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Observations on Artificial Insemination of Sheep with Fresh and Stored Semen.

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THE economic possibilities of artificial insemination in the production of livestock have stimulated intensive research throughout the world during the past ten years. At first researches into artificial insemination were mostly confined to workers in Soviet Russia, where the urgency for the rehabilitation of livestock, subsequent to the termination of the 1914-1918 war, suggested the necessity for the utmost use of sires with desirable blood. Artificial insemination, if successful, would extend the use of proven sires considerably, and it would solve, to a great extent, the problem of rapid rehabilitation of the livestock industry with which Soviet Russia was faced.

The Soviet Government founded a Central Experimental Breeding Station at Moscow in 1919, and intensive study of the possibilities of artificial insemination was begun in 1923 under the direction of Prof. E. I. Ivanov.

The early publications of the workers at the Institute for Artificial Insemination, at Moscow, stimulated research which was taken up rapidly by most countries which were interested in the improvement of livestock. These researches have made considerable progress; so much so that artificial insemination in the production of livestock has long since passed the experimental stage. The technique of the operation has been so perfected that the resultant conceptions, when fresh semen is used, is comparable with that following normal copulation. Artificial insemination appears to have become a routine practice in the production of livestock in Soviet Russia, where thousands of animals are inseminated annually. It is claimed that there has been a rapid improvement in the livestock of that country since its introduction. Goetze (1939), however, points out that the visualisation in this direction has not nearly been reached. Artificial insemination has been successfully employed in the livestock industry in the United States of America, Italy, Holland, Denmark, Australia, Kenya, and to a limited extent in South Africa. Its chief adaption in most countries would appear to be to mares and cows. However, in sheep breeding countries it will be found eminently suitable for reproduction should the indications for its employment outweigh the contra-indications.

Recent researches have been practically confined to improvement in the methods of obtaining semen, its storage, and its transport.

Observations on artificial insemination, especially with sheep and horses, have been made in this country since 1932. Quinlan, Maré and Claassens (1936) published a preliminary report on their observations with fresh and stored semen in merino ewes. Quinlan (1936) amplified the previous report in an unpublished paper read at a joint meeting of the Medical Faculty of the Witwatersrand University and the Veterinary Faculty of the Pretoria University held at Onderstepoort. It has become a routine procedure in the Government flocks of large-tailed sheep, Ronderib Afrikaner and Karakul, to use artificial insemination owing to the difficulty which some rams find in serving ewes with the extreme tail-development peculiar to these breeds (Quinlan, Claassens, Bonsma and Rose, 1939, Engela, 1940). The results obtained, when fresh semen is used, compare favourably with those following controlled mating with "hand-service": 47·5 per cent. of pregnancies were obtained when using fresh semen, as compared with 57·5 per cent. by "hand service". Only on insemination and one service was allowed during a single oestrous period. With semen stored for 3 hours at 8° C. to 13° C., 33⅓ per cent. peregnancies were obtained. No pregnancies were obtained with semen stored for 6, 9, 12, 24 and 105 hours.

These results compare very unfavourably with the results obtained by other workers. However, it must be pointed out that only one insemination was made at a single oestrous period. No doubt the results would have been better if two inseminations had been given during oestrus, and if the operation had been repeated in cases of oestrous recurrence.

The time of insemination during oestrus is important as it is desirable that highly vital spermatozoa should be present in the Fallopian tubes awaiting the arrival of an available ovum. Quinlan, Maré and Roux (1932) obtained satisfactory results in merino ewes by normal matings up to the 33rd hour following the onset of oestrus. Matings at the 36th, 39th and 42nd hour resulted in only 50 per cent. of pregnancies, while only one pregnancy resulted from the matings at the 45th hour. Kelley (1937) found that spermatozoa, even from the most fertile rams, did not remain fertile in the genital tract of the ewe for longer than 34 hours. Quinlan and Maré (1931) estimated that ovulation occurred in the merino ewe about the thirty-sixth to the fortieth hour following the onset of oestrus. Therefore if sheep are mated immediately they show oestrus the spermatozoa must survive at least thirty-six to forty hours. However, it must be indicated that spermatozoa handled as for artificial insemination may have a lowered vitality in the female genitalia and consequently a lessened impregnating capacity.

The possible influence of copulation in hastening ovulation can be overlooked in Quinlan and Maré's experiments, as the sheep which were slaughtered to observe the time of ovulation had copulated with vasectomised teasers. It is not known to the authors whether observations have been made on the influence of coitus on the time

of ovulation following the onset of oestrus in sheep, but recent work done on heifers by Quinlan, Bisschop and Adelaar (1941) has shown that coitus probably hastens ovulation.

Kardymovic (1937) says that about 20 hours from the onset of oestrus is the most favourable time for the insemination of merino sheep. Winters *et alia* (1938) give the optimum time as about 10 hours from the termination of oestrus and suggest two inseminations during one heat period to raise the percentage of pregnancies. Buchanan-Smith (1939) recommends insemination towards the end of oestrus and suggests the second day as the optimum time. Since the exact time of ovulation is not known in the different breeds of sheep the necessity for further study on sex physiology is pointed out by Phillips (1939). This hiatus in the knowledge of the physiology of the genitalia has been filled in under the environmental conditions prevailing in certain sheep breeding areas in South Africa, and the results will form the subject of an early report.

The effectiveness of repeated insemination of sheep during one oestrous period has been shown by Avramov (1937), Peregon (1936) and Gavrilov (1937). When the interval between inseminations was 24 hours the lambing rate was increased by 11·7 and 14·1 per cent Gavrilov suggests that the second insemination should be carried out later than 12 hours after the first. Peregon recommends two inseminations at intervals of 20 to 30 hours.

The resulting percentage of pregnancies is easily influenced by environmental factors during storage. One of the most important of these factors is temperature. Spermatozoa do not survive long at room temperature in this country, where shade temperatures of around 90° F. and over are frequently recorded between September and April. It has been observed that a temperature not far above 0° C. is the optimum for storage of ovine spermatozoa. Storage, even for short periods, at room temperatures, reduces the impregnating capacity of spermatozoa even though they retain active motility. Attempts at storage at temperatures of between 10° C. to 12° C. have failed to maintain sperm activity for longer than 6 days. The spermatozoa from the same rams when stored at temperatures between 2° C. to 4° C. have shown activity up to 34, 46 49, 50, 51, 54, 66, 72 and 82 days. In one case longevity up to 100 days was recorded.

During recent years very extensive literature has appeared on the influence of temperature and other environmental factors on the vitality of spermatozoa. Bonadonna (1939), who sent a questionaire, requesting information, to the workers in every country where experiments on artificial insemination were being conducted, quotes the principal works relating to storage and shipment, and no attempt is made to give a complete review of all relevant literature in this report.

Bonadonna indicates that the optimum conditions for the prolonged storage of semen include low temperature, the reduction of spermatozoal movement, the maintenance of sufficient amounts of nourishing material (glucose) and of material able to neutralise, as much as possible, the toxic effects of the products of metabolism, thus effecting a balance of the H-ion concentration.

The pH of normal ovine semen, tested within half an hour after ejaculation, is acid. The pH range lies within 6·3 to 5·8. When fresh semen is stored at room temperature there is an increasing acid reaction. This change is apparently due to the products of metabolism, because it is less rapid when the sperm activity is lowered by reduction in temperature. Our tests have been made with a Beckmann pH meter with glass electrodes. The following is a typical example of the observations made. The semen of ram 45106 just after ejaculation on 17.5.40, showed a pH of 6·3; on 24.5.40 it was 5·9, and after 32 days it was 4·4 with sperm motility still fairly active. According to Webster (1938) the semen of highly fertile rams is acid with very rare exceptions. When testing the semen of rams for fertility by means of artificial insemination the most successful results were obtained with semen having a pH range between 5·00 and 6·50. A very slight acidity indicated low fertility, while alkaline samples were from infertile rams. Moskoitis (1934) states that the best pH for dilutors is 7·3 to 7·5, while Lardy and Phillips (1939) give the optimum-pH for storage as 6·72 to 6·80. The experience under the environment prevailing at Onderstepoort is that there is a gradual increasing acidity with stored normal ram semen until active motility of the spermatozoa is reduced by cooling in the refrigerator.

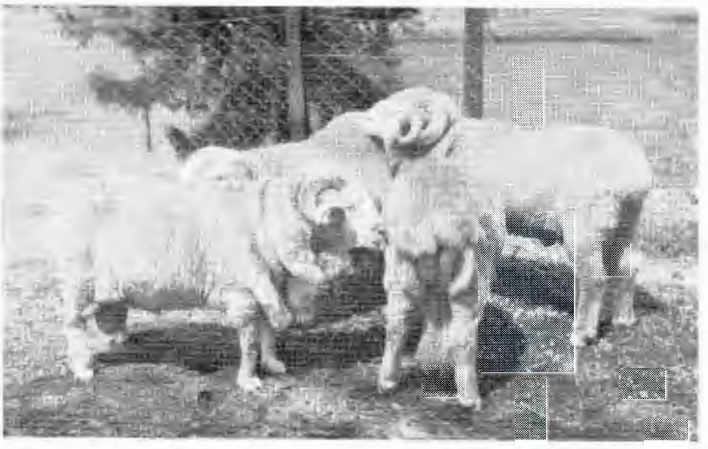

Fig. 1.—Photograph of some of the merino rams used.

The method of obtaining semen is also most important for the optimum longevity in storage. The utmost care must be taken to ensure absence of contamination. The method at present most favoured in sheep is the artificial vagina, which for practical purposes appears to supply all requirements.

Williams and Savage (1927) have remarked upon the resistance of spermatozoa to decomposition. In one instance the longevity of spermatozoa in contaminated semen was 43 days. Baker (1929, 1931, 1931, 1932) thinks that contamination imposes a definite but not very marked limitation on the longevity of spermatozoa in vitro.

In our experiments an increase in the bacterial content of stored semen, although not incompatible with sperm activity, has quite definitely limited longevity when compared with semen in which bacterial life was not so active.

In our observations the semen has been stored by means of the Cambridge method described by Walton (1936). Attempts have been made to correlate longevity in vitro with the fertilising power of spermatozoa. During these studies it has been noted that active motility is no criterion in determining the fertilising power of stored semen. Habibullin (1938) has made similar observations. The longest period during which spermatozoa have remained motile was 100 days. Several specimens were found active up to forty-six to fifty-four days. Ovine spermatozoa in vitro have been observed by workers in other countries to be active after several days: Lebedeva (1934) 29 days, Gunn (1936) 28 days, Bonadonna (1939) 569½ hours.

Fig. 2.—Photograph showing the type of merino ewe used: Taken just after shearing.

The results of artificial insemination with stored sperm, in our hands, have been disappointing since highly active, morphologically unchanged spermatozoa have failed to impregnate after 72 hours. Other workers have had more encouraging results: Goetze (1933) was successful with ovine semen stored for 94 hours, Phillips, Schott and Gildow (1938) 115 hours, Bonadonna (1939) 53 to 69 hours. Gunn (1936) was successful with semen stored for 24 hours at -5° C., 6 hours up to 18° C. and 2 hours at 18° C., followed by 30 hours at 6° C. (total 32 hours). Winters et alia (1938) state that at Minnesota several ewes have been successfully inseminated with sperm four and five days old and two sheep with sperm stored for six days.

Attempts to transport stored semen have been successfully accomplished, but to a limited extent. Successful transportation of sheep semen has been done by Walton and Prawochenski (1936). Sheep semen was sent from Cambridge to Warsaw. Two of five

sheep artificially inseminated became pregnant. The duration of storage was 51 hours. Bonadonna (1939) succeeded in impregnating four out of seven ewes with semen sent from Milan to Monza, the journey taking seven hours. Phillips (1939) (the work was done co-operatively by Dr. R. W. Phillips, Dr. E. M. Gildow, Dr. R. I. Schott and Dr. C. E. Terrill) records the transportation of ram semen by air express from Dubois, Idaho to Moscow, Idaho (700 miles), Beltsville, Maryland to Moscow (2600 miles), and in both directions between Beltsville and Dubois (2250 miles). The time in transit occupied 19 to 74 hours. Of 309 inseminations 31 resulted in pregnancies. The oldest semen used successfully was 115 hours. Kulesenko (1937) successfully transported semen 300 Kilometres. All the semen used was not transported, but his resulting fertility is remarkable: 15,000 sheep were inseminated with the semen obtained from one ram and the percentage of barrenness was not more than 1-2. Phillips says that his results indicate that the practicable use of ram semen, where storage periods of one day or more are necessary, will be limited until technique for storage is improved. The methods employed in packing for transport are more or less similar to that recommended by Walton and Prawochenski (1936). The semen is put into narrow test tubes, covered with a layer of sterile liquid paraffin and corked, no air space being left. This tube is placed in a container to prevent breaking. The container is then put into a thermos flask filled with ice and water. Bonadonna (1939) suggests the addition of a gluco-phosphate solution (0·1 to 0·2 c.c. for each c.c. of semen) for sheep semen.

Successful attempts have been made to transport gelatinised spermatozoa in capsules: Milovanov, Najarnyi, Sivokorj and Malohov (1937) and Razumov (1938).

No attempts have been made to transport ovine spermatozoa for use in artificial insemination in this country.

In our observations it has been noted that only highly active spermatozoa will establish pregnancy. It has been suggested that stored spermatozoa used for artificial insemination may produce weak offspring (Bonadonna, 1939). One deformed lamb was born in our experiments (see Table 4) but all others were healthy lambs, of normal weight and their post-natal development was excellent (Fig. 3).

It appears highly probable that stored spermatozoa capable of fertilisation will produce healthy offspring. Bonadonna (1939) recommends, from a practical point of view, that ovine sperm should not be stored for longer than 30 to 50 hours prior to use. This recommendation was made after a review of the most important publications published up to 1939.

The methods of collecting ram semen for artifical insemination have been so frequently described within recent years that it is unnecessary to repeat them: Anderson 1937, Winters et al. 1938, Walton 1938, Webster 1938, Gunn 1936, Goetze 1939, Brady and Gildow 1939 and others. Most workers are in agreement that collection by means of the artificial vagina is best suited for the ram. It involves little labour and trouble and a specimen free from

contamination can be obtained. Brady and Gildow (1939) say that the artificial vagina is the most satisfactory for rapid and easy collection. However, electric stimulation is most useful when semen has to be collected from several rams or when a ram will not or cannot copulate with the artificial vagina. A most suitable collector is that described by Walton (1938). The pattern described by

Fig. 3.—Photograph showing some of the lambs born from experimental ewes.

Fig. 4.—Photograph of ewe in service bail.

Webster (1938) is also very suitable, but with Walton's artificial vagina there is no necessity for insertion into the vagina of the ewe. Rams trained to serve sheep in a service bail, as shown in Fig. 4, will use the artificial vagina without difficulty. Rodin (1934) indicates that the temperature of the artificial vagina must be maintained at

approximately 40 – 41° C. to ensure ejaculation. Brady and Gildow (1939) and Goetze (1939) have also made similar observations. Walton (1938) says that the temperature of the artificial vagina at the moment of collection should be between 40° C. and 45° C. At a lower temperature ejaculation may not take place, while a higher temperature may be detrimental to the spermatozoa. When using semen obtained by electric stimulation, as suggested by Gunn (1936), the resultant conceptions do not appear to be so satisfactory as those obtained by collection in the artificial vagina. Kelley (1937) had only twelve pregnancies from fifty-five sheep when using semen obtained by electric stimulation, as compared with forty-nine out of seventy-nine " hand served ", and seventy-two out of ninety-three " paddock " mated. Kelley, however, points out that his ewes were not inseminated under ideal conditions.

Material and Method.

Two hundred young sexually mature merino ewes between the ages of $1\frac{3}{4}$ and 3 years, were placed under observation for oestrus. They were tested for oestrus daily at 8.30 a.m. by using vasectomised teasers. The sheep were run under " dry lot " conditions in two kraals, each measuring 75 yards by 60 yards; a wood and iron shelter was provided in each kraal. Fresh water from the tap was always available. The ration consisted of crushed yellow maize, 3/4 lb.; crushed oats, $\frac{1}{4}$ lb.; lucerne and veld hay *ad lib.;* green food, either lucerne or barley, *ad lib.;* bone meal, $\frac{1}{4}$ oz. and salt $\frac{1}{8}$ oz. The concentrate ration was given in equal amounts twice daily. Green food was fed once daily in the forenoon. The hay was always available.

All the sheep had been subjected to routine vaccination against Blackleg, Anthrax and Bluetongue. At the commencement of the observation the sheep were in moderate condition, but they improved rapidly and maintained excellent condition throughout (Fig. 2). They carried approximately six months wool when the observations began.

All ewes were marked over the rump with the sequence numbers, and their group number was marked over the back. The laboratory registration number, which was recorded by ear-tagging, acted as a check. Nine vasectomised teasers were used for testing. These were half-bred Welsh-Mountain × Ronderib Afrikaners, a cross which makes an excellent teaser.

Testing for oestrus was done daily, except Sunday, at 8.30 a.m. The sheep were driven into a collecting pen, and then passed in batches of about twenty through a teasing pen in which four or five vasectomised teasers were placed. The teasers were rested periodically when it was considered necessary. Ewes showing oestrus were taken out and placed in a small kraal, which contained a brick-walled shelter with a galvanised iron roof, where the inseminations were carried out. Only sheep which accepted coitus willingly were considered as being in oestrus. All inseminations or normal matings, in the case of the control group, were done within six hours from the time of testing.

It is not possible to estimate with any degree of accuracy the duration of oestrus at the time of insemination or normal mating. It may have begun at any time within the previous twenty-four hours.

After insemination or normal mating the ewes were returned to the original camp where they were submitted to the daily routine testing until they were considered pregnant or had been inseminated or mated for the third time at successive recurrences of oestrus.

The ewes were divided into ten groups of twenty sheep each: Group 1 was a control group in which normal mating was carried out: Group 2 was inseminated with fresh semen within fifteen minutes following ejaculation. The remaining seven groups 3 to 9, were inseminated with stored semen, kept in vitro at a temperature of between 2° C. to 4° C. for 6, 12, 18, 24, 48, 72 and 96 hours. Group 10 was inseminated with semen stored from 5 to 11 days. The data are recorded in Tables 1-10. Thirteen merino rams were used for mating and as donors for obtaining semen for insemination. Prior to the commencement of the observations these rams' semen and spermatozoa had been tested and proved to be physically and morphologically normal. The semen was collected in an artificial vagina (Holborn Instrument Coy. type, as recommended by Walton, 1936). Prior to use the rubber lining was washed with soap and water. It was then rinsed thoroughly with running water to remove all traces of soap and finally swabbed with 65 per cent. alcohol and allowed to dry before being used.

Before use warm water was placed in the jacket so that the temperature was about 104° F. to 105° F. Air was pumped in to bring it up to the required pressure. If the pressure of the artificial vagina was not adjusted correctly, or if the temperature was too high or too low ejaculation did not usually take place. Both factors can be controlled quickly and accurately by an experienced worker.

When collecting semen by means of the artificial vagina it is necessary to have the ewe well controlled in a service bail, such as that shown in Fig. 4. The ewe stands firmly secured with the hind quarters sufficiently clear for the ram to mount.

When training rams to use the artificial vagina it is advisable to allow them to copulate normally with ewes showing oestrus, which are confined in the service bail. With trained rams any ewe can be used for collection whether she is in oestrus or not.

After fastening the ewe in the service bail the artificial vagina was prepared for use. The ram was then admitted to the house. He usually tried to mount immediately. When mounting the penis was deflected into the artificial vagina which was placed alongside the right flank of the ewe, as shown in Fig. 5. The penis was not touched with the hand, the deflection being done by grasping the sheath. After ejaculation the pressure in the artificial vagina was immediately released by opening the stop-cock. It was held upright so that the semen ran into the glass container.

If the semen was to be used for immediate insemination it was diluted in the container and withdrawn from there. If it was to be stored it was removed from the container with a sterile pipette and transferred to narrow test tubes, 3 inches by $\frac{3}{4}$ inch, and covered with sterile medicinal liquid paraffin. This is the treatment for storage of semen recommended by Walton (1936). It was then left to cool to room temperature for about twenty minutes. The tubes were then placed in a water bath at room temperature and transferred to the refrigerator.

Fig. 5.—Photograph of ram copulating with the artificial vagina.

The influence of various temperatures on the longevity of the spermatozoa was tried. It was found that the optimum temperature for the preservation of life and activity of ovine spermatozoa *in vitro* was between 2° C. and 4° C.

An extensive series of observations were made in order to ascertain the optimum environmental conditions for the preservation of maximum activity after storage, and those above described have given the most successful results. By this method live sperms were seen after a storage period of 100 days, and several specimens showed activity for varying periods up to 82 days.

The semen when being removed from the refrigerator for use was allowed to stand for about twenty minutes until room temperature was again attained. Before mixing the semen with the dilutor care was taken that both were of equal temperature.

The motility and sperm density of all specimens of semen used for artificial insemination were controlled, both at the time of collection and immediately prior to using. Only specimens considered to be of high quality were retained. The longevity of spermatozoa in specimens which were not entirely used for insemination was almost invariably observed after they were returned to the refrigerator. The semen used in group 2 was not subjected to microscopic examination prior to use as it was used immediately after collection and was known to come from highly fertile rams which were used throughout the observations.

The sperm dilutor used was made up according to the formula of Winters et al. (1938). The formula is as follows:

Glucose	50·00 grams.	pH 7.
KH_2PO_4	0·32 ,,	
Na_2HPO_4 $12H_2O$	1·54 ,,	
Dist. H_2O	1000 c.c.	
$CaSO_4$	Saturate.	

The semen was diluted four times for each insemination immediately before using. The instruments used were those recommended by Walton (1938): They were purchased from the Holborn Instrument Coy. Ltd., London. The syringe was an all glass type fitted to a vulcanite nozzle, 10 inches long. The vagina of the ewe was dilated with a vaginal speculum or a pyrex test tube, $\frac{3}{4}$ inch in diameter and 6 inches long, from which the end had been removed; the rough edge was smoothed in a bunsen flame. The speculum before insertion was lubricated with sterile liquid paraffin. It was inserted sufficiently deep to expose the opening of the cervical canal. Illumination of the interior of the vagina was obtained by means of an electric forehead lamp. The vulcanite nozzle of the syringe, containing the desired quantity of diluted semen, was inserted into the cervical canal to a depth of 3 to 4 mm. and the injection made into the lumen. The quantity of diluted semen used was 0·2 cc. to 0·4 cc. The whole injection does not remain in the cervical canal; a portion usually returns to the vagina. When penetration of the ostium externum was difficult the semen was deposited at the opening to the canal and on the pars vagina of the cervix; this method proved equally successful as injection directly into the cervical canal.

During insemination it is necessary that the ewe remains quiet. This can be effected by the use of an insemination crate, as shown in Fig. 6. This method is comfortable for the operator as the crate can be adjusted to the desired height. However, it necessitates raising the ewe into the crate for each insemination.

Fig. 6.—Photograph of ewe in insemination crate showing actual insemination in standing position.

A simple and less laborious method of holding the ewe is shown in Fig. 7. The sheep is held in the dorsal or dorso-lateral position on the sack placed on the ground. The operator kneels behind the ewe during the operation. By this method less assistance is needed and control is equally effective.

Fig. 7.—Photograph of ewe being inseminated in dorso-lateral position.

After each insemination the speculum was cleaned and wiped with gauze soaked in 65 per cent. alcohol. It was allowed to dry before being used for the next insemination. The outside of the vulcanite nozzle was similarly treated by wiping with gauze, moistened with 65 per cent. alcohol, after which it was wiped off with gauze moistened with dilutor.

Sheep which did not become pregnant to the first mating or insemination were subjected to similar treatment when they again showed oestrus after the termination of an ovarian cycle. These sheep were mated normally or inseminated three times in their respective group, once during each successive oestrus. In groups 8 and 9 some ewes were inseminated only once or twice, although they did not become pregnant. Failure to carry out the usual three inseminations was due to the fact that there was no semen of suitable age at the time oestrous recurrence appeared. After the third service or insemination, whether they became pregnant or not, the ewes were placed in a camp where they were kept under the closest observation for abortions. Towards the termination of the gestation period all ewes showing udder development were placed in a shelter where they were allowed to lamb. Records were kept of the percentage of conceptions from each service or artificial insemination, the number of services or inseminations required to establish pregnancy, the duration of gestation and the sex and weight of the lamb.

The following Tables 1 to 10 show in summarised form the results obtained.

Table 1.

Group 1.—Normal Service.

Ewe.		1st Service.		2nd Service.		3rd Service.		Date of Parturition.	Sex of Lamb.	Weight of Lamb. lb.	Duration of Gestation: Days.	No. of Services to Establish Pregnancy.	Remarks.
No.	D.O.B. No.	Date.	No. of Ram.	Date.	No. of Ram.	Date.	No. of Ram.						
43	55928	6/2/40	45219	24/2/40	45103	—	—	21/7/40	Male.....	8½	148	2	—
30	53334	6/2/40	45135	—	—	—	—	4/7/40	Female....	6½	149	1	—
110	55801	6/2/40	45135	—	—	—	—	6/7/40	Male.....	8½	151	1	Dystokia; lamb born dead during night.
72	55909	7/2/40	45301	26/2/40	45301	—	—	30/7/40	Male.....	9¾	155	2	—
24	55763	7/2/40	45301	24/2/40	45129	—	—	25/7/40	Male.....	9	150	2	—
112	53362	7/2/40	45301	20/4/40	45129	—	—	16/9/40	Female....	6½	149	2	—
113	53353	8/2/40	45301	26/2/40	45129	21/6/40	45106	—	—	—	—	—	Did not conceive.
114	55787	8/2/40	45301	13/3/40	45103	—	—	11/8/40	Male.....	6¾	151	2	—
115	55708	8/2/40	45301	27/2/40	45301	16/7/40	45106	20/7/40	Female....	7½	144	2	*N.B.*—This ewe allowed copulation 4 days prior to parturition: Lamb born dead during night.
15	55865	9/2/40	45301	27/2/40	45129	—	—	27/7/40	Female....	10	151	2	—
65	55888	9/2/40	45301	17/2/40	45135	6/5/40	45103	6/10/40	Female....	8	153	3	20/2/40 inseminated with fresh semen from ram 45129.
116	56980	9/2/40	45301	26/2/40	45103	—	—	26/7/40	Male.....	10	151	2	—
117	55903	9/2/40	45301	26/2/40	45129	—	—	27/7/40	Female....	10½	152	2	—
118	55779	10/2/40	45301	27/2/40	45129	8/5/40	45103	5/10/40	Male.....	8¾	150	3	—
119	49384	10/2/40	45301	27/2/40	45129	—	—	29/7/40	Female....	9	153	2	—
80	55716	10/2/40	45301	29/2/40	45307	24/4/40	45129	23/9/40	Female....	7¼	152	3	—
7	55882	14/2/40	45135	—	—	—	—	15/7/40	Female....	8	153	1	—
222	55835	21/6/40	45106	—	—	—	—	—	—	—	—	—	Did not conceive.

Males..... 7 43·75 per cent.
Females..... 9 56·25 per cent.
} Average weight, 7·1 lb.; Average gestation period, 150·75 days; Average number of services, 2.

Note:—

Percentage Conceptions for—

	Per cent.
1st Service	16·66
2nd Service	55·55
3rd Service	16·66
Non-pregnant	11·11

88·87 per cent. ewes lambed.
11·11 per cent. ewes did not conceive.

Ram.	*Service.* +	*Service.* —
45103	5	—
45106	—	2
45135	3	1
45219	6	3
45301	2	13
45307	—	1
	16	20

+ = Fertile. — = Not fertile.

TABLE 2.

Group 2.—0 hour: Inseminated immediately after the collection of Semen. Diluted 4 times.

EWE.		1ST INSEMINATION.			2ND INSEMINATION.			3RD INSEMINATION.			PARTURITION.			Gestation Period: Days.	No. of Inseminations to Establish Pregnancy.
No.	D.O.B. No.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Sex of Lamb.	Weight of Lamb. lb.		
45	33612	12/2/40	45131	7·34	29/2/40	45129	7·34	—	—	—	30/ 7/40	Female	9½	152	2
36	56977	12/2/40	45131	7·34	1/3/40	45103	6·99	23/4/40	45301	6·99	19/ 9/40	Female	6¾	149	3
120	51135	12/2/40	45131	7·34	29/2/40	45129	7·34	—	—	—	29/ 7/40	Female	8	151	2
28	55794	12/2/40	45131	7·34	29/2/40	45129	7·34	—	—	—	31/ 7/40	Male..	9½	153	2*
84	55707	12/2/40	45131	7·34	—	—	—	—	—	—	—	—	—	—	—
99	55751	12/2/40	45131	7·34	4/4/40	45103	6· 9	—	—	—	1/ 9/40	Female	8	150	2
51	55923	13/2/40	45131	7·34	2/3/40	45103	6·99	—	—	—	29/ 7/40	Male..	8½	149	2
32	55682	14/2/40	45135	6·99	20/3/40	45301	6·99	25/4/40	45219	6· 9	23/ 9/40	Female	8	151	3
70	55692	14/2/40	45135	6·99	1/3/40	45103	6·99	27/4/40	45308	6· 9	23/ 9/40	Male..	7¾	149	3
1	53599	14/2/40	45135	6·99	28/3/40	45103	6·99	—	—	—	25/ 8/40	Female	7½	150	2
4	55723	14/2/40	45135	6·99	2/3/40	45103	6·99	—	—	—	29/ 7/40	Female	8½	148	2
9	55810	15/2/40	45301	6·99	6/6/40	×	7·21	24/6/40	45095	6· 9	18/11/40	Male..	7½	147	3
2	55855	15/2/40	45301	6·99	17/2/40	45135	6·99	6/3/40	45219	6·99	6/ 8/40	Female	7¾	153	3
101	51110	15/2/40	45301	6·99	4/3/40	45103	6·99	13/5/40	45106	6· 9	30/ 9/40	Male..	8½	140	3
102	52583	15/2/40	45301	6·99	—	—	—	—	—	—	13/ 7/40	Female	8½	149	1
47	55904	16/2/40	45301	6·99	—	—	—	—	—	—	14/ 7/40	Male..	6½	149	1
104	55722	16/2/40	45301	6·99	—	—	—	—	—	—	14/ 7/40	Male..	7½	149	1
105	53172	17/2/40	45301	6·99	—	—	—	—	—	—	14/ 7/40	Female	8	148	1
106	55881	17/2/40	45301	6·99	—	—	—	—	—	—	15/ 7/40	Male..	8½	149	1
100	55876	17/2/40	45301	6·99	—	—	—	—	—	—	15/7/40	Male..	8½	149	1

* Dystokia: Lamb born dead during night.

Females..... 10 52·6 per cent.
Males..... 9 47·3 per cent.
} Average weight, 8 lb.; Average gestation period, 149·21 days; Average number of inseminations, 2.

NOTE:—

Percentage Conceptions for:—

1st Insemination, 30 per cent
2nd Insemination, 35 per cent.
3rd Insemination, 30 per cent.
Non-pregnant, 5 per cent...

95 per cent. Ewes lambed.
5 per cent. did not conceive

Ram.	Insemination. +	Insemination. −
45095	1	—
45103	4	3
45106	1	—
45129	3	—
45131	—	7
45135	—	5
45219	2	—
45301	7	4
45308	1	—
X	—	1
	19	20

Conceptions for dilutor pH.:—

	Conceptions. +	Conceptions. −
7·34	3	7
7·21	—	1
6·99	11	12
6·9	5	—
	19	20

\+ = Fertile. − = Not fertile.

TABLE 3.

Group 3.—Inseminated with Semen stored for 6 hours. Diluted 4 times.

EWE.		1ST INSEMINATION.			2ND INSEMINATION.			3RD INSEMINATION.			PARTURITION.			Gestation Period: Days.	No. of Inseminations to Establish Pregnancy.
No.	D.O.B. No.	Date.	Ram No.	Diluter pH.	Date.	Ram No.	Diluter pH.	Date.	Ram No.	Diluter pH.	Date.	Sex of Lamb.	Weight of Lamb. lb.		
17	55940	19/2/40	45135	7·21	20/2/40	45301	7·21	9/3/40	45129	6·9	—	—	—	—	—
18	55851	19/2/40	45135	7·21	8/3/40	45301	6·99	13/6/40	45095	6·9	—	—	—	—	—
107	55749	19/2/40	45135	7·21	6/3/40	45103	6·99	15/5/40	45103	6·9	14/11/40	Male..	6½	147	4*
108	55758	19/2/40	45135	7·21	8/3/40	45301	6·99	18/4/40	45103	6·9	21/10/40	Male..	7½	151	4†
109	57131	19/2/40	45135	7·21	6/3/40	45103	6·99	—	—	—	3/ 8/40	Male..	8½	150	2
42	55915	19/2/40	45135	7·21	9/3/40	45129	6·99	—	—	—	—	—	—	—	—
		20/2/40	45301	7·21	—	—	—	—	—	—	6/ 8/40	Female	8	150	2
155	53220	8/3/40	45301	6·99	12/4/40	45129	6· 9	—	—	—	8/ 9/40	Female	7¼	149	2
156	55678	8/3/40	45301	6·99	7/6/40	45219	6· 5	—	—	—	—	—	—	—	—
37	55733	13/3/40	45129	6·99	21/5/40	45301	6·99	—	—	—	—	—	—	—	—
66	47291	13/3/40	45129	6·99	—	—	—	—	—	—	13/ 8/40	Female	9	153	1
164	55866	13/3/40	45129	6·99	7/6/40	45219	6· 5	—	—	—	—	—	—	—	—
163	55859	13/3/40	45129	6·99	—	—	—	—	—	—	13/8/40	Female	10	153	1
14	55685	13/3/40	45129	6·99	20/4/40	45129	6·99	—	—	—	—	—	—	—	—
60	55818	1/4/40	45129	6·99	18/4/40	45103	6·99	—	—	—	14/ 9/40	Female	7½	149	2
173	55837	1/4/40	45129	6·99	—	—	—	—	—	—	28/ 8/40	Female	6½	149	1
174	49678	1/4/40	45129	6·99	18/4/40	45103	6·99	—	—	—	16/ 9/40	Male..	7¼	151	2
171	53473	1/4/40	45129	6·99	—	—	—	—	—	—	25/ 8/40	Female	7¾	147	1
172	55795	1/4/40	45129	6·99	—	—	—	—	—	—	28/ 8/40	Female	6	149	1
175	57296	1/4/40	45129	6·99	—	—	—	—	—	—	—	—	—	—	—
181	55729	19/4/40	45129	6·99	14/6/40	45095	6· 9	29/6/40	45301	6· 9	—	—	—	—	—

* 20/6/40 Insemination from 45301, pH. 6·9.

† 23/5/40 Insemination from 45103, pH 6·99. In these two sheep 4 inseminations were done. Both became pregnant to the 4th insemination.

Females.....8 = 66–2/3 per cent.
Males.....4 = 33–1/3 per cent.
} Average weight, 7·8 lb.; Average gestation period, 149·83 days; Average number of inseminations, 1·9.

NOTE:—

Percentage Conceptions for:—

	Ram.	Insemination. +	Insemination. −
1st Insemination, 25 per cent.	45095.....	—	2
2nd Insemination, 25 per cent.	45103.....	5	1
3rd Insemination, 0 per cent.	45129.....	7	9
4th Insemination, 10 per cent.	45135.....	—	6
Non-pregnant, 40 per cent..	45219.....	—	2
	45301.....	—	6
		12	26

60 per cent. ewes lambed.
40 per cent. ewes did not conceive.

Conceptions for dilutor pH:—

	Inseminations. +	Inseminations. −
6·5.....	—	2
6·9.....	1	4
6·99.....	11	12
7·21.....	—	8
	12	26

\+ = Fertile.
− = Not fertile.

TABLE 4.

Group 4.—Inseminated with Semen stored for 12 hours. Diluted 4 times.

EWE.		1ST INSEMINATION.			2ND INSEMINATION.			3RD INSEMINATION.			PARTURITION.			Gestation Period: Days.	No. of Inseminations to Establish Pregnancy.
No.	D.O.B. No.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Sex of Lamb.	Weight of Lamb. lb.		
200	55929	20/5/40	45095	6·99	5/6/40	45103	7·0	—	—	—	—	—	—	—	—
199	55936	20/5/40	45095	6·99	5/6/40	45095	7·0	6/6/40	45219	7·2	5/11/40	Male..	7¾	152	3
201	45774	20/5/40	45106	6·99	29/6/40	45301	6·85	—	—	—	24/11/40	Female	7½	148	2
202	55671	20/5/40	45095	6·99	7/6/40	45129	6·5	—	—	—	3/11/40	Male..	10¼	149	2*
203	49307	20/5/40	45106	6·99	—	—	—	—	—	—	8/10/40	Female	7	140	1†
		21/5/40	45106	6·99	—	—	—	—	—	—	—	—	—	—	—
204	55714	21/5/40	45106	6·99	—	—	—	—	—	—	16/10/40	Female	8½	148	1
205	53013	30/5/40	45131	6·99	17/6/40	45301	6·85	—	—	—	15/11/40	Female	5¾	151	2
206	55768	30/5/40	45131	6·99	—	—	—	—	—	—	28/10/40	Male..	7	151	1
207	50524	3/6/40	45095	7·21	—	—	—	—	—	—	30/10/40	Female	9½	148	1‡
		4/6/40	45103	7·21	—	—	—	—	—	—	—	—	—	—	—
208	55725	3/6/40	45095	7·21	—	—	—	—	—	—	—	—	—	—	—
209	53352	4/6/40	45103	6·99	—	—	—	—	—	—	30/10/40	Male..	7¾	148	1
210	55687	5/6/40	45095	6·99	9/7/40	45106	6·9	—	—	—	—	—	—	—	—
211	55819	7/6/40	45129	6·5	13/7/40	45106	6·9	—	—	—	9/12/40	Female	6¾	149	2
		6/6/40	45219	7·21	—	—	—	—	—	—	—	—	—	—	—
69	55745	7/6/40	45129	6·5	—	—	—	—	—	—	2/11/40	Female	8	148	1
19	55869	8/6/40	45129	6·5	12/7/40	45106	7·2	29/7/40	45549	6·9	25/12/40	Female	7	149	3
212	?	8/6/40	45129	6·5	12/7/40	45106	7·2	—	—	—	—	—	—	—	—
213	55674	11/6/40	45106	6·9	—	—	—	—	—	—	9/11/40	Female	8	151	1
214	55747	13/6/40	45106	6·9	—	—	—	—	—	—	8/11/40	Male..	7½	148	1
215	55939	13/6/40	45095	6·9	—	—	—	—	—	—	8/11/40	Male..	7	148	1
39	55753	13/6/40	45106	6·9	—	—	—	—	—	—	—	—	—	—	—

* Dystokia. Lamb died during night.

† Dystokia. Lamb born dead showed ascites and atresia of anus and vulva.
‡ Dystokia. Lamb born dead.

Males..... 6 = 40 per cent.
Females..... 9 = 60 per cent.
} Average weight, 7·68 lb.; Average gestation period, 148·53 days; Average number of inseminations, 1·53.

NOTE:—

Percentage Conceptions for :—

	Ram.	Insemination. +	Insemination. —
1st Insemination, 45 per cent.	45095.....	3	6
2nd Insemination, 20 per cent.	45103.....	2	1
3rd Insemination, 10 per cent.	45106.....	5	5
Non-pregnant, 25 per cent.	45129.....	2	3
	45219.....	1	1
	45301.....	2	—
	45549.....	1	—
		15	16

75 per cent ewes lambed.
25 per cent. did not conceive.

Conceptions for dilutor pH. :—

	Inseminations. +	Inseminations. —
6·5.....	1	3
6·85.....	2	—
6·9.....	5	2
6·99.....	4	6
7·0.....	—	2
7·2.....	1	2
7·21.....	2	1
	15	16

+ = Fertile.
— = Not fertile.

Table 5.

Group 5.—Inseminated with Semen stored for 18 hours. Diluted 4 times.

Ewe.		1st Insemination.			2nd Insemination.			3rd Insemination.			Parturition.			Gestation Period: Days.	No. of Inseminations to Establish Pregnancy.
No.	D.O.B. No.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Sex of Lamb.	Weight of Lamb. lb.		
57	49247	9/3/40	45129	6·99	30/3/40	45103	6·99	23/5/40	45095	6·9	—	—	—	—	—
16	55858	9/3/40	45129	6·99	27/3/40	45103	6·99	—	—	—	—	—	—	—	—
157	51681	9/3/40	45129	6·99	27/3/40	45103	6·99	30/4/40	45308	6·9	—	—	—	—	—
6	55884	14/3/40	45103	6·99	18/4/40	45103	6·99	22/5/40	45301	6·9	—	—	—	—	—
25	55863	14/3/40	45103	6·99	18/4/40	45103	6·99	—	—	—	—	—	—	—	—
139	51641	14/3/40	45103	6·99	18/4/40	45103	6·99	18/7/40	45106	6·9	14/12/40	Female	6	149	3
27	55734	16/3/40	45129	6·99	4/4/40	45129	6·99	—	—	—	4/ 9/40	Male..	5¾	153	2
34	55895	28/3/40	45103	6·99	16/4/40	45103	6·99	21/5/40	45106	6·9	—	—	—	—	—
169	56975	28/3/40	45103	6·99	—	—	—	—	—	—	26/ 8/40	Male..	7¾	151	1
170	53528	30/3/40	45103	6·99	—	—	—	—	—	—	—	—	—	—	—
23	55695	30/3/40	45103	6·99	14/6/40	45095	6·9	—	—	—	—	—	—	—	—
71	55657	30/3/40	45103	6·99	—	—	—	—	—	—	—	—	—	—	—
176	53995	2/4/40	45301	6·99	—	—	—	—	—	—	—	—	—	—	—
59	55891	4/4/40	45129	6·99	15/6/40	45095	6·9	2/7/40	45095	6·9	—	—	—	—	—
		5/4/40	45129	6·99	—	—	—	—	—	—	—	—	—	—	—
185	55868	23/4/40	45129	6·99	11/5/40	45106	6·9	18/8/40	45095	6·9	—	—	—	—	—
184	55946	23/4/40	45129	6·99	11/5/40	45106	6·9	5/7/40	45095	6·9	—	—	—	—	—
196	55756	4/5/40	45106	6·9	—	—	—	—	—	—	29/9/40	Male..	8½	148	1
26	55661	11/5/40	45106	6·9	28/6/40	45106	6·9	—	—	—	—	—	—	—	—
197	53986	16/5/40	45103	6·9	17/5/40	45103	6·9	—	—	—	12/10/40	Female	8	148	1
198	55680	16/5/40	45103	6·9	17/5/40	45103	6·9	4/6/40	45103	6·9	30/10/40	Female	8¼	148	2
67	55845	14/5/40	45103	6·9	18/5/40	45095	6·9	—	—	—	—	—	—	—	—
14	55685	18/7/40	45106	6·85	—	—	—	—	—	—	10/12/40	Female	6¾	145	1

Females..... 4 = 57·15 per cent.
Males..... 3 = 42·85 per cent.
} Average weight, 7·28 lb.; Average gestation period, 148·85 days; Average number of inseminations, 1·57.

Note :—

Percentage Conceptions for :—

1st Insemination, 18·18 per cent.
2nd Insemination, 9·09 per cent.
3rd Insemination, 4·54 per cent...
Non-pregnant, 68·18 per cent...

Ram.	Insemination. +	Insemination. −
45095.....	—	7
45103.....	3	18
45106.....	3	5
45129.....	1	8
45301.....	—	2
45308.....	—	1
	7	41

Conceptions for dilutor pH.:—

	Inseminations. +	Inseminations. −
6·85.....	1	—
6·9.....	4	18
6·99.....	2	23
	7	41

31·81 per cent. ewes lambed.
68·18 per cent ewes did not conceive.

+ = Fertile.
− = not fertile.

TABLE 6.

Group 6.—Inseminated with Semen stored for 24 hours. Diluted 4 times.

EWE.		1ST INSEMINATION.			2ND INSEMINATION.			3RD INSEMINATION.			PARTURITION.			Gestation Period: Days.	No. of Inseminations to Establish Pregnancy.
No.	D.O.B. No.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Sex of Lamb.	Weight of Lamb. lb.		
103	50384	16/2/40	45301	7·21	5/3/40	45129	6·9	27/4/40	45308	6·99	—	—	—	—	—
77	55886	16/2/40	45301	7·21	—	—	—	—	—	—	—	—	—	—	—
		17/2/40	45301	7·21	—	—	—	—	—	—	16/ 7/40	Female	8½	151	1
61	55942	16/2/40	45301	7·21	—	—	—	—	—	—	—	—	—	—	—
73	55690	20/2/40	45301	7·21	—	—	—	—	—	—	—	—	—	—	—
		21/2/40	45301	7·21	—	—	—	—	—	—	17/ 7/40	Male..	8½	148	1
48	55878	20/2/40	45301	7·21	7/3/40	45103	6·9	16/5/40	45103	6·9	12/10/40	Female	7¾	149	3
123	55853	20/2/40	45301	7·21	13/4/40	45103	6·9	4/6/40	X	6·9	—	—	—	—	—
124	53531	20/2/40	45301	7·21	—	—	—	—	—	—	—	—	—	—	—
		21/2/40	45301	7·21	9/3/40	45129	6·99	7/6/40	45106	6·9	—	—	—	—	—
140	55860	24/2/40	45129	6·99	12/3/40	45129	6·99	—	—	—	10/ 8/40	Female	7½	151	2
12	55783	24/2/40	45129	6·99	30/3/40	45129	6·99	2/5/40	X	6·9	—	—	—	—	—
10	55735	27/2/40	45103	6·99	7/5/40	45095	6·9	26/6/40	45106	6·9	—	—	—	—	—
143	53368	27/2/40	45103	6·99	16/3/40	45129	6·99	5/6/40	X	6·9	—	—	—	—	—
5	55846	1/3/40	45129	6·99	18/3/40	45129	6·99	—	—	—	—	—	—	—	—
146	57134	1/3/40	45103	6·99	—	—	—	—	—	—	—	—	—	—	—
		2/3/40	45129	6·99	6/4/40	45103	6·9	25/4/40	45219	6·9	23/ 9/40	Female	8¼	151	3*
147	55715	1/3/40	45129	6·99	—	—	—	—	—	—	—	—	—	—	—
		2/3/40	45103	6·99	18/3/40	45129	6·99	28/5/40	X	6·9	—	—	—	—	—
150	57135	2/3/40	45129	6·99	27/4/40	45308	6·9	14/5/40	X	6·9	—	—	—	—	—
149	55677	2/3/40	45301	6·99	—	—	—	—	—	—	30/7/40	Male..	8	150	1
91	55824	2/3/40	45301	6·99	18/3/40	45129	6·99	7/5/40	45095	6·9	—	—	—	—	—
13	55911	2/3/40	45301	6·99	—	—	—	—	—	—	—	—	—	—	—
148	51723	2/3/40	45301	6·99	19/3/40	45103	6·99	24/4/40	—	—	—	—	—	—	—
95	55696	5/3/40	45129	6·99	17/5/40	45106	6·9	—	—	—	11/10/40	Female	8¼	147	2
152	55850	5/3/40	45129	6·99	29/5/40	X	6·9	2/7/40	45095	6·9	—	—	—	—	—
82	55812	5/3/40	45129	6·99	11/4/40	45129	6·99	18/5/40	45095	6·9	—	—	—	—	—
151	53210	5/3/40	45129	6·99	—	—	—	—	—	—	31/ 7/40	Male..	8¾	148	1

* Dystokia. Lamb born dead during the night.

Females..... 5 = 62·5 per cent.
Males..... 3 = 37·5 per cent.
} Average weight, 8·2 lb.; Average gestation period, 149·37 days; Average number of inseminations, 1·75.

NOTE :—

Percentage of Conceptions for :—

1st Insemination, 17·39 per cent.
2nd Insemination, 8·7 per cent.
3rd Insemination, 8·7 per cent.
Non-pregnant, 65·21 per cent.

Ram.	Inseminations. +	Inseminations. —
45095.....	—	4
45103.....	1	8
45106.....	1	2
45129.....	2	17
45219.....	1	—
45301.....	3	9
45308.....	—	2
X.....	—	6
	8	48

34·79 per cent. ewes lambed.
65·21 per cent. did not conceive.

Conceptions for dilutor pH. :—

	Inseminations. +	Inseminations. —
6·9.....	3	17
6·99.....	3	25
7·21.....	2	6
	8	48

\+ = Fertile.
— = Not fertile.

TABLE 7.

Group 7.—Inseminated with Semen stored for 48 hours. Diluted 4 times.

EWE.		1ST INSEMINATION.			2ND INSEMINATION.			3RD INSEMINATION.			PARTURITION.			Gestation Period: Days.	No. of Inseminations to Establish Pregnancy.
No.	D.O.B. No.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Sex of Lamb.	Weight of Lamb. lb.		
79	55681	21/2/40	45301	6·99	28/3/40	45103	6·99	18/5/40	45103	6·9	—	—	—	—	—
75	55791	21/2/40	45301	6·99	13/4/40	45103	6·99	18/5/40	45095	6·9	—	—	—	—	—
125	55672	21/2/40	45301	6·99	29/3/40	45301	6·99	3/6/40	X	6·9	—	—	—	—	—
126	49386	21/2/40	45301	6·99	28/3/40	45103	6·99	—	—	—	26/8/40	Male..	9¼	151	2
127	47292	21/2/40	45301	6·99	9/3/40	45129	6·99	18/5/40	45103	6·9	—	—	—	—	—
128	53199	21/2/40	45301	6·99	9/3/40	45129	6·99	18/5/40	45095	6·9	—	—	—	—	—
129	57132	21/2/40	45301	6·99	9/3/40	45129	6·99	15/5/40	45106	6·9	—	—	—	—	—
56	55849	22/2/40	45129	6·99	11/3/40	45129	6·99	7/6/40	X	6·9	—	—	—	—	—
132	47727	22/2/40	45129	6·99	11/3/40	45129	6·99	6/5/40	45106	6·9	—	—	—	—	—
3	55786	22/2/40	45129	6·99	11/3/40	45129	6·99	20/5/40	45095	6·9	17/10/40	Male..	8¼	150	3
130	55770	22/2/40	45129	6·99	29/3/40	45301	6·99	3/5/40	45100	6·9	—	—	—	—	—
131	55710	22/2/40	45129	6·99	18/5/40	45103	6·9	—	—	—	—	—	—	—	—
11	55861	22/2/40	45129	6·99	11/4/40	45103	6·9	—	—	—	—	—	—	—	—
		23/2/40	45129	6·99	12/4/40	45103	6·9	15/5/40	45106	6·9	—	—	—	—	—
54	55712	23/2/40	45129	6·99	12/3/40	45129	6·99	5/6/40	45301	6·9	—	—	—	—	—
135	47315	23/2/40	45129	6·99	—	—	—	—	—	—	—	—	—	—	—
		24/2/40	45129	6·99	13/3/40	45103	6·99	—	—	—	12/ 8/40	Female	11	152	2
134	47731	23/2/40	45129	6·99	12/3/40	45129	6·99	18/4/40	45103	6·9	—	—	—	—	—
62	55896	23/2/40	45129	6·99	12/3/40	45129	6·99	—	—	—	—	—	—	—	—
32	46978	23/2/40	45129	6·99	11/3/40	45103	6·99	7/6/40	X	6·9	—	—	—	—	—
133	56979	23/2/40	45129	6·99	11/3/40	45129	6·99	18/5/40	45095	6·9	—	—	—	—	—
83	55948	23/2/40	45129	6·99	11/3/40	45103	6·99	22/5/40	45301	6·9	—	—	—	—	—
220	52570	19/6/40	45129	6·9	—	—	—	—	—	—	—	—	—	—	—
		20/6/40	45301	6·9	8/7/40	45549	6·9	27/7/40	45106	6·9	—	—	—	—	—
221	55914	21/6/40	X	6·9	—	—	—	—	—	—	—	—	—	—	—
		22/6/40	45106	6·9	9/7/40	45095	6·9	—	—	—	—	—	—	—	—
46	55782	22/6/40	45106	6·9	—	—	—	—	—	—	—	—	—	—	—

Females..... 1 = 33–1/3 per cent.
Males..... 2 = 66–2/3 per cent.
} Average weight, 9·5 lb.; Average gestation period, 151·0 days; Average number of inseminations, 2·33.

NOTE :—

Percentage Conceptions for :—

1st Insemination, 0 per cent.
2nd Insemination, 8·7 per cent.
3rd Insemination, 4·35 per cent.
Non-pregnant, 86·95 per cent.

Ram.	Inseminations. +	−
45095.....	1	4
45100.....	—	1
45103.....	2	10
45106.....	—	6
45129.....	—	26
45301.....	—	12
45549.....	—	1
X.....	—	4
	3	64

13·05 per cent. ewes lambed.
86·95 per cent. ewes did not conceive.

Conceptions for dilutor pH. :—

	Inseminations +	−
6·9.....	1	26
6·99.....	2	38
	3	64

\+ = Fertile.
− = Not fertile.

TABLE 8.

Group 8.—Inseminated with Semen stored for 72 hours. Diluted 4 times.

EWE.		1ST INSEMINATION.			2ND INSEMINATION.			3RD INSEMINATION.			PARTURITION.			Gestation Period: Days.	No. of Inseminations to Establish Pregnancy.
No.	D.O.B. No.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Sex of Lamb.	Weight of Lamb. lb.		
137	55703	23/2/40	45129	7·21	—	—	—	—	—	—	—	—	—	—	—
		24/2/40	45129	7·21	11/3/40	45103	6·99	21/5/40	45106	6·9	—	—	—	—	—
136	51088	23/2/40	45129	7·21	14/5/40	45095	6·9	20/6/40	45103	6·9	—	—	—	—	—
138	53170	23/2/40	45129	7·21	30/3/40	45103	6·99	—	—	—	—	—	—	—	—
6	55884	26/2/40	45103	6·99	—	—	—	—	—	—	—	—	—	—	—
25	55862	26/2/40	45103	6·99	—	—	—	—	—	—	—	—	—	—	—
141	57133	26/2/40	45103	6·99	—	—	—	—	—	—	—	—	—	—	—
		27/2/40	45103	6·99	14/3/40	45103	7·21	—	—	—	10/8/40	Female	10½	149	2
142	41839	26/2/40	45103	6·99	29/3/40	45301	6·99	30/4/40	X	6·99	—	—	—	—	—
49	55938	26/2/40	45103	6·99	20/4/40	45129	6·99	—	—	—	—	—	—	—	—
60	55818	26/2/40	45103	6·99	—	—	—	—	—	—	—	—	—	—	—
97	55797	26/2/40	45103	6·99	—	—	—	—	—	—	—	—	—	—	—
58	55737	11/3/40	45103	6·99	15/4/40	45129	6·99	20/5/40	45129	6·9	—	—	—	—	—
74	55817	11/3/40	45103	6·99	30/3/40	45103	6·99	10/5/40	45106	6·9	—	—	—	—	—
22	55664	11/3/40	45103	6·99	12/4/40	45301	6·9	17/6/40	45095	6·9	—	—	—	—	—
161	45128	11/3/40	45103	6·99	15/4/40	45129	6·9	4/5/40	45095	6·9	—	—	—	—	—
160	51111	11/3/40	45103	6·99	15/4/40	45129	6·9	20/5/40	45301	6·9	—	—	—	—	—
158	55898	11/3/40	45103	6·99	3/5/40	45106	6·9	—	—	—	—	—	—	—	—
159	52402	11/3/40	45103	6·99	29/3/40	45301	6·99	20/5/40	45129	6·9	—	—	—	—	—
49	55938	15/3/40	45103	6·99	2/4/40	45103	6·99	19/4/40	45129	6·99	—	—	—	—	—
165	55679	15/3/40	45103	6·99	1/4/40	45103	6·99	6/5/40	45106	6·9	—	—	—	—	—
35	55750	19/3/40	45103	6·99	4/4/40	45103	6·99	22/4/40	45129	6·99	—	—	—	—	—
								23/4/40	45129	6·99	—	—	—	—	—
218	55935	18/6/40	45095	6·9	8/7/40	45095	6·9	9/7/40	45106	6·9	—	—	—	—	—
312	55769	18/6/40	45095	6·9	6/7/40	45095	6·9	—	—	—	—	—	—	—	—
219	55730	18/6/40	45095	6·9	—	—	—	—	—	—	—	—	—	—	—

Females..... 1 = 4·35 per cent. Weight, 10½ lb.; Gestation period, 149 days; Number of inseminations, 2.

NOTE :—

Percentage Conceptions for :—

1st Insemination, 0 per cent.
2nd Insemination, 4·35 per cent.
3rd Insemination, 0 per cent.

4·35 per cent. ewes lambed.
95·65 per cent. ewes did not conceive.

Ram.	Inseminations. +	Inseminations. —
45095.....	—	8
45103.....	1	25
45106.....	—	5
45129.....	—	13
X.....	—	1
45301.....	—	4
	1	56

Conceptions for dilutor pH. :—

	Inseminations. +	Inseminations. —
6·9.....	—	20
6·99.....	—	32
7·21.....	1	4
	1	56

\+ = Fertile.
— = Not fertile.

TABLE 9.

Group 9.—Inseminated with Semen stored for 96 hours. Diluted 4 times.

EWE.		1ST INSEMINATION.			2ND INSEMINATION.			3RD INSEMINATION.			PARTURITION.			Gestation Period: Days.	No. of Inseminations to Establish Pregnancy.
No.	D.O.B. No.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Ram No.	Dilutor pH.	Date.	Sex of Lamb.	Weight of Lamb. lb.		
63	55892	12/3/40	45103	6·99	30/3/40	45129	6·99	21/5/40	45106	6·9	—	—	—	—	—
162	53480	12/3/40	45103	6·99	—	—	—	—	—	—	—	—	—	—	—
32A.	55682	20/3/40	45301	6·99	—	—	—	—	—	—	—	—	—	—	—
166	52584	20/3/40	45301	6·99	6/4/40	45103	6·9	1/6/40	45095	6·9	—	—	—	—	—
167	53364	20/3/40	45301	6·99	18/6/40	45106	6·9	—	—	—	—	—	—	—	—
88	55947	20/3/40	45301	6·99	8/4/40	45129	6·99	25/4/40	45129	6·99	—	—	—	—	—
53	55857	8/4/40	45129	6·99	—	—	—	—	—	—	—	—	—	—	—
177	55905	8/4/40	45129	6·99	26/4/40	45129	6·9	13/5/40	45095	6·9	—	—	—	—	—
179	55864	15/4/40	45301	6·99	—	—	—	—	—	—	—	—	—	—	—
		16/4/40	45301	6·99	—	—	—	—	—	—	—	—	—	—	—
180	55666	15/4/40	45301	6·99	3/5/40	45095	6·9	21/5/40	45106	6·9	—	—	—	—	—
		16/4/40	45301	6·99	—	—	—	—	—	—	—	—	—	—	—
188	55756	26/4/40	45129	6·99	15/5/40	X	6·9	1/6/40	45095	6·9	—	—	—	—	—
76	55838	27/4/40	X	6·99	15/5/40	X	6·9	—	—	—	—	—	—	—	—
20	42042	27/4/40	X	6·99	5/6/40	45301	6·9	24/6/40	45219	6·9	—	—	—	—	—
189	55921	27/4/40	X	6·9	14/5/40	45095	6·9	—	—	—	—	—	—	—	—
					15/5/40	X	—	—	—	—	—	—	—	—	—
190	44914	27/4/40	X	6·99	13/5/40	45095	6·9	3/7/40	45095	6·9	—	—	—	—	—
191	55705	27/4/40	X	6·99	14/5/40	45095	6·9	1/6/40	45095	6·9	—	—	—	—	—
192	55802	29/4/40	45129	6·9	—	—	—	—	—	—	—	—	—	—	—
193	57615	29/4/40	45129	6·9	—	—	—	—	—	—	—	—	—	—	—
		30/4/40	45095	6·9	—	—	—	—	—	—	—	—	—	—	—
194	52590	3/5/40	45095	6·9	21/5/40	45106	6·9	14/6/40	45106	6·9	—	—	—	—	—
195	46979	3/5/40	45095	6·9	—	—	—	—	—	—	—	—	—	—	—

NOTE:—No pregnancy occurred in this group.

7 ewes inseminated, once only.
3 ewes inseminated, twice only.
10 ewes inseminated, three times only.

Ram.	Inseminations. +	Inseminations. —
45095	—	12
45103	—	3
45106	—	5
45129	—	9
45219	—	1
45301	—	9
X	—	8
	0	47

Dilutor pH.:—	Inseminations. +	Inseminations. —
6·9	—	26
6·99	—	21
	0	47

+ = Fertile.
— = Not fertile.

TABLE 10.

Group 10.—*Inseminated with Semen kept in vitro up to* 11 *days. Diluted* 4 *times.*

Ewe.		1st Insemination.				2nd Insemination.				3rd Insemination.				Parturition.			Gestation Period : Days.
No.	D.O.B. No.	Date.	Ram.	Dilutor pH.	Age of Semen. Days.	Date.	Ram.	Dilutor pH.	Age of Semen. Days.	Date.	Ram.	Dilutor pH.	Age of Semen. Days.	Date.	Sex of Lamb.	Weight of Lamb. lb.	
55	55669	13/2/40	45301	7·21	7	26/4/40	45301	6·99	9	3/6/40	45301	6·9	9	—	—	—	—
121	42051	13/2/40	45301	7·21	7	1/3/40	45135	6·99	6	22/4/40	45129	6·99	7	—	—	—	—
										23/4/40	45129	6·99	10				
122	48585	13/2/40	45301	7·21	7	1/3/40	45135	6·99	6	22/4/40	45129	6·99	7	—	—	—	—
144	53365	28/2/40	45129	7·21	8	20/4/40	45129	6·99	9	7/5/40	45095	6·9	7	—	—	—	—
41	55760	29/2/40	45129	7·21	8	19/3/40	45301	6·99	6	24/4/40	45129	6·99	7	—	—	—	—
		1/3/40	45135	7·21	6												
145	50522	29/2/40	45129	7·21	8	18/3/40	45129	6·99	6	24/4/40	45129	6·99	11	—	—	—	—
		1/3/40	45135	7·21	6												
153	55976	7/3/40	45129	7·21	5	11/4/40	45129	6·99	9	29/4/40	45301	6·99	7	—	—	—	—
154	57138	7/3/40	45129	7·21	5	11/4/40	45129	6·99	9	29/4/40	45301	6·99	7	—	—	—	—
		8/3/40	45301	7·21	8												
38	55943	7/3/40	45129	7·21	5	10/4/40	45301	6·99	9	16/5/40	X	6·9	8	—	—	—	—
96	53709	7/3/40	45129	7·21	5	14/5/40	45106	6·9	8	1/6/40	X	6·9	9	—	—	—	—
168	50496	26/3/40	45103	7·21	5	12/4/40	45103	6·99	8	15/5/40	X	6·9	8	—	—	—	—
64	55739	27/3/40	45301	6·99	6	25/5/40	X	6·9	7	—	—	—	—	—	—	—	—
52	55912	27/3/40	45301	6·99	6	30/4/40	45219	6·9	5	17/5/40	45106	6·9	8	—	—	—	—
94	49642	27/3/40	45301	6·99	6	30/4/40	45219	6·9	5	18/5/40	X	6·9	8	—	—	—	—
81	55811	10/4/40	45301	6·99	9	16/5/40	X	6·9	8	3/6/40	45095	6·9	9	—	—	—	—
178	55934	11/4/40	45129	6·99	9	29/4/40	45301	6·9	7	17/5/40	45106	6·9	8	—	—	—	—
182	55793	20/4/40	45129	6·99	9	24/6/40	45095	6·9	7	—	—	—	—	—	—	—	—
183	55919	20/4/40	45129	6·99	9	7/5/40	45095	6·9	7	25/5/40	X	6·9	8	—	—	—	—
186	55740	24/4/40	45129	6·99	7	29/5/40	45301	6·9	8	—	—	—	—	—	—	—	—
						30/5/40	45301	6·9	8								

TABLE 10—(*continued*).

Ewe.		1st Insemination.				2nd Insemination.				3rd Insemination.				Parturition.			Gestation Period: Days.
No.	D.O.B. No.	Date.	Ram.	Dilutor pH.	Age of Semen. Days.	Date.	Ram.	Dilutor pH.	Age of Semen. Days.	Date.	Ram.	Dilutor pH.	Age of Semen. Days.	Date.	Sex of Lamb.	Weight of Lamb.	
187	57614	26/4/40	45301	6·99	7	—	—	—	—	—	—	—	—	—	—	—	—
92	55697	13/5/40	45095	6·9	10	17/6/40	45129	6·9	7	—	—	—	—	—	—	—	—
		14/5/40	45166	6·9	8												
216	55675	17/6/40	45095	6·9	9	6/7/40	45095	6·9	8	—	—	—	—	—	—	—	—
		18/6/40	45219	6·9	8												
217	52982	17/6/40	45301	6·9	9	5/7/40	45301	6·9	8	—	—	—	—	—	—	—	—
223	50853	3/7/40	45103	6·9	6	19/7/40	45095	6·9	8	—	—	—	—	—	—	—	—
189	55921	8/7/40	45549	6·9	5	—	—	—	—	—	—	—	—	—	—	—	†

† Transfer from Group 9.

NOTE:—No ewes in this group became pregnant.

2 ewes inseminated, once only.
7 ewes inseminated, twice only.
16 ewes inseminated, three times only.

Ram.	Inseminations. +	Inseminations. —
45095	—	8
45103	—	3
45106	—	4
45129	—	21
45135	—	4
45129	—	3
45301	—	20
45549	—	1
X	—	7
	—	71

Dilutor pH.:—	Inseminations. +	Inseminations. —
6·9	—	31
6·99	—	26
7·21	—	14
	—	71

+ = Fertile.
— = Not fertile.

The following table shows the details of results obtained by normal service and by semen used for artificial insemination from individual rams:

TABLE 11.

Duration of storage of Semen.

No. of Ram.	N. Service. Group 1.		0 Hour. Group 2.		6 Hours. Group 3.		12 Hours. Group 4.		18 Hours. Group 5.		24 Hours. Group 6.		48 Hours. Group 7.		72 Hours. Group 8.		96 Hours. Group 9.		5–11 Days. Group 10.		TOTAL.	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
45095	—	—	1	—	—	2	2	6	—	7	—	4	1	4	—	8	—	12	—	8	4 + 51	55
45100	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	1
45103	5	—	4	3	5	1	2	1	3	18	1	8	2	10	1	25	—	3	—	3	23 + 72	95
45106	—	2	1	—	—	—	5	5	3	5	1	2	—	6	—	5	—	5	—	4	10 + 34	44
45129	—	—	3	—	7	9	2	3	1	8	2	17	—	26	—	13	—	9	—	21	15 106	121
45131	—	—	—	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	7
45135	3	1	—	5	—	6	—	—	—	—	—	—	—	—	—	—	—	—	—	4	3 + 16	19
45219	6	3	2	—	—	2	1	1	—	—	1	—	—	—	—	—	—	1	—	3	10 + 10	20
45301	2	13	7	4	—	6	2	—	—	2	3	9	—	12	—	4	—	9	—	20	14 + 79	93
45307	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
45308	—	—	1	—	—	—	—	—	—	1	—	2	—	—	—	—	—	—	—	—	1 + 3	4
45549	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	—	1	1 + 2	3
X	—	—	—	1	—	—	—	—	—	—	—	6	—	4	—	1	—	8	—	7	27	27
	—	20	—	20	—	26	—	16	—	41	—	48	—	64	—	56	—	47	—	71	409	490
	16	—	19	—	12	—	15	—	7	—	8	—	3	—	1	—	0	—	0	—	81 —	—

Artificial inseminations and services..... Total..... 490
Artificial inseminations and services..... +..... 81
Artificial inseminations and services..... −..... 409

NOTE.— + = Fertile; − = Non fertile; N. services = Normal service.

The following table shows the results of inseminations at different levels of pH. The table includes all inseminations where a dilutor was used, including those groups in which not a single pregnancy resulted.

TABLE 12.

Dilutor pH.

pH.	Group 2. 0 Hour.		Group 3. 6 Hours.		Group 4. 12 Hours.		Group 5. 18 Hours.		Group 6. 24 Hours.		Group 7. 48 Hours.		Group 8. 72 Hours.		Group 9. 96 Hours.		Group 10. 5–11 Days.		TOTAL.		Total.
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	
6·5	—	—	—	2	1	3	—	—	—	—	—	—	—	—	—	—	—	—	1	5	6
6·85	—	—	—	—	2	—	1	—	—	—	—	—	—	—	—	—	—	—	3	—	3
6·9	5	—	1	4	5	2	4	18	3	17	1	26	—	20	—	26	—	31	19	144	163
6·99	11	12	11	12	4	6	2	23	3	25	2	38	—	32	—	21	—	26	33	195	228
7·0	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2
7·2	—	—	—	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	1	2	3
7.21	3	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	7	10
7·34	—	1	—	8	2	1	—	—	2	6	—	—	1	4	—	—	—	14	5	34	39
−	—	20	—	26	—	16	—	41	—	48	—	64	—	56	—	47	—	71	—	389	454
+	19	—	12	—	15	—	7	—	8	—	3	—	1	—	0	—	0	—	65	—	—

Total artificial inseminations..... 454
Total artificial inseminations.....+ 65
Total artificial inseminations.....− 389

NOTE.— + = Fertile; − = Non fertile.

Field Observations.

During 1940 a field observation was carried out in South West Africa in co-operation with Mr. R. L. Rabie, a farmer in the Rehoboth district. He used one Karakul ram to inseminate a large number of Afrikaner ewes, because, he said this ram was an exceptionally prepotent sire. The ewes were mostly about 4 years old. Some of the sheep were suckling lambs, while others were dry, consequently it was considered desirable to divide them into two groups. All the ewes had lambed during December, 1939 and January, 1940. One group of ewes suckled their lambs during the period when the inseminations were done, while the other group was dry, their lambs having been killed shortly after birth to obtain their pelts. The ewes in the former group were in good condition but were not fat; the ewes in the latter group were fat.

The two groups were treated similarly and had always been under the same environment. They were kept in separate camps on the same farm.

The teasers were "raddled" every morning and all ewes marked by them during the following 24 hours were inseminated the next morning. Ewes from both groups which had been marked by the teasers were placed together in a shed until insemination was completed. The following morning they were returned to their respective groups. They were inseminated with semen obtained from the only Karakul ram used. This ram was six years old, a proved sire of high quality with an excellent breeding record. His ejaculate was frequently controlled during the course of the observations. The ram was stabled, and the only exercise he was allowed was his daily walk to the inseminating room and back to his stable, about 300 yards each way. He was fed on 2 lbs. crushed oats morning and evening, green lucerne every night and grass hay *ad lib.* He maintained excellent condition throughout and copulated readily with the artificial vagina.

The technique employed was similar to that already described, but the dilutor used was purchased from The Holborn Instrument Coy. in ampoules (R242, Dilutors for Ram's Sperm, Moscow formula). All inseminations were done within ½ hour from the time the semen had been collected. The duration of oestrus was not known. The following tables show in summarised form the results obtained:

Table 13.

Group 1. *Ewes Suckling Lambs.*

Insemination.	Fertilised.	Not Fertilised.	Percentage of Ewes Fertilised.
First	220	262	45·6456
Second	65	132	13·4854
Third	33	47	6·8464
Fourth	1	3	0·2074
	319	444	66·1848

Total inseminations 763.

Number of ewes inseminated 482; number of lambs, including twins, 324; mean gestation period 150·0625 days.

TABLE 14.

Group 2. Ewes without Lambs.

Insemination.	Fertilised.	Not Fertilised.	Percentage Fertilised.
First	29	169	14·6464
Second	17	94	8·5885
Third	5	46	2·5250
Fourth	—	11	—
	51	320	25·7599

Total insemination 371.

Number of ewes inseminated 198; number of lambs, including twins, 54; mean gestation period 150·5 days.

The following table shows details of the combined results shown in tables 13 and 14.

TABLE 15.

Groups 1 and 2.

Insemination.	Fertilised.	Not Fertilised.	Percentage Fertilised.
First	249	431	36·6176
Second	82	226	12·0588
Third	38	93	5·5882
Fourth	1	14	0·1470
	370	764	54·4116

Total inseminations 1134;

Number of ewes 680; number of lambs, including twins, 378; mean gestation period 150·1 days.

DISCUSSION.

The results obtained indicate that the technique of storage will have to be greatly improved to make insemination with stored semen of great practical value. From a practical point of view it must be considered that these results are unsatisfactory when semen stored for longer than twelve hours was used. The pregnancies resulting from insemination with fresh semen and with semen stored up to twelve hours are comparable with those obtained by normal mating.

The average number of inseminations required to establish pregnancy was no greater than the average number of normal services. That the technique of insemination was satisfactory is shown by the results of Group 2, Table 2, where 95 per cent. of the ewes lambed to insemination with fresh semen. The less successful results of insemination with semen stored for varying periods can be attributed to deterioration in the fertilising capacity of the spermatozoa, although active motility was maintained. Brady and Gildow (1939) say " To date no sure and simple method of ascertaining the fertilising capacity of the sperm cell has been determined ". Active motility has been no indication as to what can be expected in fertilising capacity. Fertility has been obtained only with semen in which the spermatozoa were highly motile, but equally active specimens failed to fertilise.

There was a marked fall in the resulting pregnancies with semen stored longer than twelve hours. Approximately a third of the ewes inseminated with semen stored for eighteen and twenty-four hours lambed. The percentage of pregnancies fell to thirteen at 48 hours, while after 72 hours storage, only one out of twenty-three sheep lambed. This was the longest period of storage at which a pregnancy was obtained. Failures followed insemination with semen stored from five to eleven days, although the specimens of semen used showed actively motile spermatozoa.

In the field observations four hundred and eighty-two ewes suckling their lambs required a total of seven hundred an sixty-three inseminations to establish 66·1848 per cent. fertility. This would not be considered satisfactory for normal mating with Karakul sheep, but since only one high quality ram was used, instead of the usual practice of putting 2 per cent. of rams with the flock, the results, judged by the large number and high standard of the lambs, are justified.

These observations were made on sheep which were suckling lambs and although in good condition were not fat. It is interesting to compare the results from this group with the other group on the same farm. The lambs of the second group had been killed shortly after birth to obtain the pelts and the ewes became very fat. One hundred and ninety-eight ewes required three hundrel and seventy-one inseminations to produce 25·7599 per cent. fertility. There was no difference in the treatment of the two flocks. Consequently it appears justified to conclude that the failure to breed was due to the fat condition of the sheep which had not had the constitutional drain of milk production.

In this observation a total of six hundred and eighty sheep required eleven hundred and thirty-four inseminations to establish 54·4116 per cent. fertility. The number of lambs produced was three hundred and seventy-eight, with an average gestation period of 150·1 days. This is the first attempt at a large-scale field observation in this country, and although the results from the ewes without lambs were disappointing the 66·1848 per cent. pregnancies from the sheep suckling lambs indicate what may be attained with selected material and improved facilities.

Owing to the environmental conditions prevailing in the sheep raising districts in this country, where high temperatures are experienced during many months of the breeding season, it is highly probable that fertilisation by artificial insemination will never reach the high percentages recorded by workers in Europe and Soviet Russia. The conditions which prevail over the greater portion of South Africa appear to be incompatible with the optimum environment associated with spermatozoal life outside the genitalia. Field observations are usually carried out in kraals from which dust cannot be eliminated, and where shade temperatures of over 90° F. are common. It has been proved that spermatozoa do not survive room temperature in the laboratory at Onderstepoort, during the summer months, for longer than a few hours. Consequently it must be expected that the very adverse temperature conditions under which the work must be carried out detrimentally influences the vitality of the spermatozoa and lowers the expectancy of fertilisation when compared with European standards.

In the study of the conditions which influence spermatozoal life in the female genital tract the body-temperature of sheep would appear to merit investigation. It is possible that high body-temperatures influence the fertilising capacity of the spermatozoa. Quinlan and Maré (1932) observed that the temperature of the normal merino ewe in a restful state will vary during the warm summer months: The mean temperature at 6 a.m. was 101·77° F. and at 7·30 p.m. 102·95° F. Temperatures up to 105·4° F. were recorded. Exercise and excitement caused a marked rise in temperature.

Since sheep under field conditions have to be driven to the kraals to be tested for oestrus, and thereafter handled during insemination, it appears probable that any rise of temperature may detrimentally influence the fertilizing capacity of spermatozoa already weakened by *in vitro* treatment. Enlightenment on this question may throw some light on the poor results obtained from artificial insemination under field conditions in this country.

The gestation periods recorded in the different groups at the laboratory were normal for merino sheep in this country. There was a range of 148·53 to 151 days as compared with 150·75 days for the control group. This gestation period does not differ from that recorded for the merino in this country by Quinlan, Maré and Roux (1932) and Roux (1936). The lambs born, with one exception, were normal in weight and their post-natal development was excellent (Fig. 3). The abnormal lamb was born dead. There was marked ascites and atresia of the anus and the vulva.

There were thirty-eight female lambs and twenty-seven males; rather an unproportionate sex ratio.

The range of pH values of the dilutor used varied from 6·5 to 7·34. Owing to the nature of the work it cannot be said that there was an optimum value within this range. However, if observations on the most suitable pH of the dilutor were confined to those groups where high fertility was obtained, that is groups 2, 3 and 4, 6·9 and 6·99 would appear to have given the best results.

It is doubtful, under the conditions of sheep farming which exist in South Africa, if artificial insemination will become an operation of widespread practical importance in sheep husbandry. There is no scarcity of high quality rams, which can be purchased at a reasonable price. The carrying capacity of the veld is low, and flocks are large, necessitating very large farms. Driving, testing for oestrus and handling for the operation involve much labour and detrimentally influence the condition of the sheep, especially during warm weather. The visualisation of its value would appear to be confined to stud flocks that could be confined to small paddocks, and handled without unreasonable difficulty. A proved stud sire could be used more extensively to produce high class rams for sale at a cheaper price. The sheep breeding industry would eventually benefit by this larger distribution of desirable blood far more than is possible by normal breeding. With normal mating a merino ram can be expected to mate with fifty ewes, and by " hand-service " with a hundred ewes during the breeding season. By using artificial insemination a ram can produce more lambs during one breeding season than he could during a life time with normal mating. Consequently a proven sire, of the highest quality, could be of inestimable value to the sheep breeding industry within very few years. Similarly the ewes produced would improve the sheep breeders stud flock in a far shorter time than could be expected by normal mating. The position regarding Karakul breeding is somewhat different. The number of available high quality rams is very limited. Consequently some Karakul breeders have begun extending the use of proven sires by using artificial insemination.

Conclusions.

(1) One hundred and ninety-six merino sheep, maintained under dry lot conditions, were submitted to artificial insemination with fresh and stored semen obtained from highly fertile merino rams. The resulting pregnancies were compared with eighteen control sheep which were mated normally. Table 16 shows the results in summarised form.

(2) The technique employed in the operation was satisfactory, as indicated by the results in groups 2, 3 and 4.

(3) The cause of failure to impregnate by stored semen is due to deterioration in fertilising capacity. The activity of the spermatozoa is not always an indication of their capacity to impregnate, although active motility is necessary for fertilisation.

(4)The number of inseminations required to establish pregnancy, the gestation periods and the weights of the lambs born do not differ significantly from normal mating.

(5) There were five cases of dystokia in the groups treated by artificial insemination as compared with two in the normal service group. One deformed lamb was born in the inseminated groups. No importance is attributed to these occurrences.

(6) The lambs born were of normal weight and their post-natal development was excellent.

TABLE 16.

Group Number.	Number of Ewes per Group.	Treatment: Normal Service or Artificial Insemination.	Duration of Storage of Semen.	Percentage Pregnancy.	Average Number of Normal Services or Inseminations.	Average Gestation Period. Days.	Average Weight of Lambs. lb.
1.....	18	Normal service.....	—	88·87	2	150·75	7·1
2.....	20	Artificial Insemination*.....	Fresh.....	95	2	149·21	8·0
3.....	20	Artificial Insemination*.....	6 hours.....	60·0	1·91	149·83	7·81
4.....	20	Artificial Insemination*.....	12 hours.....	75·0	1·53	148·53	7·68
5.....	22	Artificial Insemination*.....	18 hours.....	31·81	1·57	148·85	7·28
6.....	23	Artificial Insemination*.....	24 hours.....	34·79	1·75	149·37	8·2
7.....	23	Artificial Insemination*.....	48 hours.....	13·05	2·33	151·0	9·5
8.....	23	Artificial Insemination*.....	72 hours.....	4·35	2·0	149·0	10·5
9.....	20	Artificial Insemination*.....	96 hours.....	—	—	—	—
10.....	25	Artificial Insemination*.....	5–11 days...	—	—	—	—

* Dilution—× 4.

(7) A field observation was carried out on 680 Karakul sheep; 378 lambs were born with a mean gestation period of 150·1 days. These sheep were divided into two groups because of their condition: 482 sheep were suckling lambs; of these 319 became pregnant and 324 lambs were born. The percentage pregnancy was 66·1848. The other group was not suckling lambs and was fat. There were 198 ewes inseminated of which only 51 became pregnant and gave birth to 54 lambs. The percentage pregnancy was only 25·7599.

There is an indication that the fat condition of the ewes rendered conception more difficult.

(8) The artificial insemination of sheep with fresh semen or with semen stored up to 12 hours will give satisfactory results in sheep husbandry in South Africa. However, it is suggested that the employment of artificial insemination has a limited value in reproduction of sheep when the local conditions are compared with those in countries where insemination has become a routine measure in the production of livestock.

(9) No pregnancies followed insemination with semen stored longer than 72 hours.

(10) Artificial insemination overcomes the difficulty experienced in mating sheep with extreme tail development. It has proved satisfactory with the Karakul and Ronderib-Afrikaner.

(11) Dilutor with pH value of 6·9 to 6·99 gave the most satisfactory results.

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