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Sex Physiology of Sheep.

The Hydrogen-Ion Concentration of the Vaginal Secretion of Merino Sheep during Oestrus, Dioestrus, and Pregnancy, with some remarks on its Influence on Sex-Determination, and the Influence of the Vaginal Temperature at the time of Mating on Conception.

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WITH the progress of studies on sex physiology of sheep and the semen and spermatozoa of rams, under the environmental conditions prevailing in South Africa, various questions, which have not yet been answered by scientific research, arise. Low fertility in sheep has become a matter of considerable economic importance in this country. The aetiology of many factors associated with low fertility remains unsolved. Consequently, any addition to the knowledge of sex physiology of sheep, under our particular environmental and nutritional conditions, may be helpful in developing improved methods of management.

For practical purposes, it may be said that pathological genital lesions in the ewe constitute a minor rôle in infertility. Low sexual activity, influenced by changeable environmental factors of temperature and nutrition, as well as the tendency for most breeds of sheep to have a seasonal anoestrus during the late spring and early summer months, is the major factor in the female. In the ram, infertility, or complete sterility, is frequently encountered, and it appears that spermatogenesis and the spermatozoa are easily influenced by environmental conditions which are considered to be incompatible with high vitality. These include obesity, low condition, cold rainy weather, extreme cold or extreme heat, lack of exercise, unsuitable diet, excessive sexual use, sudden changes in altitude, etc.

The exact changes in the animal body, occurring as a result of these environmental factors, which produce deviation from normal sex physiology, need investigation. Further, the immediate aetiology of low fertility in sheep, in which youth is a predisposing factor, remains unexplained.

The question arises what changes are produced in those ductless glands, associated with genital physiology, and the genitalia by various environmental factors? What is the reason for the extraordinary low fertility encountered in

South Africa in first season merino sheep? The local or general factors resulting from various environmental conditions need elucidation, and it is in an attempt to fill this hiatus gradually that these observations were instituted.

It appears possible that spermatozoal vitality may be lowered by unfavourable environment during or subsequent to spermatogenesis. Such spermatozoa would be further detrimentally influenced by an unfavourable reaction of the vaginal secretion subsequent to copulation, especially if their sojourn in the female genital tract was of long duration while awaiting the arrival of an available ovum.

Quinlan, Marè, and Roux (1937) showed that the spermatozoa survive longer in the cervical canal than in the vagina, uterus, or the fallopian tubes. Perhaps this longer survival is dependent upon the reaction of the cervical secretion.

Considerable study has been carried out on the reaction of the semen of rams, but the pH of the genital secretion in the ewe has not received much attention. Consequently, the object of the present observation was to ascertain the pH of the vagina of the ewe during oestrus, dioestrus, and pregnancy. As a concurrent observation the influence of vaginal pH, within a very limited range, on sex-determination was observed. Studies on sex-determination within a wider pH range have been conducted as a separate observation. These will be recorded when the results are available.

The detrimental effect of temperatures, higher than intra-testicular temperature, on spermatozoa *in vitro* is well-known. Consequently, the study was amplified by mating sheep, in which the temperature had been raised by forced exercise, immediately after the determination of the pH. These results were compared with those obtained from resting sheep in which the vaginal temperature was also recorded just prior to normal mating or artificial insemination. Quinlan and Maré (1932) recorded the normal temperature of merino sheep under the conditions prevailing in the Karroo during summer, and showed that exercise appreciably raised the body-temperature.

An acid reaction of the vaginal secretion at the time of mating has been considered, by breeders of cattle and horses, to have some influence on fertility. Consequently, it has become a routine treatment amongst breeders to use vaginal douches of sodium bicarbonate solution whenever breeding difficulties are encountered. Recent publications have suggested a connection between the reaction of the vaginal secretion at the time of mating and the determination of sex. (Unterberger, 1932, Mulder, 1935, Warren, 1940, Roberts, 1940.) These observations, however, need to be repeated under carefully controlled experimental conditions, as indicated by Cole, Waletzky, and Shackelford (1940), and Quisenberry and Chandiramani (1940).

This is a further reason why the vaginal pH of normal animals, under various environmental conditions, at different periods of the ovarian cycle, should be studied.

McNutt, Schwarte and Eveleth (1939) have studied the hydrogen-ion concentration of the vaginal secretion of cows. Cows during late pregnancy showed a pH variation between 7·0 and 8·8. After calving the range was somewhat lower, but the actual difference was insignificant. During early pregnancy the range was between 6·0 and 7·0. At the time of oestrus all measurements were at neutral point or above, with one exception, which was 6·75. The authors suggest the possibility of a seasonal variation, although it was not possible to draw definite conclusions from their limited material. Dougherty (1941) records *in vivo* determinations of the hydrogen-ion concentration of the vaginas of dairy cows. He used a different technique from that employed by McNutt and his co-workers in order to exclude the possibility of a change in reaction due to exposure of the vaginal secretion to the air.

The pH showed a range between 5·52 and 8·00; 78·50 per cent. of the measurements were at neutral point or below; 21·50 per cent. gave an alkaline reaction. The greater majority of cases fell within the range 6·60 to 6·90. For pregnant cows the average pH value was 6·58, and for non-pregnant cows 6·68. During oestrus the range was between 6·72 and 7·08; five out of six cows showing oestrus were lower than 7·00.

Oberst and Plass (1936) have observed that the vaginal discharge of women during the intermenstrual period is acid, ranging from 4·0 to 4·5. During menstruation it approaches neutrality or may become alkaline. These observations are of considerable importance since women become impregnated only during the mid-intermenstrual period, when, according to these workers, the secretion of the vagina is markedly acid in reaction. During gestation the vaginal acidity was somewhat less than in non-pregnancy.

Mason (1929) states that the cervical secretion of women is alkaline, while the vaginal secretion is acid. He suggests the difference in reaction of these two divisions, so closely situated anatomically, may be the means by which spermatozoa are directed towards the cranial extremity of the female genital tract.

Hall and Lewis (1938) found the pH range for vaginal washings of normal children between 7·0 to 7·2. Ranson and Zuckerman (1937) record a range between 5·2 to 8·7, with a slight rise at or before menstruation, in monkeys.

Material.

One hundred mature merino ewes, in good breeding condition, were selected. They were kept, under " dry lot " conditions, in a sheltered kraal. Their ration consisted of crushed oats, ¼ lb., crushed yellow maize, ¼ lb., green feed *ad lib.* when available (several times weekly), and veld hay *ad lib.* Water was always available. All the sheep were similarly treated throughout the observation.

Nine rams, of known high fertility, were used for mating or as donors for artificial insemination.

Method.

The sheep were tested for oestrus by using vasectomised teasers once daily. Ewes showing oestrus were placed in a separate shelter until mating or artificial insemination was carried out, when they were returned to the flock.

The ewes were divided into three groups: (1) Numbers 1-50, (2) 51-75, (3) 76-100. The pH of the vagina was determined during oestrus, mid-dioestrus, and in a few cases during pregnancy.

Groups 1 and 3 were artificially inseminated twice during the day on which they showed oestrus, at 10 a.m. and 4 p.m. The technique used was similar to that described by Quinlan, Steyn, and de Vos (1941).

Group 2 was similarly treated, but normal service was used instead of artificial insemination, services being allowed at 10 a.m. and 4 p.m.

Group 3 was driven for five minutes, after the determination of the vaginal pH, so as to cause a rise in body temperature. After forced exercise the vaginal temperature was recorded and they were artificially inseminated immediately.

The vaginal pH during oestrus, dioestrus, and pregnancy was recorded. In addition, the vaginal temperature, just prior to artificial insemination or normal mating, the date of normal mating or artificial insemination, the date of parturition, the period of gestation, and the sex of the lamb were recorded. Table 1 shows all the data recorded in summarized form.

Determination of the Vaginal pH.

(1) *Selection of a Suitable dilutor for the Vaginal Secretion.*

In most cases the vaginal secretion was not sufficient for testing and it was necessary to flush the vagina with a suitable fluid. For this purpose an isotonic saline solution was used, as it did not materially change the pH. For each test five ml. of the dilutor was used.

(2) *Technique.—(a) Sampling.*

Five ml. of the isotonic saline solution were drawn into a glass syringe, fitted with a vulcanite nozzle, and squirted into the cranial extremity of the vagina. This fluid was repeatedly withdrawn and injected to insure thorough flushing. The liquid finally drawn into the syringe for testing was watery and clear to opalescent. Some samples contained a large quantity of more or less viscid material, not unlike white of egg. [No originality is claimed for this method: It is similar to that employed by McNutt, Schwarte and Eveleth (1939).]

(*b*) Measurement of the pH was recorded wth the Beckman Glass Electrode pH meter.

The fluid obtained from the vagina was immediately run into the glass cup and the pH measured directly on the Beckman apparatus. After such measurement the electrodes were carefully washed with a saturated solution of potassium chloride followed with double distilled water.

As there was a danger of film formation on the glass electrode, it was found necessary, as a precautionary measure, to wash it, after each measurement, with the saturated solution of potassium chloride.

Every precaution was taken to prevent contamination of the fluid withdrawn from the vagina until measurement was completed.

Discussion.

The vaginal pH of ninety-seven ewes, measured during oestrus, gave a mean value of 6·648, with a range of 5·85 to 7·40. Twenty-six samples gave a pH measurement above 7; two samples measured 7, and 69 below 7.

The following summary shows the results of different pH measurements on pregnancy, and the sex of the offspring:—

Table 2.

pH.	Number.	Percentage of Total.	Percentage ♂.	Percentage ♀.	Percentage Twins.	Percentage Non-pregnant.
Above 7............	26	26·81	50	23·08	—	26·92
7................	2	2·06	50	—	—	50·0
Below 7............	69	71·13	43·48	36·23	2·90	17·39
Total.......	97	100	—	—	—	—

TABLE I.

Date.	Ewe No.	D.O.B. No.	pH Oestrus.	pH Mid-dioestrus.	pH during Advanced Pregnancy.	Vaginal Temperature, First Insemination. °F.	Vaginal Temperature, Second Insemination. °F.	Date of Parturition.	Sex of Lamb.	Gestation Period (days).
3.2.41........	1	50496	6·98	—	—	—	—	2.7.41	Male	149
	2	50545	6·99	—	—	—	—	—	—	—
	3	55943	7·01	—	—	—	—	4.7.41	Female	151
	4	55791	7·01	—	—	—	—	—	—	—
	5	55858	7·00	—	—	—	—	—	—	—
	6	52590	6·99	—	—	—	—	7.7.41	Female	154
	7	57132	7·01	—	—	—	—	3.7.41	Female	150
	8	55891	7·01	—	—	—	—	1.7.41	Male	148
	9	55892	7·02	—	—	—	—	—	—	—
	10	53365	7·00	—	—	—	—	5.7.41	Male	152
	11	53170	7·02	—	—	—	—	5.7.41	Male	152
7.2.41.........	12	55846	7·21	—	—	103·0	102·4	10.7.41	Male	153
	13	55895	7·18	—	—	104·4	103·2	6.7.41	Male	149
	14	53013	7·18	—	—	103·4	103·6	—	—	—
	15	57615	7·21	—	6·18	102·6	103·4	10.7.41	Male	153
	16	55657	7·20	—	—	103·0	103·2	—	—	—
	17	(?)	7·18	—	—	103·4	103·2	6.7.41	Male	149
	18	55850	7·15	—	—	103·4	102·6	8.7.41	Male	151
	19	55712	7·12	—	—	102·8	102·6	7.7.41	Male	150
	20	55797	7·18	—	—	103·8	103·4	7.7.41	Female	150
10.2.41........	21	50384	6·00	—	—	103·0	103·0	15.7.41	Female	155
	22	(?)	7·02	—	—	100·2	103·8	13.7.41	Female	153
	23	55901	6·02	—	—	103·2	103·2	10.7.41	Female	150
	24	55851	6·50	—	—	103·4	103·0	—	—	—
	25	55750	6·32	—	6·09	103·0	103·4	7.7.41	Female	147
11.2.41........	26	52570	6·92	6·68	—	104·0	103·5	17.7.41	Male	156
	27	55921	6·96	6·15	—	103·2	104·0	—	—	—
	28	55760	6·39	6·70	—	103·2	102·8	9.7.41	Male	148
	29	55669	7·16	6·51	—	103·5	103·5	15.7.51	Male	154
	30	53368	7·21	6·00	—	104·4	104·4	12.7.41	Male	151
	31	55783	7·38	6·82	—	103·8	103·6	17.7.41	Male	156
	32	49247	6·49	6·71	—	104·0	104·3	14.7.41	{Female, Male}	153
13.2.41........	33	47292	6·50	6·50	—	104·2	103·4	19.7.41	Female	156
	34	53528	5·96	6·21	—	105·4	105·2	17.7.41	Male	154
	35	55697	6·30	6·16	6·00	103·4	103·6	16.7.41	Female	153
17.2.41........	36	53334	6·25	6·40	—	103·4	103·8	19.7.41	Female	152
	37	55756	6·29	6·70	—	104·8	103·5	—	—	—

REMARKS.—1–50 : pH determined during oestrus before artificial insemination. In some sheep pH also done in mid-dioestrus, and in a few in advanced pregnancy.

All ewes inseminated at ± 10 a.m. and again ± 4 p.m. of the day on which oestrus was present.

The vaginal temperatures were taken immediately before artificial insemination morning and afternoon.

TABLE 1—(*continued*).

Date.	Ewe No.	D.O.B. No.	pH Oestrus.	pH Mid-dioestrus.	pH during Advanced Pregnancy.	Vaginal Temperature, First Insemination. °F.	Vaginal Temperature, Second Insemination. °F.	Date of Parturition.	Sex of Lamb.	Gestation Period (days).
20.2.41........	38	47727	6·38	6·55	—	103·6	103·0	22.7.41	Male	152
	39	50853	5·89	6·82	—	Thermometer	103·0	23.7.41	Female	153
	40	56976	6·50	7·01	—	broken	103·6	20.7.41	Male	150
	41	55946	6·60	7·19	—	101·8	102·6	23.7.41	Male	153
21.2.41........	42	55860	6·22	6·50	—	103·0	103·2	22.7.41	Male	151
	43	55940	5·85	6·36	—	102·4	103·0	23.7.41	Male	152
	44	47731	6·55	6·29	—	103·4	103·0	23.7.41	Male	152
26.2.41........	45	45737	6·13	6·68	6·98	102·6	103·0	28.7.41	Male	152
	46	55938	6·48	6·86	—	102·4	103·2	29.7.41	Female	153
	47	55904	6·56	6·80	—	103·6	103·0	28.7.41	Male	152
	48	55865	6·24	6·90	—	103·0	102·6	30.7.41	Male	154
	49	55935	6·18	6·74	—	103·8	103·4	—	—	—
27.2.41........	50	33612	6·00	6·80	—	102·8	102·2	30.7.41	Female	153
	51	55666	5·92	6·60	—	104·0	104·0	27.7.41	Female	150
	52	55859	6·89	6·31	—	103·2	102·8	29.7.41	Female	152
	53	53199	6·36	7·20	—	104·2	103·6	26.7.41	Female	149
	54	51723	6·92	6·69	—	103·0	103·5	27.7.41	Male	150
28.2.41........	55	55909	6·70	6·98	6·13	102·3	102·0	30.7.41	Female	152
	56	55722	5·96	6·70	—	103·8	102·8	—	—	—
	57	55903	6·38	7·01	—	102·0	102·5	1.8.41	Male	154
	58	55723	6·81	6·46	—	103·0	102·4	27·7.41	Male	149
	59	56980	6·59	7·60	—	103·4	102·2	29.7.41	Female	151
	60	55795	5·96	6·50	—	102·6	102·5	29.7.41	Male	151
3.3.41.........	61	55172	6·75	7·00	—	103·6	103·4	31.7.41	Female	150
	62	55923	6·38	6·26	—	102·6	103·5	31.7.41	Female	150
	63	44914	7·18	6·90	—	103·8	103·4	—	—	—
	64	53531	6·10	6·80	—	103·6	103·5	29.7.41	Female	148
	65	46979	6·40	7·13	7·00	104·2	103·6	31.7.41	Female	150
5.3.41.........	66	55677	6·50	7·01	—	102·4	103·0	5.8.41	Male	153
	67	55730	6·90	6·45	—	102·6	102·6	3.8.41	Female	151
	68	55735	6·56	6·70	—	103·6	103·5	1.8.41	Female	149
	69	55855	6·02	6·35	—	102·6	103·2	4.8.41	Female	152
	70	55835¶	5·89	6·96	—	104·0	102·8	13.7.41	Female	130
	71	55824	6·68	6·85	—	103·4	103·3	4.8.41	Male	152

REMARKS.—51–75: Normal service group.
Treated exactly the same as 1–50, but instead of artificial insemination, the ewes were served normally, once in the morning and once in the afternoon.
¶ Aborted.

TABLE 1—(*continued*).

Date.	Ewe No.	D.O.B. No.	pH Oestrus.	pH Mid-dioestrus.	pH during Advanced Pregnancy.	Vaginal Temperature, First Insemination. °F.	Vaginal Temperature, Second Insemination. °F.	Date of Parturition.	Sex of Lamb.	Gestation Period (days).
6.3.41.........	72	55672	6·40	6·49	—	103·7	103·0	6.8.41	Male	153
	73	55810	6·56	6·59	—	103·1	103·4	—	—	—
	74	55886	6·12	7·24	—	103·4	103·5	1.8.41	Male	148
	75	55915	6·50	6·89	6·00	103·0	103·4	6.8.41	Male	153
14.3.41........	76	55884	6·70	—	—	104·0	103·4	—	—	—
	77	41839	6·56	—	—	103·6	103·7	16.8.41	Female	155
	78	55675	6·28	—	—	103·6	104·0	—	—	—
	79	53364	7·26	—	—	104·2	104·8	—	—	—
	80	(?)	6·78	—	—	103·4	103·6	11.8.41	Male	150
	81	49462	6·61	—	—	102·2	103·0	13.8.41	Male	152
	82	49384	6·78	—	—	104·2	104·4	11.8.41	Female / Male	150
	83	55682	7·40	—	—	104·1	103·0	13.8.41	Male	152
	84	55751	6·41	—	—	103·2	103·4	11.8.41	Female	150
	85	55818	6·40	—	5·76	103·4	104·2	10.8.41	Male	149
17.3.41........	86	(?)	7·35	—	—	104·4	104·0	16.8.41	Male	152
	87	56977	7·01	—	—	104·6	104·2	14.8.41	Female	150
18.3.41........	88	55714	—	—	—	104·4	104·0	11.8.41	Male	146
	89	53599	—	—	—	105·3	104·8	16.8.41	Male	151
	90	55936	—	—	—	103·4	104·0	16.8.41	Female	151
19.3.41........	91	51681	7·01	—	—	104·6	105·4	17.8.41	Female	151
	92	49678	7·28	—	—	104·2	104·5	—	—	—
	93	55942	6·62	—	—	103·2	103·8	17.8.41	Female	151
21.3.41........	94	55708	6·16	—	—	104·8	104·8	—	—	—
	95	(?)	6·70	—	6·31	106·2	104·8	21.8.41	Male	153
24.3.41........	96	55716	6·68	—	—	104·0	104·2	25.8.41	Male	154
	97	57131	6·40	—	—	103·4	103·8	22.8.41	Male	151
	98	53210	6·18	—	—	103·4	103·8	28.8.41	Male	149
	99	55782	6·86	—	—	104·4	105·0	—	—	—
	100	55817	6·10	—	—	104·4	104·4	—	—	—

REMARKS.—76–100: To observe the effect of high temperature on artificially inseminated sheep. After the pH was determined, and immediately before insemination, the ewes were driven for about 5 minutes, morning and evening, to raise the temperature.

Analysis of the data appears to indicate that there is a slight tendency for male offspring to be associated with a pH approaching neutral or alkaline measurements. However, this evidence cannot be regarded as conclusive with such small groups of sheep.

When the pH was on the alkaline side 26·92 per cent. of ewes failed to become impregnated, while 17·39 per cent. of those showing an acid reaction failed to conceive, an indication that an alkaline reaction of the vagina at the time of mating may be detrimental to the life or vitality of the spermatozoa in the female genital tract.

Measurements of the vaginal secretion during mid-dioestrus showed a mean pH, for 50 samples, of 6·694, with a range of 6·00 to 7·60.

Owing to the small number of samples tested the data do not allow conclusive deductions, but there appears to be a tendency towards increase in the acidity of the reaction of the vaginal secretion during oestrus. This would appear to be significant in view of the pregnancies resulting from matings, where the semen is ejaculated into an acid or alkaline media, as shown in Table 2.

Nine samples, measured during advanced pregnancy, gave a mean pH value of 6·272, with a range of 6·00 to 7·00. From the few samples tested it would appear that there is a tendency to increased acidity of the vaginal secretion during pregnancy.

The results show that exercise for five minutes increases the temperature of the vagina, as the following group mean-temperatures indicate:—

TABLE 3.

Group.	Mean Temperature, First Insemination or Normal Mating. °F.	Mean Temperature, Second Insemination or Normal Mating. °F.
1	103·286	103·264
2	103·244	103·096
3 (exercised)	104·017	104·108

The temperature of the vagina at the time of normal mating or artificial insemination appears to have no influence on the sex of the lamb.

Matings or artificial inseminations were carried out on sheep showing a range of vaginal temperature between 100·2° F. and 106·2° F. Within this range there was no significant difference in the fertility of the three groups of sheep. These temperatures refer only to the condition of the vagina at 10 a.m. and 4 p.m. just prior to artificial insemination or normal mating. The duration of the recorded temperatures was not ascertained.

CONCLUSIONS.

(1) The pH of the vaginal secretion of mature merino ewes, measured during oestrus, gave a mean value of 6·648, with a range of 5·85 to 7·40. During mid-dioestrus the mean value was 6·694, with a range of 6·00 to 7·60. During pregnancy the mean value was 6·272, with a range of 6·00 to 7·00. There are indications that there is a tendency to slightly increased acidity during oestrus and pregnancy.

(2) There are indications that there is a slight tendency for male offspring to be associated with a pH approaching neutral or alkaline measurements.

(3) The percentage fertility was higher with pH measurements below 7 (82·51 per cent.) than with those above 7 (73·18 per cent.). There are indications that an alkaline medium may be detrimental to the vitality of the spermatozoa in the vagina when pregnancy is used as an indicator.

(4) Forced exercise increased the vaginal temperature of ewes when they are compared with ewes, kept under similar environmental conditions, which were allowed voluntary exercise.

(5) The temperature of the vagina (within a range of 100·2° F. to 106·2° F.), at the time of mating or artificial insemination did not influence the sex of the lamb.

(6) The temperature of the vagina, within a range of 100·2° F. to 106·2° F., did not make a significant difference in the resulting pregnancies in three groups of sheep with mean temperatures, at 1st and 2nd matings or artificial inseminations, of: (1) 103·286, 103·264, (2) 103·244, 103·096, and (3) 104·017, 104·108 (temperatures are recorded in °F.).

(7) The gestation period, for 79 normal pregnancies in merino sheep, showed a range of 146 to 156 days, with an average of 151·4 days.

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