EFFECT OF TIME OF INSEMINATION ON THE DISTRIBUTION OF SPERMATOZOA IN THE GENITAL TRACT IN EWES

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Summary

Merino ewes were artificially inseminated during either early or late oestrus and were killed 4 or 24 hr after insemination. Insemination during late oestrus resulted in fewer spermatozoa in the cervix, the uterus, and the fallopian tubes and in a lower fertilization rate. Increased resistance of the cervical mucus to penetration by spermatozoa during late oestrus appeared to be responsible for these effects.

I. INTRODUCTION

In ewes, ovulation occurs at or soon after the end of oestrus (Kelley 1937; Robinson 1959) and the fertile life of the egg lasts for 12–15 hr (Dauzier and Wintenberger 1952). Kelley (*loc. cit.*) suggested that insemination of ewes toward the end of oestrus would ensure maximal numbers of viable spermatozoa in the fallopian tubes at about the time of ovulation and thus result in maximum fertility. However, it has generally been found that lambing percentages are depressed if ewes are inseminated late in oestrus (Sinclair 1957; Morrant and Dun 1960; Dun and Restall 1961).

In ruminants, the establishment of a large population of spermatozoa in the cervix is necessary to ensure continuity of migration of spermatozoa to the fallopian tubes and good fertility (Quinlan, Maré, and Roux 1932; Mattner 1963*a*). In women, the penetrability of cervical mucus to spermatozoa decreases with increasing viscosity of the mucus (Lamar, Shettles, and Delfs 1940; Abarbanel 1946) and the evidence available (Turnbull, Shutt, and Braden 1967; Lindsay and Francis 1968) suggests that the viscosity of the cervical mucus in sheep increases toward the end of oestrus. The present study was undertaken, therefore, to determine whether the physical state of the cervical mucus in ewes at the time of insemination affects the development of a population of spermatozoa within the cervix and the subsequent migration of spermatozoa to the fallopian tubes.

II. MATERIALS AND METHODS

Vasectomized rams fitted with harnesses and marking crayons (Radford, Watson, and Wood 1960) were used to detect the onset of oestrus in 5-year-old, parous Merino ewes. Ewes not marked by the rams at 6 a.m. but marked at 9.30 a.m. the same day were removed from the flock. At this time and 4 or 24 hr later, an indication of the consistency of the cervical mucus was obtained by assessing the Spinnbarkeit (Clift 1945) and the apparent chloride concentration (Turnbull, Shutt, and Braden 1967).

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Thirty-two ewes were assigned at random to four groups (eight per group):

(1) Inseminated at 10 a.m. on the day of onset of oestrus and killed 4 or 24 hr later.

(2) Inseminated (while still in oestrus) on the day following onset of oestrus and killed 4 or 24 hr later.

For convenience, the groups are designated 0–4, 0–24, 24–4, and 24–24 hr groups. Ewes in the two latter groups were rechecked for oestrus immediately prior to insemination. Each ewe was inseminated with 0·2 ml of freshly collected ram semen ($4–7 \times 10^8$ live sperm) deposited within the first fold of the cervix.

Slaughter of the ewes, removal and flushing of the genital tracts, and assessments of the number of spermatozoa in the flushings were carried out as described previously (Mattner and Braden 1963) except that mucus in flushings from the cervix was dispersed with a few drops of dilute sodium hypochlorite solution. The cervices were flushed from the cranial to the caudal end and were then fixed in 10% buffered formalin. The number of spermatozoa remaining in the cervix was estimated by examination of sections (Mattner 1966).

Eggs recovered in flushings from the fallopian tubes were examined for the presence of spermatozoa on the zona pellucida and were then fixed, cleared, and stained (Mattner 1963*b*) and re-examined for evidence of fertilization.

TABLE 1

NUMBERS OF SPERMATOZOA IN THE GENITAL TRACTS OF EWES INSEMINATED EARLY OR LATE IN OESTRUS AND KILLED 4 OR 24 HR LATER

Time from Insemination to Slaughter (hr)	Time of Insemination (group)	Mean No. of Spermatozoa* in:			Ratio†
		Cervix	Uterus	Fallopian Tubes	
4	Early (0–4 hr)	17,220,000	101,000	13	1:170
	Late (24–4 hr)	525,000	1,000	2	1:525
Significance of difference between means‡		$P<0·001$	$P<0·01$	$P<0·05$	$P<0·05$
24	Early (0–24 hr)	3,169,000	166,300	4,960	1:18
	Late (24–24 hr)	37,000	400	7	1:91
Significance of difference between means‡		$P<0·025$	$P<0·001$	$P<0·001$	$P<0·05$

* Antilogarithm of mean $\log_{10}$ of number of spermatozoa. All means based on eight animals.

† Ratio of number of spermatozoa in uterus and fallopian tubes to number in cervix.

‡ By Student's *t*-test.

III. RESULTS

At both 4 and 24 hr after insemination, there were significantly greater numbers of spermatozoa in the cervices, the uteri, and the fallopian tubes of the ewes inseminated early in oestrus than in the corresponding parts of the genital tracts of the ewes inseminated late in oestrus (see Table 1). Further, the number of

spermatozoa in the uterus and fallopian tubes relative to the number in the cervix was significantly higher in the early-inseminated ewes at both examination times. There was also a significant difference between the early- and the late-inseminated ewes in the number of spermatozoa in the cervix, the uterus, and the fallopian tubes at about the time of ovulation (0–24 hr group *v.* 24–4 hr group, $P<0\cdot01$ by *t*-test).

There was no significant difference between ewes inseminated either early or late in oestrus in either the proportion of spermatozoa that remained in the crypts between the villi of the cervical mucosa after the cervices had been flushed (44–52%) or the distribution of these spermatozoa along the length of the cervix. In the ewes killed 4 hr after insemination (0–4 and 24–4 hr groups), quarters 1, 2, 3, and 4 of the cervices contained 38 ± 5 (S.E.), 35 ± 5, 20 ± 3, and $7\pm2\%$, respectively, of the total number of spermatozoa remaining in the cervix (the quarters were numbered from the caudal end). The corresponding proportions in ewes killed 24 hr after insemination (0–24 and 24–24 hr groups) were 31 ± 6, 33 ± 5, 21 ± 3, and $15\pm3\%$ respectively.

Early in oestrus, the cervical mucus was usually clear and copious, the Spinnbarkeit was high (>10 cm), and the apparent chloride concentration was equivalent to 0·6–0·8% NaCl. There was no obvious change in the characteristics of the mucus in ewes examined 4 hr later, but by 24 hr after the first observation the cervical mucus was cloudy, less profuse, and more cohesive. At this time, the Spinnbarkeit was low (<4 cm) and the apparent chloride concentration was equivalent to 0·1–0·3% NaCl. In one ewe in the 24–4 hr group and another from the 24–24 hr group, the physical characteristics of the cervical mucus at the time of insemination (24 hr) resembled those of mucus during early oestrus and the apparent chloride content was equivalent to 0·5% NaCl. When killed, each ewe had a far greater number of spermatozoa in the cervix than any other ewe in the same group ($11\cdot7\times10^6$ in the 24–4 hr group ewe and $3\cdot5\times10^6$ in the 24–24 hr group ewe).

Ovulation had not occurred prior to slaughter in any of the ewes from the 0–4 hr group. One ovum was recovered from each of five ewes from the 24–4 hr group but none of the ova was fertilized or had spermatozoa attached to the zona pellucida. Eight ova were recovered from seven ewes in the 24–24 hr group; spermatozoa were attached to the zona pellucida of only two of them (both from the same ewe) and only these two ova were fertilized (two pronuclei). In contrast, all eight ova recovered from six ewes in the 0–24 hr group had spermatozoa attached to the zona pellucida (6–30 spermatozoa per ovum) and all were fertilized as evidenced by the presence of two pronuclei and a spermatozoal tail within the vitellus. The difference between the 0–24 and the 24–24 hr groups in both the proportion of ewes with fertilized ova and the proportion of ova fertilized was statistically significant [$P<0\cdot01$ using the Fisher–Yates test of significance (Finney 1948)].

IV. Discussion

The present findings indicate that the relatively poor fertility of ewes inseminated late in oestrus compared with that of ewes inseminated early in oestrus (Sinclair 1957; Morrant and Dun 1960; Dun and Restall 1961) is due to a low fertilization rate. Fewer spermatozoa were found in the fallopian tubes at about the time of ovulation in the former ewes and this was evidently caused by both a decrease in the

number of spermatozoa entering the cervix and a decrease in the proportion of the "cervical" spermatozoa entering the uterus.

The smaller number of spermatozoa entering the cervix may be attributed to the greater viscosity of the cervical mucus of the late-inseminated ewes, for Lamar, Shettles, and Delfs (1940) and Abarbanel (1946) have shown that human cervical mucus becomes more resistant to penetration by spermatozoa as its viscosity increases. Evidently, the progression of spermatozoa within the substance of the mucus was not severely retarded, for the distribution of the spermatozoa within the cervix was similar in the early-inseminated and the late-inseminated ewes. Nevertheless, an increase in the viscosity of the mucus could, by retarding the progress of spermatozoa, decrease the rate of escape of spermatozoa from the cervix into the uterus.

An alternative explanation for fewer spermatozoa in the uterus and fallopian tubes relative to the number in the cervix in the late-inseminated than in the early-inseminated ewes may be that there was a more rapid loss of spermatozoa from the uterus and fallopian tubes of the former ewes. In ruminants, there is an increase in the number of leucocytes in the lumen of the uterus with time during oestrus (Mattner 1968) and consequently the losses of spermatozoa by phagocytosis may have been greater in the late-inseminated ewes. It does appear, however, that the difference between the fertility of ewes inseminated early or late in oestrus may be attributed mainly to differences in the physical state of the cervical mucus.

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