant forms found in semen, coiled tails were the most frequent and their proportion was most readily altered, but those that were closely coiled round the head were not present in large numbers except when the general sperm picture showed that spermatogenesis had been affected. The two rams, Romney Marsh 56939 and Dorset Horn 56956, both poor breeders (5 pregnancies out of 23 ewes served and 8 out of 19 respectively), almost invariably gave semen with high abnormality counts

including large numbers of coiled tails. Many had the tails closely coiled round their heads. The Karakul ram 62544 served 26 ewes and twenty lambs were born (76·9 per cent.). In March his semen showed a gradual increase in coiled tails whereas other aberrant forms did not then increase correspondingly (see Appendix). In June there was a big increase, the figure reaching 378 per thousand and it went as high as 446 in July. A high proportion of coiled tails was maintained up to the middle of October. On 10th July this ram served a ewe which conceived and subsequently lambed. His semen on that day showed an abnormality count of 328 per thousand of which 288 (88 per cent.) were coiled tails. The motility rating was two. The coiling involved practically only the lower parts of the tails, and this was a feature of the coiled tails in this ram's semen (Fig. 21).

A sharp kink in the middle piece was occasionally seen; and in the angle of the kink was a mass of protoplasm. Spermatozoa with this type of deformity mostly also had abnormal heads (Fig. 7).

Enlarged Middle Pieces.—In this classification are included spheroidal swellings (Fig. 8) and a uniform thickening of the middle pieces. The former were the more common. These spheroidal swellings which are associated only with the middle pieces, appear most commonly just below the neck and were rarely seen in any other position. They differ from middle piece heads by being apparently *inside* the protoplasmic sheath and not wrapped round it.

Williams and Savage (1925) regarded spheroidal swellings as an indication of disturbed spermatogenesis in the bull. They did not generally exceed twenty per thousand and in that proportion were not found to be of immediate clinical significance, but more serious affections of the spermatogenic tissue might be expected to follow. W. L. Williams (1923) referred to a stallion which ejaculated abundant, highly motile spermatozoa most of which, however, had an elliptical enlargement in the middle piece. The stallion was sterile. Williams believed the ripening process to have been interrupted with premature ejaculation of these cells.

Enlarged middle pieces were present only occasionally and in small numbers, generally two to six per 1,000, in semen from our normal rams. In specimens from the rams with poor and indifferent fertility, they were encountered quite frequently. They increased more or less as the total abnormality count increased and thus contributed to the bad semen picture. This was especially the case with the Romney Marsh ram 56939 (21·7 per cent. fertility) whose semen on one occasion showed enlarged middle pieces in the proportion of 82 per 1,000.

Double Middle Pieces and Tails.—In the final stages of spermatogenesis each secondary spermatocyte divides into two spermatids. The spermatid does not divide, but a metamorphosis into a single spermatozoon takes place. Williams and Savage (1925) explain the formation of double middle pieces as being due to incomplete division of the secondary spermatocytes.

In this study on rams double middle pieces (Fig. 9), and double middle pieces *and* tails were most commonly observed in connection with normal-looking heads. Less frequently, they were associated with large heads (Fig. 10). One wonders, therefore, whether this anomaly could not equally well be explained by a duplication of centrosomes during the metamorphosis of the spermatid into a spermatozoon.

Complete division of the middle piece and tail into two separate organs was rarely seen. Usually the upper part of the middle piece was more or less clearly divided and the remainder plus the tail in a state of incomplete division.

No relationship could be seen between this type of abnormality and the general sperm pictures or between it and fertility. Double middle pieces were found in insignificant numbers in three ejaculates from ram 56939 (21·7 per cent. pregnancies) and in about equal numbers and frequency in the semen of rams 56956 (42 per cent. pregnancies) and 50735 (78 per cent. pregnancies). In thirteen out of twenty-seven ejaculates from the Romney Marsh ram 56947 (55·9 per cent. pregnancies) double middle pieces could be demonstrated, and they ranged in numbers from 2 to 14 per 1,000. Every ram ejaculated double middle pieces on at least one occasion, but the numbers were very small. A few spermatozoa with double middle pieces were observed in wet preparations and they showed fair motility.

Middle Piece Beads.—These are delicate, globular, protoplasmic structures surrounding the middle piece. In ejaculated semen they were seen either in the region of the neck (Fig. 11) or near the end of the middle piece (Figs. 12 and 13), but their appearance in the former position was much less frequent. During examinations of more than one hundred semen smears a middle piece bead was seen in the middle of the middle piece on only one occasion.

Redenz (1924), quoted by McKenzie and Berliner (1937), found " protoplasmic drops " on almost all spermatozoa in the epididymis of the bull, and he observed further that in the head of the epididymis the beads were located at the neck and that they were near the caudal end of the middle piece in the tail of the epididymis. Many workers considered the presence of protoplasmic drops (middle piece beads) in ejaculations a sign of immaturity. To-day this view is not generally accepted. Gunn (1936) showed that frequent ejaculations in rams did not increase the number of spermatozoa with protoplasmic drops, and he considered that they were not an indication of exhaustion or of the age of the spermatozoa.

Middle piece beads were observed only occasionally and in small numbers, in the semen of good rams. They were almost invariably present in ejaculations of the Romney Marsh ram 56939 which had such a bad sperm picture generally and a poor breeding record. At the end of May spermatozoa with middle piece beads began to appear in the semen of the Karakul ram 62544 and reached a proportion as high as 146 per 1,000 towards the end of August. During October they decreased rapidly and by the end of the month were absent. With the appearance of the middle piece beads, the number of coiled tails began to rise accordingly, the coiling being mainly at the ends of the tails. The initial motility had been good up to the time of the increase of middle piece beads and coiled tails, but during the period of the high proportion of these abnormal forms it deteriorated to the 3 and 1 ratings. When the sperm picture had again improved the motility returned to its original good figure.

Gunn, Sanders and Granger (1942) agree with Lagerlöf (1934) that these protoplasmic drops indicate a disturbance of spermatogenesis and not merely a state of immaturity. Gunn, *et al* found them only as late results of acute degenerative changes, or in the slow recovery stages or in chronic degeneration.

Filiform Middle Pieces.- In this type of abnormality the protoplasmic sheath of the middle piece is absent leaving the axial filament bare (Fig. 7). Williams and Savage (1925) found filiform middle pieces to be of quite frequent occurrence in semen from apparently healthy bulls at the rate of one to two per 1,000. They suggest that the aberration is caused by defective development of the ends of the body (posterior end knob and end ring) with the result that the axial filament is not covered.

Filiform middle pieces were very rarely found in specimens from the good rams; and only in the very poor Romney Marsh ram 56939 did they occur in a fair number of specimens. They were found in seven out of twenty-one ejaculations from this ram, usually at the rate of two per 1,000 with a maximum of six per 1,000. In Fig. 7 it will be noticed that the head of the spermatozoon showing this abnormality has undergone a degenerative process, the usual subdivisions being absent. Head changes were observed in most spermatozoa with filiform middle pieces.

Very little can be found in the literature about this type of abnormality and it was found so seldom in semen samples examined (except in the case of the ram mentioned above) that its significance cannot be judged. It struck the author, however, as a most undesirable type of cell since it was a feature only of the semen of the ram which had a constantly bad sperm picture.

Abaxial Attachment of Middle Piece.—In the normal spermatozoon the middle piece is attached to the head in the middle of its base. Sometimes the attachment is slightly to one side, and in extreme cases it is right on the edge. Abaxialism was encountered very rarely in the semen of the rams used in this work and then only with the tail attached slightly off centre. The affected spermatozoa appeared to be healthy in all other respects. This abnormality is apparently of no significance in the ram. In the stallion extreme forms of abaxialism have frequently been observed.

(b) Abnormalities of the Sperm Head.

Defective Staining. Occasionally spermatozoa were observed in which the whole head did not stain and there was no differentiation between anterior and posterior portions (Figs. 6 and 7). Such spermatozoa were probably dead when ejaculated and their presence in appreciable numbers would indicate a serious disturbance of spermatogenesis. They were, however, only found in insignificant numbers in this experiment. More commonly defective staining was associated with the anterior head cap, which then stained faintly and the outline was not so sharply defined (Fig. 14). The nuclear portion of the head was stained normally. It was not possible to identify these aberrant forms in wet preparations.

Defectively stained spermatozoa were present in almost every ejaculation of all the rams, but in the case of the good rams their numbers were comparatively small. The lowest individual average among the good rams was 3·8 and the highest 27·6 per thousand, with a mean average of 12 per 1,000. The rams with poor breeding records averaged between 52 and 85 per 1,000 defectively stained spermatozoa.

In the poor rams this type of abnormal cell was part of a bad sperm picture. Welsh Mountain-Ronderib Afrikaner ram 62542 presented an interesting case in respect of defectively stained anterior head caps. This

abnormality was the main characteristic in all his ejacula. They averaged 65 per cent. of the total abnormality count and in one semen sample were as high as 94 per cent. of a total of 314 abnormals per 1,000. The lowest number per thousand in any one ejaculate was 20 and the highest 268; average 106. The semen had an appearance of curdled milk and precipitated more readily than normal specimens. Under the microscope the spermatozoa were invariably " clumped " which made counting very difficult. Volume and concentration were hardly influenced, but the motility was seriously affected. The highest motility rating recorded was 3, but most commonly it was either 1 or 2. Stained preparations revealed numerous neutrophiles.

Clinical examination of the genitalia failed to indicate any abnormality. Reference to Table 20 in the Appendix shows that the only other abnormality which appeared more or less constantly were tailless spermatozoa and coiled tails. This fact would appear to suggest that this abnormality may not have resulted from disturbance during the early stages of spermatogenesis, but rather that it was caused by noxious influences after the spermatozoa were fully formed. It is possible that a morbid condition existed in the accessory sex organs. This ram (62542) served thirteen ewes and produced eight (61·5 per cent.) pregnancies. Libido was normal.

Figure 15 shows an interesting phenomenon: Stain granules are deposited along the superior border of the posterior head cap of normal spermatozoa from a fertile ram. No explanation can be offered for the arrangement of these granules. It was seen only once.

Pyriform Heads.—In this classification was considered those sperm heads whose nuclear portion was reduced out of all proportion to the anterior part of the head. The construction might either be abrupt and severe as in Figure 16 or more gradual as in Figure 6. The contour of the anterior part of the head was usually also affected to a greater or a lesser degree. Most commonly the nuclear portion was reduced in its transverse diameter although not infrequently the longitudinal diameter was similarly affected, with the result that the pyriform head was smaller than normal.

It will readily be appreciated that spermatozoa whose nuclear material is so greatly reduced cannot be very vital, and that they are associated with poor fertility. Pyriform heads were observed in wet preparations but none were seen to show motility. It is, however, to be expected that the less severe forms may possess some degree of motility. Williams and Savage (1927) were so impressed by pyriform heads that they thought they indicated a " lowering of fertility out of all proportion to the numbers in which it happens to occur ". With regard to human spermatogenesis, Moench and Holt (1931) pointed out that all sperm changes were not of equal importance; thus a slight narrowing of the head, unless most cells were involved, was considered of relatively little significance, but they took a serious view of cells in which the nuclear material was distinctly reduced. McKenzie and Phillips (1934) found that in rams of affected fertility sperm heads were frequently abnormal. In a few of their rams of very low fertility the number of tapering heads was 20 and more per 1,000—an appreciable number.

Pyrifom heads appear to be associated with the more serious type of derangement of spermatogenic tissue in which regressive changes have taken place. Only in the semen of rams with poor breeding records were pyriform heads more or less constantly found. It will be seen from the detailed

classifications of abnormal spermatozoa in the appendix that these abnormal heads were closely associated with the total abnormality counts where the sperm pictures were generally bad. Fifteen out of twenty-one ejaculations from Romney Marsh ram 56939 (5 pregnancies out of 23 ewes served) showed pyriform heads ranging from two to sixty-four per thousand. Dorset Horn ram 56956 settled eight out of nineteen ewes and his semen showed pyriform heads on fifteen out of twenty-six occasions, with a range of two to seventy-six per thousand. The highest number of this type of cell was recorded in one semen sample from Romney Marsh ram 56947, namely, 172 per thousand. Pyriform heads were practically absent from the semen of Dorset Horn ram 62548, but in the last sample before he died the abnormality count rose to 352 per 1,000 and sixteen were pyriform heads. The records show that pyriform heads are correlated with poor fertility.

Narrow Heads.—It was sometimes difficult to decide whether to class a head as "narrow" or "pyriform". Narrow heads were regarded as those in which the entire head was reduced in its transverse diameter, and in which there was no distinct tapering towards the base as in pyriform heads. From a clinical point of view narrow heads and pyriform heads are no doubt equally undesirable and could conveniently be classed together.

In the two least fertile rams, 56939 and 56956, narrow heads were found in only two ejaculates of each, but in ram 56947 (55·9 per cent. fertility) they appeared in sixteen out of twenty seven samples in numbers of from two to twenty per thousand. Narrow heads, like pyriform heads are apparently not a common feature of ram semen and their presence must be viewed with concern. Figure 19 shows a narrow head in the bottom right hand corner.

Megalosperm.—Moench and Holt (1931) regarded distinctly large round cells in human semen as probably more or less immature or degenerated cells and that they were of importance, but not found in large enough numbers to correlate statistically with clinical abnormalities. The round type of large cell was not seen in this study of ram semen. Every ram on at least one occasion ejaculated a few giant spermatozoa. The majority were normal in appearance except in size, as in Fig. 17; others as in Fig. 10 were attached to middle pieces and tails which were in a state of incomplete duplication. The megalosperm in Fig. 10 also shows shreds of cytoplasm still adherent to the upper part of the middle piece.

Megalosperm could not be correlated with the semen picture in general, nor was there any relationship between the frequency with which they occurred in ejaculates and the breeding records of the rams. In fact, they were found in very few semen samples from the two worst rams and quite frequently in samples from some of the good rams in numbers from 2 to 8 per thousand. They were quite a feature of the semen of the two Ronderib Afrikaners 62418 and 62546. These were two reasonably fertile rams.

Microsperm.—Small heads which were less than half normal size were not encountered in any semen smears. Spermatozoa were classified as microsperm when they appeared to be approximately two-thirds normal size as in Fig. 18. Some of these small heads were normally proportioned;

on the other hand, the posterior portions of many were constricted giving the heads a pyriform appearance. In the latter the whole head or mainly the posterior part stained intensely.

On 13th January (Table 8 in Appendix) the semen of Merino ram 50735 had a marked increase in the total abnormality count due to an increase in nearly all the different abnormal forms listed. The number of small heads increased from nil in the previous ejaculation to fourteen per thousand. The least fertile ram in the experiment, Romney Marsh 56939, ejaculated microsperm on six out of twenty-one occasions (Table 9 in Appendix), the maximum number at a single examination being thirty-six per thousand. Because microsperm are ejaculated comparatively infrequently by rams, their contribution towards infertility is apparently small, but it would appear that they are associated with disturbed spermatogenesis. No connection could be seen between microsperm and megalosperm.

Double Heads.—McKenzie and Phillips (1934) observed double heads only rarely in ram semen. Lagerlöf (1934) also found them of infrequent occurrence in the semen of bulls. He did not consider them to play any part in the question of fertility.

Double heads were found in the semen of only six of our rams—five rams showed them on one occasion each and the sixth in three samples. In every case the number was two per thousand. These six rams included animals of good, bad and indifferent fertility. In the majority of cases there was no correlation between the double heads and the sperm picture, and in the remainder there may have been. In any case their numbers were so small that no opinion could be expressed regarding their significance. The double heads were either attached to normal looking bodies or to bodies that were thickened, giving the impression that nuclear division had taken place without cytoplasmic division of the body.

More than two heads to one body were not seen in the semen of these rams. During this work, however, semen samples were examined from a six year old stallion which had never served. Many double and also triple and quadruple heads were present in the first few ejaculations. Fig. 20 shows a four-headed spermatozoon in which incomplete division of the bodies can be clearly seen.

Variation in Head Sizes.—Williams and Savage (1925) indicated that within certain normal limits, the spermatozoa in any one ejaculation should be of uniform size, and they suggested that a spermatic study of the size variation of the heads might furnish good information with regard to the breeding potentialities of the particular animal. They showed that in really good bulls the length of the sperm heads was very uniform. In 1927, Savage, Williams and Fowler, quoted by Williams and Savage (1927) carried this study of sperm head lengths further. Measurements were made with a projection microscope at 3,000 diameters magnification. The coefficients of variation of samples from 53 good bulls ranged from 2·5 to 4·0. These workers concluded that the coefficient of variation in normal bulls should not exceed 4. They claimed that the practical application of this method of analysis had enabled them to recognise several unsatisfactory sires which would otherwise have escaped detection. Lagerlöf (1934) conducted a biometric study of the head lengths of spermatozoa of bulls of varying degrees of fertility and found that the coefficient of variation was 3·7 in 30 fertile bulls, 4·8 in 15 doubtful bulls and 6·2 in bulls with distinctly

poor fertility or sterile. Donham, Simms and Shaw (1931) attempted to measure the spermatozoa on stained slides and to correlate the measurements with pregnancy results. They came to the conclusion, however, that such measurements were not of any value in determining the breeding efficiency of bulls.

During this study of sperm morphology it was observed that the sperm heads of the best rams were extremely uniform in size. In certain other semen samples, however, it was noticed that there was a variation in head sizes concerning both the number of cells affected and the extent to which they were affected (Fig. 19). Biometric measurements were not possible in this work, but the extent to which this phenomenon occurred in smears was arbitrarily indicated. Thus when approximately 40 per cent. or more of the cells examined were different in size the variation was considered to be marked (xxx); it was slight (x) when only a few were affected and fairly marked (xx) when the number fell in between. This is shown with the classification of abnormal spermatozoa in the Appendix. The spermatozoon in the bottom right hand corner of Fig. 19 was classified as a narrow head.

Merino ram 45307 showed a variation in the size of the sperm heads on only two occasions and on each occasion the variation was associated with a sudden and marked increase in the abnormality count. Semen samples from Dorset Horn ram 62548 did not show a variation in head sizes until the last ejaculation before he died and in that sample it was marked. The total abnormality count rose to 352 per 1,000 from 52 in the sample taken two weeks earlier. The abnormality count of Ronderib Afrikaner ram 62418 began to show an upward trend in May and the comparatively high count was maintained until September. During that period slight variation in sperm-head sizes was recorded. Dorset Horn ram 56956 had a bad sperm picture practically throughout the test period and as can be seen from Table 12 in the Appendix variations in the head sizes were very closely related to the abnormality count. The position was the same for the other two poor rams—Romney Marsh rams 56939 and 56947 (Tables 9 and 10) respectively.

A slight variation in the size of the heads of a few spermatozoa of good rams will occasionally be found, but generally they are very uniform. It seems clear that when head sizes vary markedly, a poor sperm picture and low fertility can be expected.

The results of breeding tests are shown in Table 4.

All the Ronderib Afrikaner rams, except 62418, refused to serve even when locked up with the ewes over night. Their semen was artificially inseminated.

The ewe that failed to conceive to ram 62545 had a deformed cervix and never conceived.

In the following Figures, 4 to 19, are shown normal ram spermatozoa and the different types of abnormal forms that were encountered in this work. The four-headed specimen in Figure 20 is from a stallion following his first service. In Figure 21 are shown coiled tails in which the lower parts of the tails are affected. This type of coiling has been referred to in the text, and is discussed further later, especially in connection with the Karakul ram 62544. Note the high proportion of coiled tails in the picture. Figure 22 is the sperm picture of an infertile ram.

TABLE 4.

Results of Breeding Tests.

Ram.	No. of Ewes Served.	No. Conceived and Lambed.	Percentage.
Merino Rams—			
45106	23	20	86·9
45307	25	21	84
50549	18	16	88·8
50735	9	7	78
TOTAL	75	64	85·3
Romney Marsh Rams—			
56939	23	5	21·7
56947	34	19	55·9
TOTAL	57	24	42·1
Dorset Horn Rams—			
56956	19	8	42·0
62548	22	14	63·6
TOTAL	41	22	53·7
Romney Marsh and Dorset Horns—			
TOTAL	98	46	46·9
Blackhead Persian Rams—			
62030	22	13	59
Ronderib Afrikaner Rams—			
62418	29	23	75·9
62546	5	3	60
62419	5	5	100
62549	6	2	33·3
62543	6	5	83·3
TOTAL	51	38	74·5
Welsh Mountain—Ronderib Afrikaner Rams—			
62541	18	11	61·1
62542	13	8	61·5
TOTAL	31	19	61·3
Southdown—Blackhead Persian Ram—			
62545	20	19	95
Karakul Ram—			
62544	26	20	76·9

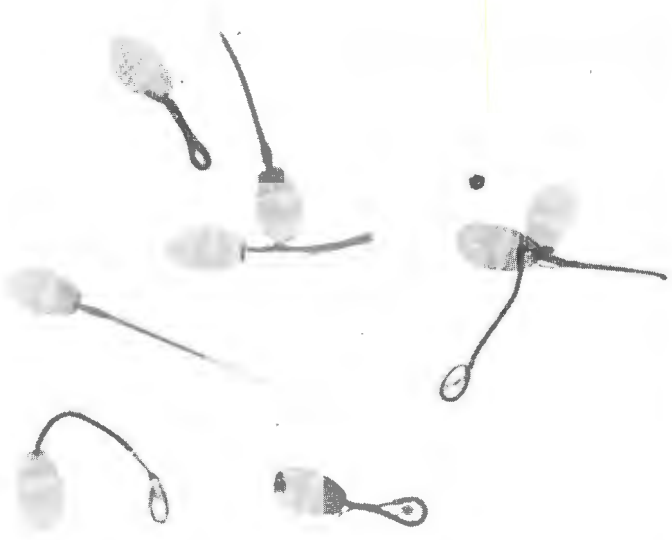


FIG. 5.—Showing different types of coiled tails and two spermatozoa with a spheroidal enlargement of the middle piece.

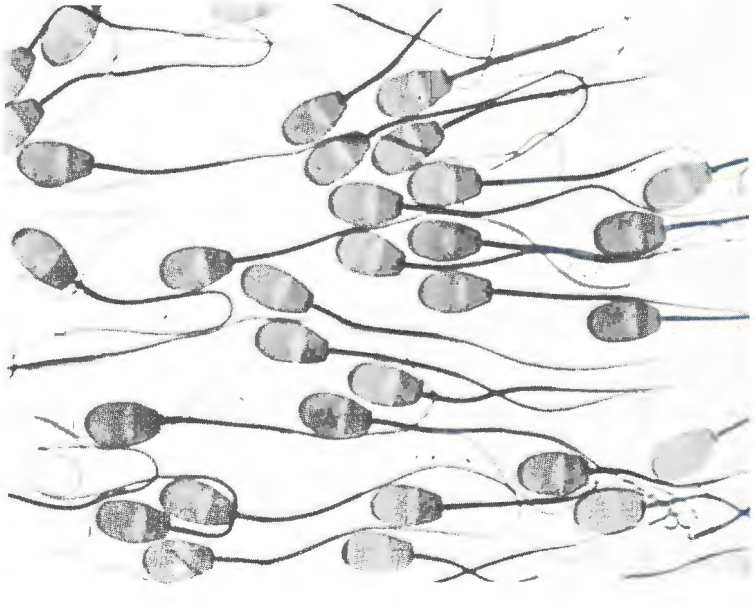


FIG 4.—Normal ram spermatozoa.

FIG. 7.—Showing a degenerated head with filiform middle piece and a pyriform head with bent middle piece.

FIG 6.—Showing coiled tails, a degenerated spermatozoon, and a pyriform head with coiled tail.

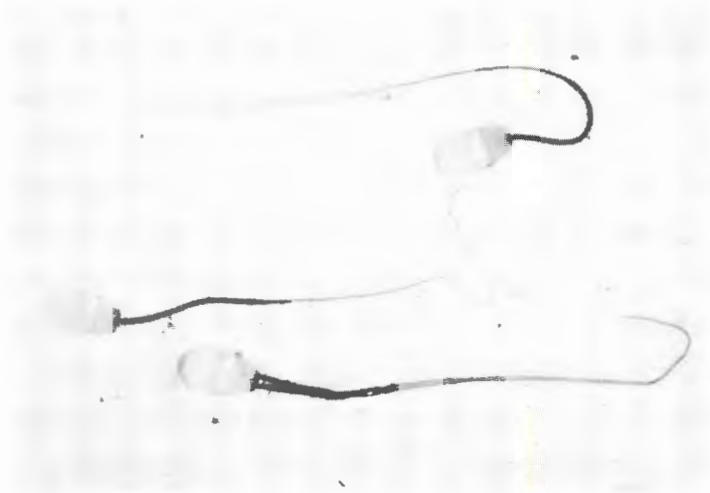


FIG. 9.—Showing a double middle piece. Incomplete division of the tail can also be seen. The head of the spermatozoon appears normal.

FIG. 8.—Showing spheroidal enlargement of the middle piece.

FIG. 11.—Showing middle piece heads situated at the neck.

FIG. 10.—Showing a large head with middle piece and tail in a state of incomplete division. Note the cytoplasmic remains on the middle piece.

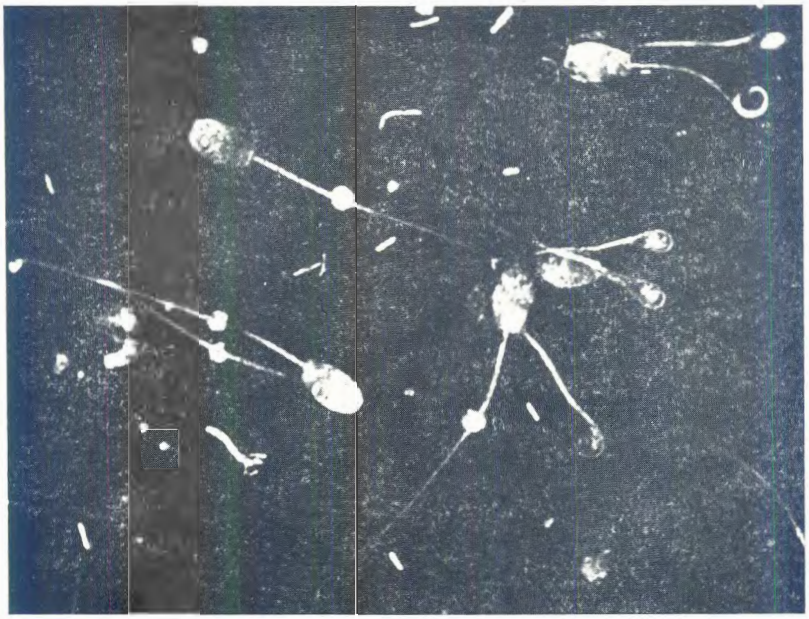


FIG. 13.—Showing middle piece beads at the junction of the middle piece and tail. The preparation was made by pouring diluted india ink over the unstained slide.

FIG. 12.—Showing middle piece beads at the junction of the middle piece and tail. In the picture is also a free head.

FIG. 14.—Showing defective staining of the anterior head cap. The outline of the affected heads is blurred. Two spermatozoa superimposed upon each other can also be seen in the picture.

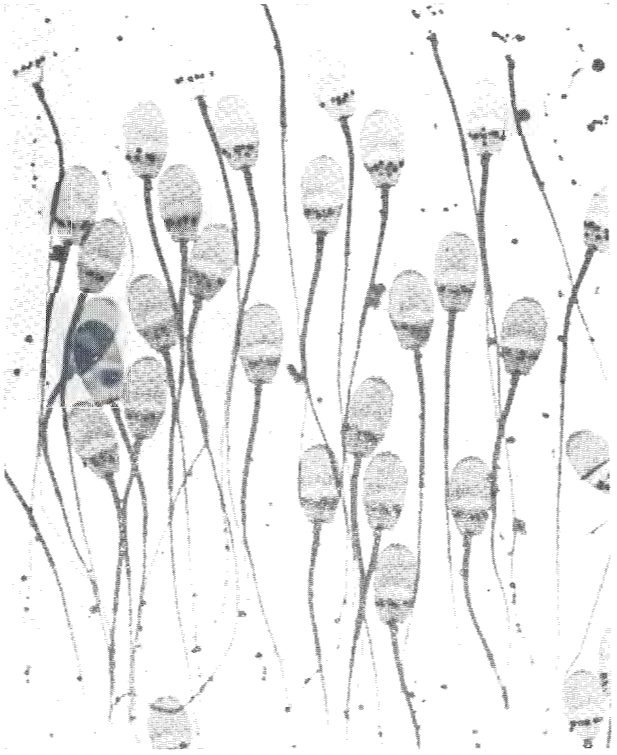


FIG. 15.—Showing a peculiar arrangement of stain deposit along the superior border of the posterior head cap. The spermatozoa appear normal.

FIG. 17.—Showing a megalo-sperm among normal spermatozoa. The megalo-sperm appears to be normal in all respects except size.

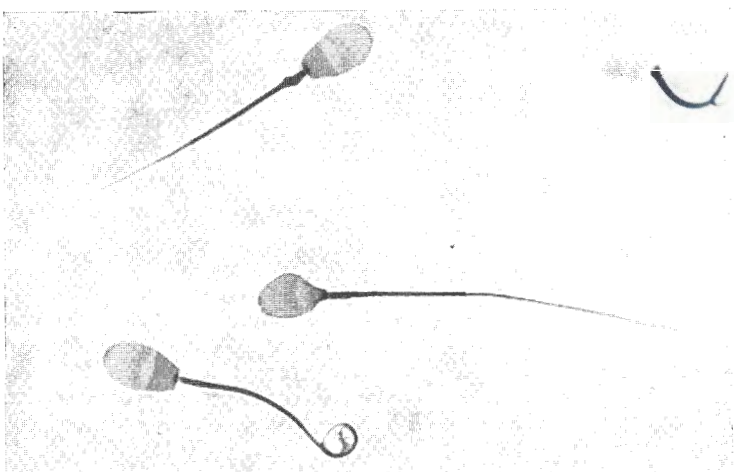


FIG. 16.—Showing an extreme type of pyriform head. Below, on the right is a free tail.

FIG. 18.—Showing a small head intensely stained.

6241—11

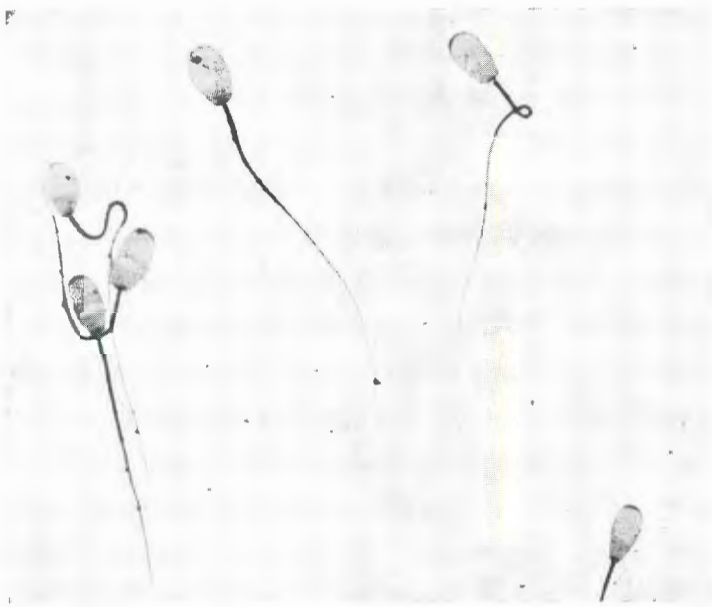


FIG. 19.—Spermatozoa showing variation in head sizes. The spermatozoon at the right bottom corner of the picture was classified as a " narrow " head.

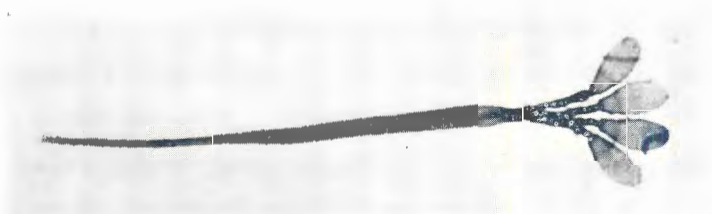


FIG. 20.—Spermatozoa of a stallion—a quadruple head. This specimen was obtained from the semen of a six year old stallion which had copulated for the first time. His semen contained many immature spermatozoa.

FIG. 21.—The sperm picture of the Karakul Ram, 62544, on 10/7/42 referred to in the text under "Abnormalities of the Tail". The coiling of the tails involes mainly the lower parts. Middle piece beads are also present.

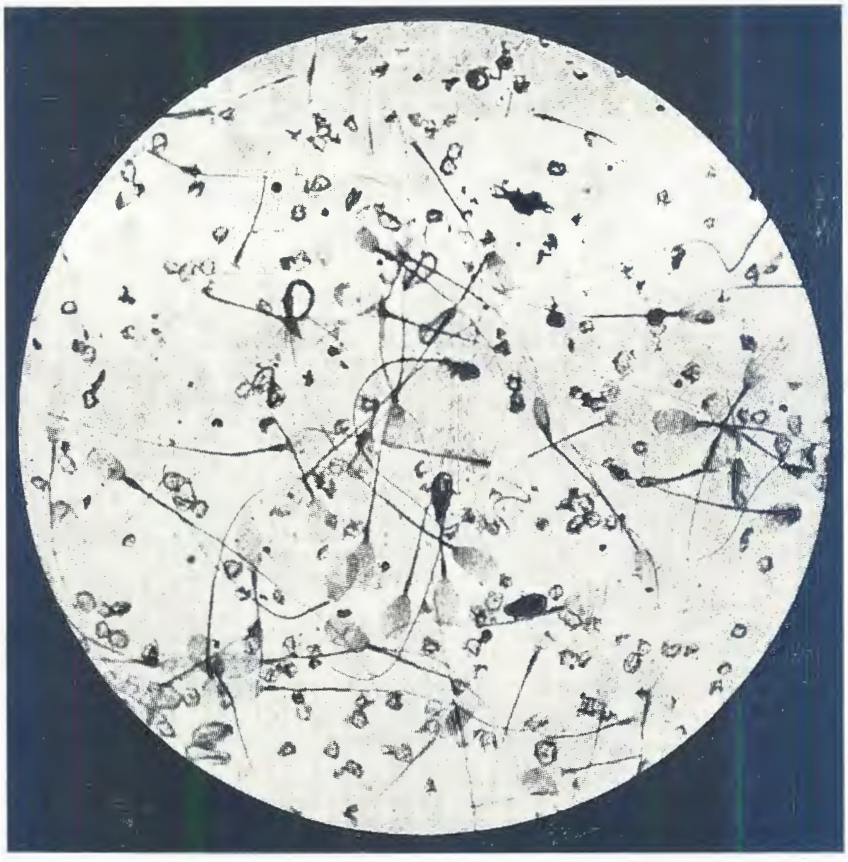


FIG. 22.—The sperm picture of the Romney Marsh Ram, 56939, during the period he was completely infertile. The spermatozoa are degenerated and numerous free protoplasmic bodies cover the picture. Coiling of the tails involves the middle piece.

VII. The Relationship of the Semen Picture to Fertility.

The relationship to fertility of the different types of abnormal spermatozoa found in semen samples has been discussed by different investigators. A study of the literature makes it clear that many factors may influence spermatogenesis, and investigators are not in general agreement with regard to the value of the study of sperm morphology in relation to fertility.

Williams and Savage (1925), Moench and Holt (1931), and others came to the conclusion that abnormalities of the sperm head were of greater importance than changes in other parts of the cell structure. On the other hand, Swanson and Herman (1941) could find no significance between pyriform heads and coiled tails in the semen of bulls; nor could pyriform heads be correlated with infertility since in some bulls of good fertility a large percentage of pyriform heads was observed, and coiled tails were predominant in the semen of bulls of both good and poor fertility. Again, Donham, Sims and Shaw (1931) showed that the microscopic picture of semen of the bull was definitely related to the percentage of conceptions that followed. McKenzie and Phillips (1934) concluded that in the ram morphological examination of spermatozoa revealed, within reasonable limits, the degree of fertility of that ram, provided the semen sample was not taken after a prolonged period of sexual rest or from the earliest ejaculates of the breeding season. Other investigators, including Walton (1933), Webster (1932), and McKenzie and Berliner (1937) also observed higher proportions of abnormal spermatozoa in the first ejaculations after sexual inactivity than in subsequent samples. To this view, Gunn (1936) does not subscribe. His work produced no evidence of an increase in the percentage of abnormal forms, or of a decrease in the percentage of motile spermatozoa, or in the degree or duration of motility after sexual rest. Ewes were inseminated successfully with a one twenty-first part of an ejaculum of a ram which had been sexually rested for several months.

As a possible explanation of the differences in these findings, Gunn offers the suggestion that much greater numbers of spermatozoa are emitted during artificial ejaculation than at natural coitus, and that the limited number of abnormal forms that may be present would be in such low proportion as to pass unobserved.

The highly fertile Southdown-Blackhead Persian ram 62545 was ejaculated electrically after a rest period of eighteen months and his semen showed few abnormal spermatozoa and the highest motility mark (5). The five Ronderib Afrikaners, the two Welsh Mountain-Ronderib Afrikaners, the Karakul, and the Southdown-Blackhead Persian had either not been used at all before they were taken into the experiment or they had not been ejaculated for many months previously. The first ejaculate from three of these rams shows fewer abnormal spermatozoa than the second two weeks later, and the differences in the abnormal counts between the first and second ejacula of the remaining six were so small as to be insignificant. Masturbation was not observed .

A number of investigators have expressed the relation between abnormal spermatozoa in semen and fertility in percentages. Thus Williams and Savage (1925) recorded that bulls with good breeding records did not "eliminate" more than 166 abnormal heads per 1,000, and Lagerlöf found 180 abnormals per 1,000 to be the limit for bulls with good fertility. In normal human semen Moench and Holt (1931) found not more than 20 per

cent. abnormal sperm heads and clinical sterility was usually present if the figure exceeded 25 per cent. Swanson and Herman (1941) by considering all the aberrant forms and not only those concerning the sperm heads, produced data to show that some individual bulls might maintain good fertility even when the abnormal count was as high as 30 per cent., but that above that value and when it approached 50 per cent., poor breeding efficiency would result. Good fertility, however, was not assured by a low percentage of abnormal spermatozoa. Anderson (1939) in Kenya, reported that the percentage of abnormal spermatozoa in fertile bulls was 8·1; in bulls of " reduced " fertility 13·1 and in sterile bulls 17·6. For the ram, McKenzie and Phillips (1934) suggests that more than 140 abnormals per thousand are associated with reduced fertility if the sample is not taken after a period of prolonged rest.

Gunn, Sanders and Granger (1942) do not agree with the authors just cited that any given percentage of morphological abnormalities is indicative of sterility, but they consider that " the percentage of morphological abnormalities compatible with fertility varies with the cause of the derangement of spermatogenesis ". These authors found no sharp line of distinction between highly fertile and sterile semens, but that there were intermediate degrees of fertility between the two. As a rough working basis they suggest the following subdivision of semen types according to their likely fertility under otherwise ideal conditions:—

Per cent. abnormal spermatozoa.	*Per cent. likely fertility.*
0·1	80 to 100
1	60
10	45
30	20
more than 50	0

On ten occasions during this study no abnormal spermatozoa could be found on microscopical examination of semen samples; otherwise some, however few, were invariably found. The highly fertile rams maintained a comparatively low total abnormality sperm count which was made up mainly by tailless spermatozoa, coiled tails, and spermatozoa with defectively stained anterior head caps. Other abnormal types appeared in occasional samples, but in small numbers. Abnormalities of the sperm head, such as pyriform heads, narrow heads, and small heads made a very small contribution towards the abnormality count of good semen when they were observed at all. The coiled tails were mainly of the " looped " variety or with the coiling in the lower part of the tails.

A very different picture was found in the semen of those rams which exhibited poor fertility. Figure 22 represents a spermatozoon picture of the almost infertile Romney Marsh ram 56939. Most of the sperm heads are degenerated and large numbers of free protoplasmic bodies cover the picture; the coiled tails are of the type with the tails wrapped about the heads.

In poor quality semen nearly all the different abnormal types listed were represented in different samples, so that the total abnormality counts did not result from only two or three types. Pyriform heads, narrow heads,

enlarged middle pieces, middle piece heads and sometimes microspermatozoa could be found in appreciable numbers. Coiling of the tails involved the middle pieces and many tails were wrapped closely round the heads.

The significance of sperm heads with defectively stained anterior head caps is not clear as they were present at times in appreciable numbers even in semen of very fertile rams. In the case of ram 62542, however, they were the main abnormal type and were associated with very poor initial motility and "clumping" of the spermatozoa. At room temperature, motility soon ceased—usually within about 90 minutes.

It has not been found possible from this work to indicate any definite percentage of abnormal spermatozoa as an index of a ram's fertility. Like Gunn, Sanders and Granger (1942), no distinct line could be shown between highly fertile and sterile semens. Between these two extremes were semens of varying degrees of fertility. It is concluded that a morphological examination of semen is a valuable guide to the degree of fertility of a ram, and that the type of abnormal cell ejaculated is of more importance than the total abnormality count.

VIII. The Effect of Seasonal Variations on Spermatogenesis.

The conditions under which the rams were kept and the method of collecting semen have already been explained. During the period of the experiment observations were made to determine the extent to which spermatogenesis was affected by seasonal changes in the climate prevailing at Onderstepoort.

It was shown by McKenzie and Berliner (1937) at Columbia, U.S.A., and by Gunn, Sanders and Granger (1942) in Australia that climatic conditions brought about seasonal variations in the semen of rams.

The part played by heat in affecting testicular derangement has been studied by several investigators. It was shown by Young (1939) that spermatogenic activity was upset by the application of heat to the testes. The first effects were seen during the division of the primary and secondary spermatocytes followed by degeneration of the older spermatids and spermatozoa. Gunn (1936) found the first degenerated spermatozoa in the vasa deferentia after 7 days of scrotal insulation and believed that these changes were produced on fully formed spermatozoa which were already in the epididymis when the insulation of the scrotum was commenced.

Hammond (1930) and Walton (1930) studied the effect of temperature on survival *in vitro* of rabbit spermatozoa obtained respectively from the vagina and from the vas deferens. The experiments were carried out over the range from 45° C. to 0° C. When allowance was made for differences in technique of the two methods, no essential difference was found in the length of life of the spermatozoa. It was determined that above body temperature (40° C.) maximal survival was about 13 hours. Lowering the temperature prolonged survival until a maximum of 7 days was reached at 15° C. As the temperature fell below the optimum (10°-15° C.) the rapidity of destruction was accelerated. The results of these experiments showed the importance of temperature on the vitality of the mammalian sperm outside the body.

Phillips and McKenzie (1934) caused heat degeneration of the testes of rams by scrotal insulation. Within the insulated scrota the temperature

was 2·2° C. above normal and that of the testes 2·1° C. above normal. During the first few days of insulation some rams showed high abnormal sperm counts which dropped considerably in a short time. This was believed to be due to " physiological degeneration " of spermatozoa in the vas deferens. Insulation resulted in an increase in the number of the various types of abnormal spermatozoa; especially tailless spermatozoa, coiled tails, tapering heads and enlarged middle pieces. The first increase in abnormal spermatozoa appeared on an average of 8·8 days after commencement of insulation.

Gunn (1936) raised the scrotal temperature of rams by insulation, and his experience of the effect on spermatogenesis aroused the suspicion that the hot dry climate of the western districts of New South Wales was responsible for the marked degenerative changes encountered in the semen of rams in those districts in summer. Thus Gunn, Sanders and Granger (1942) determined to attempt to produce those conditions artificially. They placed rams in a well insulated room in which ventilation was effected by a powerful fan. Fresh air and re-circulated air were dried by passing them over solid calcium chloride and electric radiators raised the air to the desired temperature. Food was available in the chamber, but not water. The rams were allowed outside for periods of half an hour twice a day. The experiment showed that seminal degeneration occurred in all the rams as a result of exposure to dry hot atmospheres and that the rapidity of degeneration varied with the duration of their exposure to the hot atmosphere. Extreme degeneration was produced whether the rams were exposed for a short continuous period of a few days or for a short daily period on a greater number of days. Indications of seminal changes were observed within a few days. Degeneration was progressive and the authors concluded that " the exposure of rams to hot atmospheres results in a graded departure of their semen from the normal according to the degree of the heat and the duration of the exposure ". They considered further, that the heat of summer in the hot areas of Australia was probably the underlying cause of the seasonal seminal changes that occurred in rams in those parts. The authors proceeded to investigate those changes under natural conditions and selected areas which ranged from cool districts with comparatively high rainfall to hot areas with low rainfall and low humidity.

Thus it was found that minimal seasonal variations in ram's semen occurred in the cooler localities and that in the hotter districts these changes were very marked, corresponding closely to seasonal heat. Degeneration was most marked in summer. Most normal semen was found in winter. From their results the authors anticipate that where daily recurrent maximum temperatures were above 90° F. for long spells, with occasional readings of 100° F., seminal degeneration might be noticeable as a result of the adverse effects of heat. Well marked effects could be expected if the temperature were above 100° F. for long spells. Individual susceptibility to heat among rams was experienced, and adolescent, and unmated rams were less susceptible to seasonal degenerative changes than mated rams. Also, where degenerative changes were obvious in earlier examinations they rapidly became more marked during the hot periods. The authors stress the value of shade as a means of preventing seminal degeneration due to atmospheric heat. When rams in Sydney were run in an area provided with plentiful shade they were not affected, but if kept in unshaded areas they showed definite degrees of seminal degeneration.

McKenzie and Berliner (1937) observed a distinct period of increased spermatogenic activity in Shropshire and Hampshire rams, October to January for the Shropshires and August to January for the Hampshires, coinciding with the recognised breeding season. There was a marked variation in sperm production in the two breeds and in individual rams within the breeds. Breeding desire did not change markedly throughout the year and seasonal variation in breeding capacity could not be adequately determined by it. Gunn, *et al* (1942) did not observe any difference in sperm production between breeds. They found that British breeds of sheep reacted to heat similarly to Merinos.

During the breeding season with its high spermatogenic activity (McKenzie and Berliner) the number of completed copulations, the number of spermatozoa ejaculated, and the volume of semen were large, and the absolute and relative number of abnormal spermatozoa were small. Volume of ejaculates and concentration of spermatozoa were smaller in late winter and during the spring than in the breeding season, and the number of abnormalities in the Shropshires was higher than during the breeding season. The increase in abnormalities, the authors consider, was not high enough to impair fertility. A period of decreased sperm production (June to September) preceded the onset of the breeding season with its high activity. Here again there was a marked difference between the two breeds. The authors showed from their results that the increase in the number of abnormal spermatozoa coincided with the rise in atmospheric temperature.

Erb, Andrews and Hilton (1942) observed seasonal changes in the semen of the bull. The various semen characteristics studied, except pH, showed highly significant differences between months. In the spring the quality of the semen was significantly superior to other seasons and the average sperm concentration and total sperm per ejaculate were at their maximum. In summer the average semen volume and the average initial motility were least. The average period of sperm survival was lower in summer than during other months. Highly significant differences in the various semen factors were noted between bulls.

During the summer of 1941-42, frequent temperatures of 90° F. and over were recorded at Onderstepoort, with a peak of 103° F. in November (Graph 9). The higher degrees of temperature were, however, not maintained for long continuous periods. From December 24th to February 18th inclusive, atmospheric temperatures were not recorded at Onderstepoort due to damage to the thermometers. The unstippled portion of Graph 9 therefore represents the temperatures in Pretoria, seven miles south, during that period. The rainfall during the period of the experiment and the average for the corresponding period of the past 25-27 years are shown in Graph 10.

Only one (50735) of the four Merino rams (Graph 1) showed any marked increase in sperm abnormalities during the warm season, and that was during January and February. Tailless heads, coiled tails and defectively stained spermatozoa which had always been present in previous samples, increased in number. Pyriform heads appeared in the semen in appreciable numbers and in one ejaculate in January small heads, enlarged middle pieces, double middle pieces and middle piece heads showed a marked relative increase. The remaining three Merinos had very low abnormality counts in January, and throughout the summer there was no appreciable derangement in spermatogenesis. In the autumn the Merinos, except ram

50735, ejaculated very few abnormal spermatozoa. Ram 50735 again had a relatively high count, 70 per 1,000, in April. The Ronderib Afrikaners (Graph 2) as a group gave semen with more uniform abnormality counts than the Merinos during summer and autumn. Ram 62418 had a higher abnormality count than the others in October, November and December. The abnormal types were tailless heads, defectively stained heads, coiled tails and large heads. In a single ejaculate in March the Ronderib Afrikaner ram 62546 gave semen with a dark brown colour and a very high abnormality count (276/1,000). Tailless heads, coiled tails, defectively stained heads and middle piece beads were the main abnormal types. The brown colour of the semen was due to blood from a local injury to one testicle. The semen was normal again at the next ejaculation fourteen days later. Only one of the five Ronderib Afrikaner rams was therefore affected by the warm weather.

Romney Marsh ram 56939 died in July and is not considered in the graphs. His fertility was always very poor and his semen picture generally bad. The number of abnormal spermatozoa was extremely high during the hot months and rose to 702/1,000 in a single ejaculate. A marked relative and absolute improvement was noted during March, April and May. The other Romney Marsh ram 56947 (Graph 3) showed a sharp increase in the total number of abnormal spermatozoa and in the different aberrant forms during December and January. The sizes of the sperm heads lost their uniformity and the variation became very marked. The return to a normal sperm picture in February was as sudden as the rise in the abnormality count was in December. Fairly good semen was ejaculated from February to May.

Dorset Horn ram 62548 died in the beginning of June and is not considered in the graphs. His ejaculation a few days before death contained 352 abnormal spermatozoa per 1,000, but during the months October to May inclusive, the total number of abnormal spermatozoa per ejaculate ranged from 2 to 52 per 1,000. There was no indication whatever of any seasonal effect. The Dorset Horn 56956 (Graph 4) maintained a fairly good sperm picture from October to the middle of December. At the beginning of January the number of abnormal spermatozoa rose rapidly to a high figure due almost entirely to an increase in the number of tailless heads and spermatozoa with coiled tails, middle piece beads and defectively stained heads. Variation in head sizes was slight to very marked. This unfavourable position was maintained until the middle of March when the ram became sick and his ejaculates during April were of a fluid nature with very few spermatozoa. The ram recovered in May, but although his semen improved somewhat, the number of abnormal spermatozoa continued to be high and there was a further deterioration during the spring.

Graph 5 shows the position with regard to abnormal spermatozoa for the two Welsh Mountain-Ronderib Afrikaner rams. The graph for the ram 62541, is practically a straight line from October to April. The highest number of abnormal spermatozoa in any ejaculate during that period was 14 per 1,000. The summer heat affected him not at all. It was explained elsewhere that by far the greater proportion of abnormal spermatozoa in every ejaculate obtained from the Welsh Mountain-Ronderib Afrikaner ram 62542, consisted of spermatozoa with defectively stained heads. Motility was always poor. Towards the end of October his abnormality count rose and was high in January and February with a relative fall in March and

beginning of April. In May the number of abnormals was relatively low. Spermatozoa with defectively stained heads were mainly responsible for the increase in the abnormality count.

The Blackhead Persian 62030 (Graph 6), gave semen of consistently good quality with few abnormal cells. Only in November 1941 was there an appreciable increase in the abnormality count (78/1,000) and during this month the highest atmospheric temperature was registered. During December to March a few abnormal spermatozoa were noted, but in April and May they increased slightly. This ram's excellent sperm picture was not consistent with his breeding record (59 per cent. pregnancies), but as has been explained before, his act of coitus was badly performed.

The Southdown-Blackhead Persian 62545 (Graph 7), gave no indication from his semen of any spermatogenic derangement during autumn, summer or spring. The graph shows practically a straight line for these seasons. This was a highly fertile ram.

The Karakul 62544 (Graph 8), came into the experiment as a young ram in November. His semen was of good quality throughout the summer months and few abnormal spermatozoa were observed until March and April when there was a slight increase.

At one ejaculate on January 30th no aberrant forms could be found.

With the onset of cold weather in May a decided change was observed in the number of abnormal spermatozoa ejaculated by nearly all the rams, although all were not affected to the same degree. The correlation between the spermatozoon abnormalities and the low atmospheric temperature is best shown by the minimum temperature graph (Graph 9). In May there was a tendency for the abnormality counts to rise; during the winter months they were high and remained high until the end of August or September. In the fertile rams the increase in abnormal spermatozoa was not so marked as to affect their fertility.

Three of the four Merinos, all five Ronderib Afrikaners, both the Welsh Mountain-Ronderib Afrikaners, Romney Marsh 56947, the Southdown-Blackhead Persian 62545, and the Karakul gave greater numbers of abnormal spermatozoa during the cold months than during any other season. The Romney Marsh 56947, gave similar high abnormality counts in semen collected the previous winter when collections were made by means of the artificial vagina. The Blackhead Persian ram 62030, and the Merino 50735, registered their highest abnormality counts in the hot season. Dorset Horn 56956, whose semen almost invariably contained large numbers of abnormal spermatozoa, showed distinct improvement in winter. The Romney Marsh 56939, which died in July, had an abnormality count high in summer, low in autumn, and high in June and July. The Karakul ram appeared to be physically greatly affected by the cold weather. Spermatozoa with coiled tails and middle piece beads made the greatest contribution to his high abnormality count. The latter were observed for the first time in his semen with the advent of the cold weather. Pyriform heads, which had been rare before, were found more frequently in winter. In the case of the Romney Marsh 56939, the sperm picture was very much the same in winter as in the summer, except that the variation in head sizes was more pronounced in winter. A return to normal in most of the rams was noticed by September or October.

Graph 10 shows the combined average graphs of the different semen characters of all the rams and their relation to each other and to the seasons.

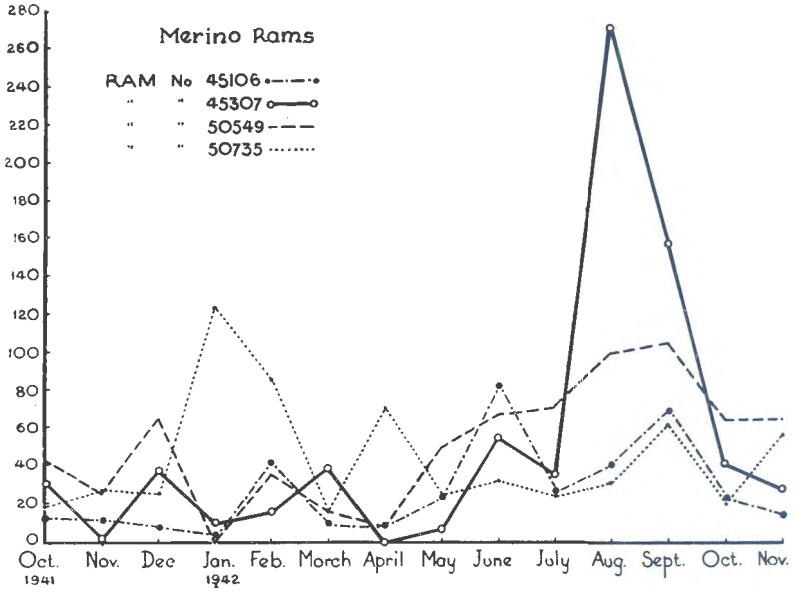
The two rams that died are not included. The distinct increase in the number of abnormal spermatozoa during the cold months can be seen. Semen volumes of individual rams showed no clear seasonal changes, but it must be admitted that no attempt was made to "empty" the rams completely at the time of collection. The combined semen volume curve shows that the largest volumes were collected in the months of February, March and July (1·1 c.c.) and the lowest in December (0·7 c.c.). Sperm concentrations were high in spring and dropped gradually as the summer advanced to the lowest mark in February (1·3 million per c. mm.). During autumn there was a steady increase up to and including June (3 million per c. mm.), and a high concentration was maintained throughout the cold weather. Some individual rams reached a peak in May. Fluctuations between different collections from individual rams were experienced at all times. The total sperm numbers were influenced to a greater extent by sperm concentration than by semen volume. This is shown by the almost parallel curves of sperm concentration and total sperm numbers. Therefore during the cold season a greater total number of spermatozoa was ejaculated; sperm concentrations were higher and more abnormal spermatozoa were observed. Seven rams gave high abnormality counts during the hot months, but they were not high enough to impair fertility except in the case of the two least fertile rams which became almost completely sterile during that period. The high atmospheric temperatures registered on some days were not continuous, and the climate conditions were modified further by the summer rains and sufficient shade. In winter there was little protection from the cold.

IX. Discussion.

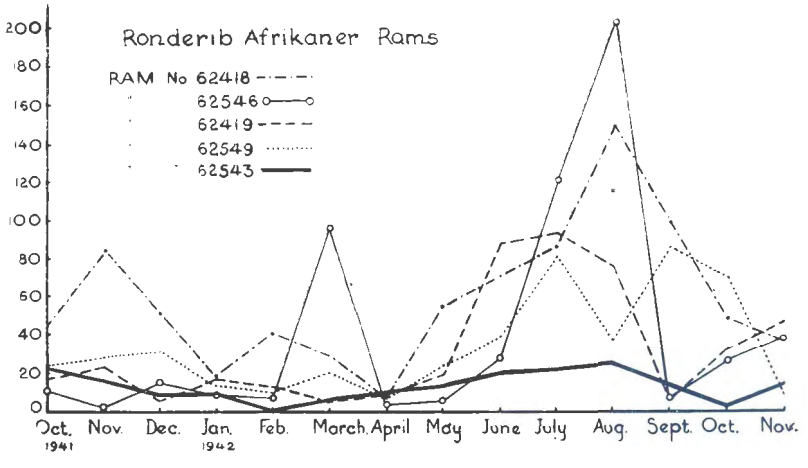
Gunn's (1936) electrical method of obtaining semen from rams was of great value in this work. Collection by any other method would have been impossible from a number of the rams used. The Ronderib Afrikaners especially, could neither be induced to serve ewes in oestrus nor to ejaculate into an artificial vagina. Training them would have taken too long for their inclusion in the experiment. Semen collected by the electrical method is well suited for detailed study and for artificial insemination.

It is important in handling semen samples from a number of animals that the same procedure and methods are adopted in order to insure uniform results during a particular experiment. Examinations for initial motility should be made as soon as possible after collection. In this experiment this was done within half an hour. Distinct differences in the various semen characteristics were noticed in ejacula of different rams. Thus some gave semen of greater volume, others higher sperm concentrations, and again others had a more constant initial motility rating. Nevertheless, the more fertile rams had a more consistent sperm picture than the less fertile ones which showed considerably more variation in semen characteristics.

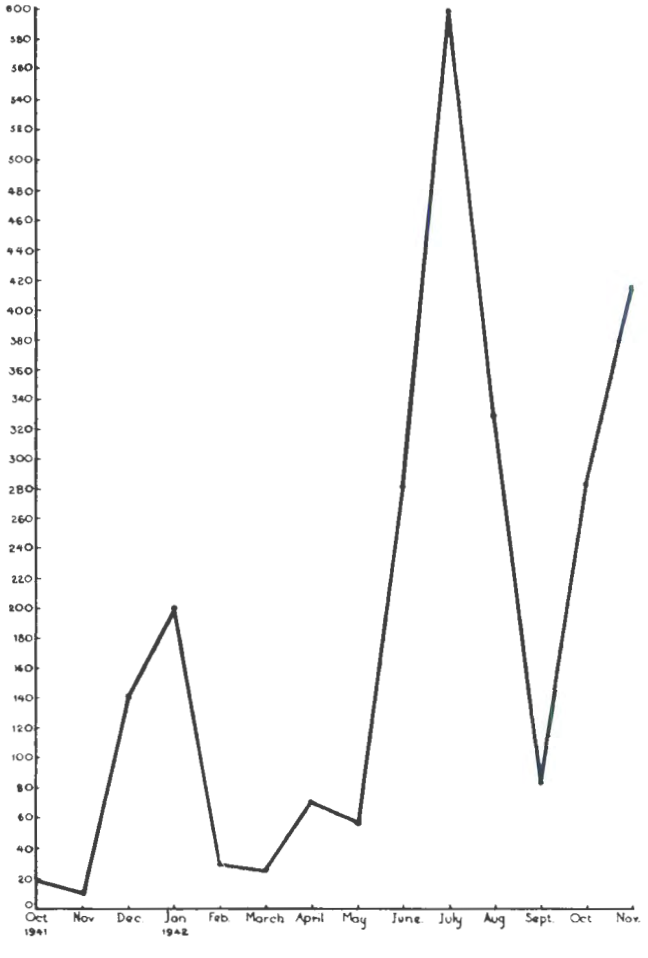
Too few rams of the different breeds were represented to justify conclusions being drawn in regard to breed differences. Only the Merinos and Ronderib Afrikaners could possibly be considered in this respect. The latter as a group were certainly more consistently good and gave a larger semen volume, higher sperm concentration and a greater total number of spermatozoa, but they were younger rams, and the possibility that the age factor might have contributed appreciably towards the better semen cannot be ignored.



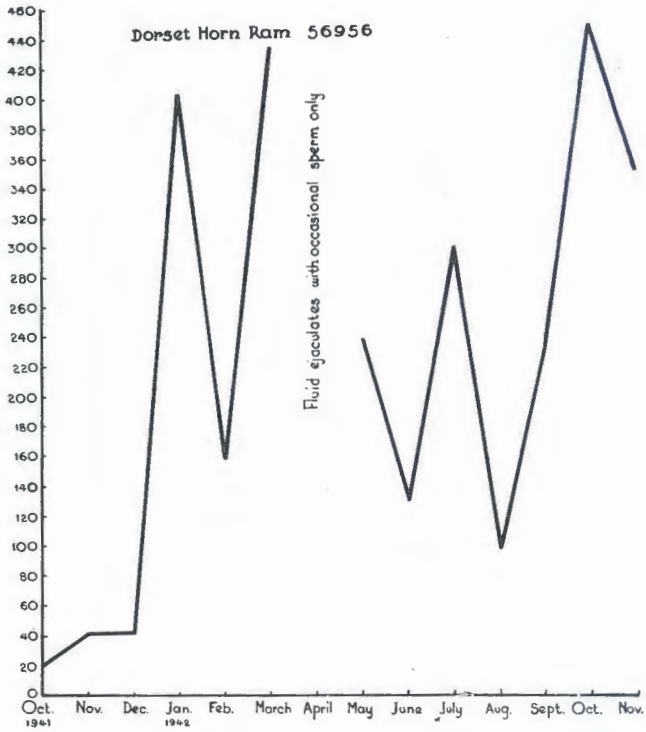
GRAPH 1.—Merino Rams. Showing seasonal variations in the proportion of abnormal spermatozoa.



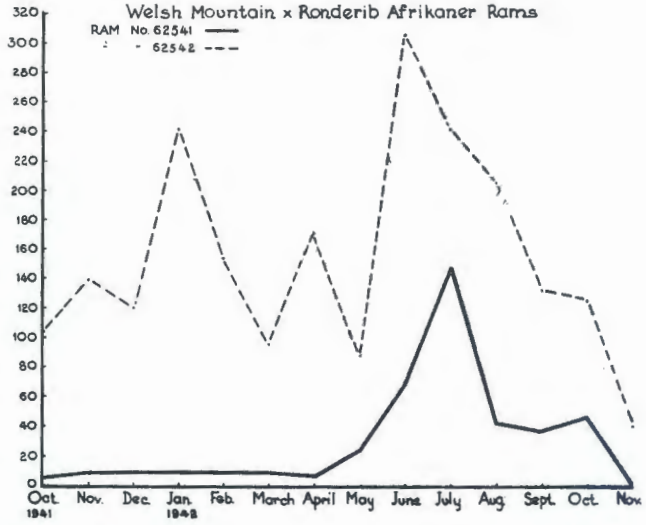
GRAPH 2.—Ronderib Afrikaner Rams. Showing seasonal variations in the proportion of abnormal spermatozoa.



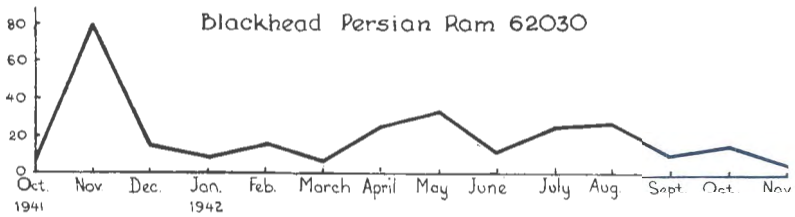
GRAPH 3.—Romney Marsh Ram No. 56947. Showing seasonal variations in the proportion of abnormal spermatozoa.



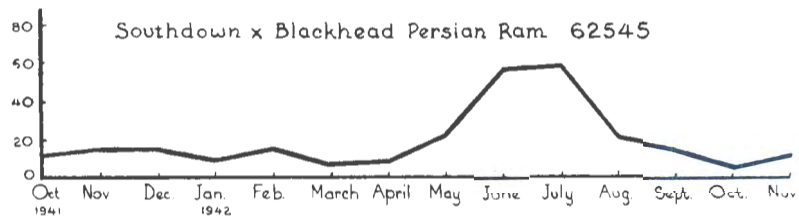
GRAPH 4.—Dorset Horn Ram No. 56956. Showing seasonal variations in the proportion of abnormal spermatozoa.



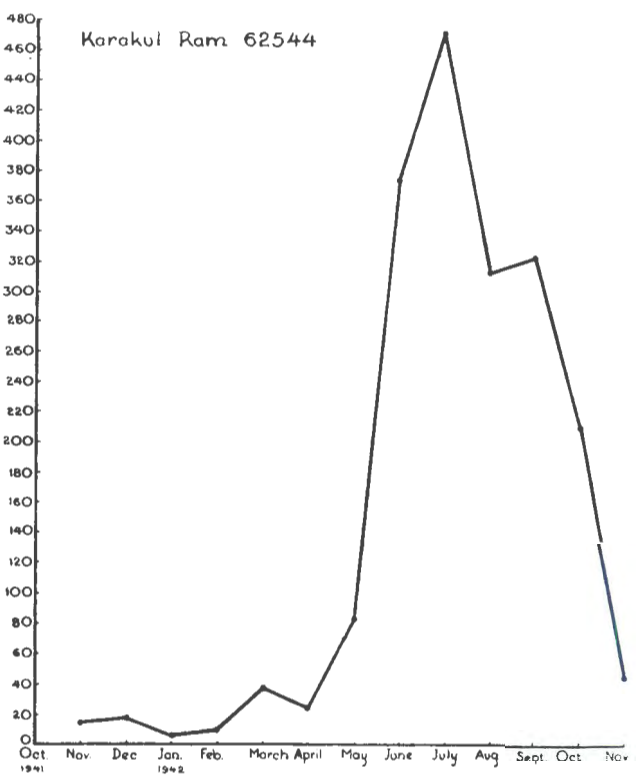
GRAPH 5.—Welsh Mountain—Ronderib Afrikaner Rams. Showing seasonal variations in the proportion of abnormal spermatozoa.



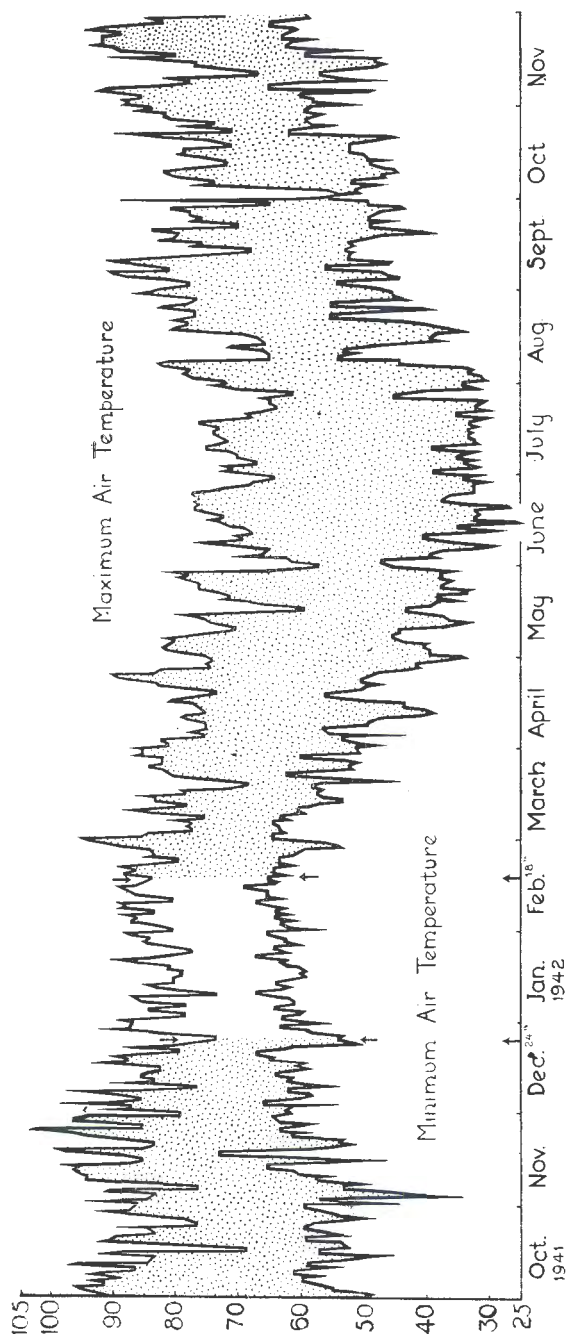
GRAPH 6.—Blackhead Persian Ram No. 62030. Showing seasonal variations in the proportion of abnormal spermatozoa.



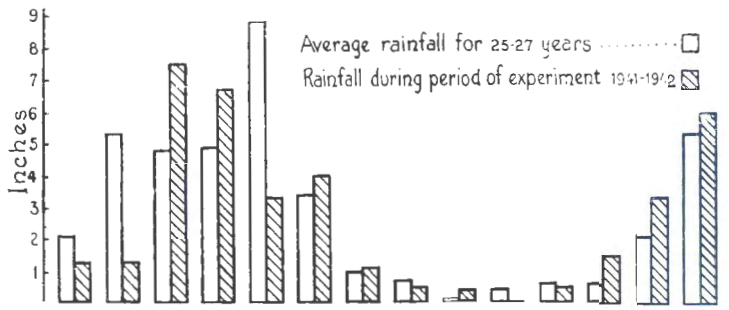
GRAPH 7.—Southdown—Blackhead Persian Ram No. 62545. Showing seasonal variations in the proportion of abnormal spermatozoa.



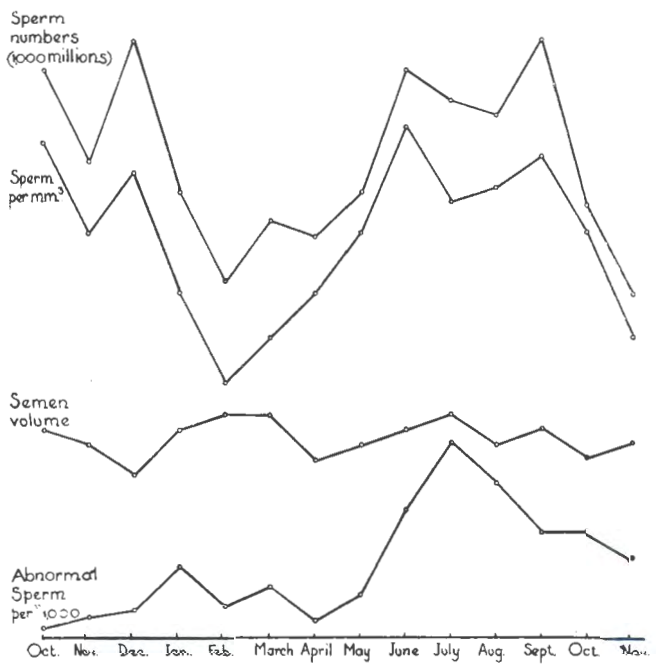
GRAPH 8.—Karakul Ram No. 62544. Showing seasonal variations in the proportion of abnormal spermatozoa.



GRAPH 9.—Showing the maximum and minimum air temperatures at Onderstepoort. The unstippled portion represents the temperatures at Pretoria, seven miles south.



GRAPH 10A.—The rainfall during the period of the experiment, and the average rainfall for 25-27 years.



GRAPH 10B.—The combined average graphs of the different semen characteristics of all the rams, their relationship to each other and to the seasons.

Good initial motility was as a rule found in semen with satisfactory sperm concentration, and poor motility could be expected when the concentration was low. The converse was, however, not necessarily the case, for some infertile ejacula were highly concentrated, but showed very poor motility. High abnormality sperm counts have been found in semen with good, as well as with low, concentration. Semen volume could be correlated with neither initial motility, sperm concentration nor with the number of abnormal spermatozoa. The total number of spermatozoa in an ejaculum, calculated from sperm concentration and semen volume, was more closely related to the former than to the latter. Longevity tests on samples of different quality semen were not carried to conclusion because of failure of the refrigeration plant and definite conclusions cannot be drawn, but the poor quality samples had either ceased to be motile sooner, or their motility rating had been more rapidly reduced, than the better samples.

In a few ejacula from different rams no abnormal spermatozoa could be found. This was, however, the exception for no matter how good the ram a few abnormal forms were as a rule found. The aberrant types most consistently present in greater or lesser numbers were tailless heads, coiled tails and defectively stained anterior head caps. Provided all due care was exercised in handling semen and in the preparation of smears, separation of the heads and tails did not readily occur in healthy spermatozoa. Large numbers of free heads were usually associated with rams whose fertility was generally poor or in cases where fertility had been reduced by a gross disturbance of testicular activity. Of all the abnormal spermatozoon types the proportion of coiled tails showed the greatest variation. Sudden significant increases and decreases in the number of coiled tails in successive ejacula of the same ram have been experienced, but when this occurred it was observed that the coiling involved mainly the free ends of the tails, and that middle piece beads were also evident. An extreme example of this kind was given by the Karakul ram 62544, in his semen from June to the middle of October, during which period an average of 350 abnormal spermatozoa per ejaculate were counted. Coiled tails and middle piece beads averaged respectively 72 per cent. and 17 per cent. of that total. During these months when the high abnormality counts were registered the ram's fertility was lowest (62 per cent. pregnancies); but if his semen were judged solely by the high total number of abnormal spermatozoa the ram should have been almost completely sterile. The more severe types of coiling which involve the middle piece, and when the tails were closely wrapped round the sperm heads were observed mainly in the semen of rams with poor fertility. It is therefore considered that the type of coiled tails is of greater significance in relation to a ram's breeding potentialities than the total number.

Although rams with reasonably good and good fertility occasionally gave semen with relatively high abnormality counts, such numbers were not maintained, and the total count was raised as a result of an increase in the few abnormal types usually present. When a ram's breeding capacity was reduced due to derangement of spermatogenesis the total number of abnormal spermatozoa was not only increased, but the different types of abnormal cells were also increased. The remarkable uniformity in the size of the sperm heads which is so characteristic in good semen was lost and different degrees of variation became apparent. Pyriform heads, narrow heads, small heads and enlarged middle pieces which are found in normal

semen in insignificant numbers, if at all, are closely associated with semen of poor quality. The total number of abnormal spermatozoa in poor semen was therefore not so much the sum of two or three abnormal types, but of a greater number of different types.

The examination of stained semen smears for sperm morphology is of great value, and when it is combined with an examination for motility and sperm concentration a very accurate prognosis of a ram's breeding capacity can be made. A single examination may lead to erroneous conclusions however, because even a good ram may give an occasional unsatisfactory ejaculum. Two or three examinations of semen collected at weekly intervals should give the true sperm picture of a particular ram. It has been shown by various investigators that high temperatures are destructive to the vitality of spermatozoa *in vitro*. Degeneration of testicular tissue occurred when the temperature of the testicles was raised by scrotal insulation and when rams were subjected to artificial hot atmospheres. Gunn and co-workers in Australia, and McKenzie and Berliner in America, showed that changes in climate conditions due to season affected spermatogenic activity in rams and that high atmospheric temperatures caused seminal degeneration. Anderson (1941 *a*) in Kenya noted a seasonal variation in the motility and volume of the ejaculates of the bull. Both these semen characters, but particularly the volume, showed a decrease from May to August.

All the rams used in this work were not affected to the same extent by seasonal changes in climatic conditions. Seven ejaculated greater numbers of abnormal spermatozoa during the period of high atmospheric temperatures: the remainder gave higher abnormality counts in winter than in summer. The mean semen volume was lowest in December and highest in the months of February, March and July. A definite correlation between semen volume and season could not be made out from the records. Sperm concentration and total sperm numbers were closely correlated with each other and with the various seasons. The high concentrations recorded during winter and spring were gradually reduced as summer advanced to their lowest in February. This low mark was followed by a steady increase through autumn to the highest peak in June. Initial motility was affected by the quality of individual ejacula and by other factors operating at the time of collection. Variations in initial motility due purely to seasonal changes could not be established.

In general, those rams whose semen characteristics varied most between ejaculates, and those that generally gave bad quality semen and whose breeding records were poor, were most readily affected by adverse seasonal changes.

X. Summary and Conclusions.

1. The sperm picture of rams of different breeds has been studied.

2. Semen was collected by Gunn's electrical method.

3. The Ronderib Afrikaners were superior to the Merinos in semen characteristics. This may have been due to the fact that the former were younger rams.

4. There were too few rams of the other breeds represented to enable an opinion with regard to breed differences to be expressed.

5. A morphological study of spermatozoa was of great value and could be correlated with a ram's fertility. It should, however, be considered together with the other semen characteristics.

6. When all the semen characteristics are considered together, a very accurate prognosis of a ram's breeding potentialities is possible.

7. The total number of abnormal spermatozoa in an ejaculum was not as good an indicator of spermatogenic activity as the *type* of abnormal cell.

8. Rams with poor fertility ejaculated more different types of abnormal spermatozoa than normal rams.

9. Variations in semen characteristics of different ejacula of the ram have been noted.

10. Semen of the less fertile rams showed greater variations than that of highly fertile rams.

11. Examination of two or three ejacula from an individual ram at weekly or fortnightly intervals is necessary in order to predict his breeding potentialities. A single examination may lead to erroneous conclusions.

12. The types of abnormal spermatozoa most consistently found in lesser or greater numbers in semen of all classes were tailless spermatozoa, coiled tails, and spermatozoa with defectively stained anterior head caps.

13. Pyriform heads, narrow heads, enlarged middle pieces, filiform middle pieces, middle piece beads and variation in spermatozoon head sizes were relatively rare in good semen, but contributed appreciably towards a poor sperm picture.

14. Spermatozoa with coiled tails, in which the coiling involved mainly the ends of the tails, have been observed in semen in large numbers, without a corresponding reduction in fertilising capacity of the semen.

15. High quality semen had good initial motility, good sperm concentration and few abnormal spermatozoa.

16. Poor quality semen may have a large volume and a high sperm concentration.

17. Seven of sixteen rams gave higher numbers of abnormal spermatozoa in summer than during any other season; in the remainder the abnormality counts were highest in winter.

18. Sperm concentration and total numbers were highest in winter and lowest in late summer.

19. Semen volume could not be definitely correlated with seasonal changes.

20. Initial motility was not affected by seasonal changes.

21. In general rams with bad sperm pictures and poor fertility were more readily affected by climatic conditions than normal rams.