DIFFERENTIAL LEUCOCYTIC RESPONSES TO SPERMATOZOA IN THE CERVIX AND THE UTERUS IN EWES

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Summary. The effect of spermatozoa within the cervix and of spermatozoa within the uterus on the infiltration of polymorphonuclear leucocytes (polymorphs) into the lumen of the genital tract at oestrus was examined in ewes. Ligation of the cranial end of the cervix resulted in an accumulation of polymorphs in the lumen of the uterus and of fewer polymorphs in the lumen of the cervix. In genital tracts so ligated, spermatozoa within either the cervix or the uterus had no material effect on the number of polymorphs found in the cervix. The presence of spermatozoa in ligated uterine horns caused an increase in the number of polymorphs migrating into the lumina of the horns, the response being manifest within 5 hr. The results indicate that drainage of polymorphs from the uterus into and through the cervix is a normal occurrence in ruminants which may reduce the incidence of phagocytosis of spermatozoa in the uterus.

INTRODUCTION

In small laboratory animals, the presence of spermatozoa in the female genital tract does not have a strong stimulatory effect on the migration of leucocytes into the lumen of the uterus until after ovulation has occurred (McDonald, Black, McNutt & Casida, 1952; Austin, 1957; Yanagimachi & Chang, 1963). The same may not be true in ruminants. Mating of goats does result in greater numbers of leucocytes in the uterus and cervix at about the time of ovulation (Mattner, 1968), but Howe & Black (1963) found that a marked increase in the number of leucocytes entering the vagina occurred within 4 to 8 hr after insemination in hormone-treated, prepuberal calves. However, neither of the latter studies revealed whether the leucocytic response to spermatozoa was rapid enough for phagocytosis of spermatozoa by the leucocytes to reduce the number of spermatozoa in the uterus (thus the number of spermatozoa passing to the Fallopian tubes) before ovulation.

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In hamsters in which the uterine horns had been ligated near the cervix, Yanagimachi & Chang (1963) found that mating with a fertile male had no effect on the number of leucocytes entering the lumina of the horns. However, in ruminants, the cervix is a relatively large and highly developed structure in which are located the majority of the spermatozoa that remain in the genital tract after mating. Thus, it is possible that spermatozoa in either the uterus or the cervix might stimulate the migration of leucocytes into the uterus in ruminants.

Studies on these aspects in sheep are presented in this paper.

MATERIALS AND METHODS

Thirty parous Herdwick ewes, aged 3 to 5 years were used in two experiments during the normal breeding season. To facilitate operational procedure, oestrus was controlled by administering 10 mg of progesterone in oily solution i.m. daily for 10 days and 800 i.u. pregnant mare serum gonadotrophin given s.c. 24 hr after the last injection of progesterone. Oestrus was detected using an aproned ram.

Ram semen was obtained by electro-ejaculation. For intra-uterine inseminations, the semen was collected aseptically as follows. The glans and urethral process of the penis were washed with sterile saline and dried with sterile gauze. After the first portion of the ejaculate had been produced, the glans and urethral process were washed and dried as before and the final portion of the ejaculate was then collected with only the urethral process contained within a sterile collecting tube. The semen was held at 36° C and was diluted with sterile saline immediately before use.

Laparotomy, ligation of the genital tracts and uterine inseminations were performed while the ewes were under cyclopropane anaesthesia. When intra-uterine inseminations were performed, each uterine horn was ligated near the junction of the horn with the body of the uterus and a second ligature was placed loosely around the horn near the utero-tubal junction. A 26-gauge hypodermic needle was inserted through the wall of each horn of the uterus near its apex and either a suspension of spermatozoa or sterile saline was injected into the lumen with a micro-syringe. The second ligature was tied firmly about 0.5 cm caudal to the site of puncture as the needle was withdrawn.

The methods used for recovery and flushing of the genital tracts and assessment of the numbers of polymorphonuclear leucocytes (polymorphs) in the lumina and the endometrial stroma of the genital tracts have been described previously (Mattner, 1968).

Data were converted on the log₁₀ scale to give homogeneous variability and were analysed by analysis of variance.

Experiment 1

The cervix was ligated at the cranial end in each of ten ewes as soon as possible after commencement of oestrus. Five of these ewes were inseminated approximately 1 hr later by flooding 0.5 ml of freshly collected ram semen over the external orifice of the cervix. The genital tracts of the ten ewes were removed

25 hr post-operatively. The genital tracts of five control ewes which received no treatment were removed 25 hr after commencement of oestrus.

Experiment 2

The cranial and caudal extremities of each uterine horn were ligated in fifteen ewes during early oestrus. In each ewe, twenty million spermatozoa in 0.2 ml containing 500 i.u. crystalline penicillin and 500 i.u. streptomycin per ml were injected into the lumen of one horn and 0.2 ml of sterile saline containing similar concentrations of penicillin and streptomycin was injected into the lumen of the other horn. The genital tracts were removed from five ewes at 5, 15 and 25 hr after this procedure.

RESULTS

Experiment 1

Ovulation of at least one egg had occurred shortly before recovery of the genital tracts in three control ewes and in four ewes from each group in which

TABLE 1
THE EFFECT OF LIGATION OF THE CRANIAL END OF THE CERVIX AND OF SPERMATOZOA IN THE CERVIX ON THE NUMBER OF LUMINAL POLYMORPHONUCLEAR LEUCOCYTES IN THE CERVIX AND THE UTERUS IN SHEEP

Treatment of ewes	Cervix		Uterus	
	Mean $\log_{10}$ no. polymorphs (with antilog)	Difference between means	Mean $\log_{10}$ no. polymorphs (with antilog)	Difference between means
A. Nil	5.178 (151,000)	A-B, 1.027***	5.808 (0.64 $\times 10^6$)	A-B, -0.748**
B. Cervix ligated	4.151 (14,000)	A-C, 1.386***	6.556 (3.60 $\times 10^6$)	A-C, -0.700**
C. Cervix ligated + vaginal insemination	3.792 (6000)	B-C, 0.359 ^{N.S.}	6.508 (3.22 $\times 10^6$)	B-C, 0.048 ^{N.S.}

All means based on five ewes.

S.E. of difference between means = 0.205, d.f. = 12.

N.S., difference not significant; ** difference significant, $P < 0.01$; *** difference significant, $P < 0.001$.

the cervix had been ligated. In each of the remaining ewes, there was at least one large preovulatory follicle.

The mean $\log_{10}$ numbers of polymorphs recovered from the lumina of the cervixes and uteri of the ewes from the three groups are shown in Table 1. For both groups of ewes in which the cervix had been ligated, the number of polymorphs in the lumen of the cervix was considerably lower than that for the ewes in the untreated group ($P < 0.001$). On the other hand, in both groups of ewes in which the ligation had been performed, the number of polymorphs in the lumen of the uterus was greater than the number in the uteri of the untreated ewes ($P < 0.01$).

When the cervixes of the ewes that had been inseminated were examined, live spermatozoa were present in all instances. The mean number of spermatozoa flushed from the cervixes of these ewes was 290,000 (S.E. 57,000), and 53 (S.E. 5)% of these were motile. Insemination did not have any significant effect on the number of polymorphs in the lumen of either the cervix or the uterus in ewes in which the cervix had been ligated (see Table 1).

To verify that ligation of the cervix had not influenced the numbers of polymorphs infiltrating from the vascular system into the endometrial stroma of the cervixes and uteri, and thus the number appearing in the lumina of these organs, the numbers of polymorphs in the cervical and uterine endometrial stroma of the ewes in all three groups were determined. There was no significant

TABLE 2

THE EFFECT OF LIGATION OF THE CRANIAL END OF THE CERVIX AND OF SPERMATOZOA IN THE CERVIX ON THE NUMBER OF POLYMORPHONUCLEAR LEUCOCYTES IN THE ENDOMETRIAL STROMA OF THE CERVIX AND UTERUS IN SHEEP

Treatment of ewes	Cervix		Uterus	
	Mean $\log_{10}$ no. polymorphs (with antilog)	Difference between means	Mean $\log_{10}$ no. polymorphs (with antilog)	Difference between means
A. Nil	5.412 (0.61×10^6)	A-B, 0.034 ^{N.S.}	7.484 (6.85×10^6)	A-B, -0.056 ^{N.S.}
B. Cervix ligated	5.378 (0.58×10^6)	A-C, -0.044 ^{N.S.}	7.540 (7.32×10^6)	A-C, -0.037 ^{N.S.}
C. Cervix ligated + vaginal insemination	5.456 (0.66×10^6)	B-C, -0.078 ^{N.S.}	7.521 (7.17×10^6)	B-C, 0.019 ^{N.S.}

All means based on five ewes.

S.E. of difference between means = 0.197, d.f. = 12.

N.S., difference not significant.

difference between any of the three groups of ewes in the number of polymorphs in the endometrial stroma in either the cervix or the uterus (see Table 2).

Experiment 2

None of the five ewes examined at 5 hr, and only one of the five ewes examined 15 hr post-operatively, had ovulated. At 25 hr, four of the five ewes had ovulated at least one egg and the ovaries of the remaining animal contained large preovulatory follicles.

At each of the three examination times, a proportion of the polymorphs flushed from the spermatozoa-injected uterine horns contained engulfed spermatozoa. The mean percentages ($\pm$ S.E.) of the polymorphs that contained spermatozoa at 5, 15 and 25 hr were 22 ± 4 , 20 ± 3 and $28 \pm 5\%$, respectively.

There was a progressive and highly significant increase in the number of luminal polymorphs with time in the spermatozoa-injected and the saline-injected uterine horns, but the number of luminal polymorphs was greater in the former than in the latter horn in four of five ewes at 5 hr, and in all five ewes at both 15 and 25 hr post-operatively. Analyses showed that the difference

between the mean $\log_{10}$ numbers of luminal polymorphs in the differently treated horns was significant at each examination time (see Table 3).

There was also an increase, with time, in the number of polymorphs in the endometrial stroma in each group of uterine horns. The mean $\log_{10}$ numbers (with antilog) of 'stromal' polymorphs at 5, 15 and 25 hr were 7.109 (12.8 million), 7.242 (17.5 million) and 7.324 (21.1 million), respectively, for the spermatozoa-injected horns, and 7.082 (12.1 million), 7.118 (13.1 million) and 7.207 (16.1 million), respectively, for the saline-injected horns. However, neither of the increases in the number of polymorphs in either group of horns, nor the difference between the mean $\log_{10}$ numbers in the two groups of horns at 5, 15 or 25 hr was significant ($P > 0.05$).

A difference between spermatozoa-injected and saline-injected uterine horns was found, however, when the total number of polymorphs (luminal and stromal) or the percentage of the total numbers of polymorphs present in the

TABLE 3
THE EFFECT OF SPERMATOZOA IN LIGATED UTERINE HORNS OF EWES ON
THE NUMBER OF LUMINAL POLYMORPHONUCLEAR LEUCOCYTES

Treatment of horn	Mean $\log_{10}$ no. of polymorphs (with antilog) at:		
	5 hr	15 hr	25 hr
Spermatozoa and saline introduced	5.653 (0.45×10^6)	6.773 (5.93×10^6)	6.962 (9.16×10^6)
Saline introduced	5.200 (0.16×10^6)	6.364 (2.31×10^6)	6.458 (2.87×10^6)
Difference between means	0.453**	0.409**	0.504***

All means based on five horns.

S.E. of difference between means = 0.109, d.f. = 12.

** Difference significant, $P < 0.01$; *** difference significant, $P < 0.001$.

lumen at either 15 or 25 hr post-operatively was considered. The mean $\log_{10}$ values (with antilog) for the total numbers of polymorphs in spermatozoa-injected and saline-injected horns were 7.373 (23.6 million) and 7.195 (15.7 million), respectively, at 15 hr and were 7.488 (30.8 million) and 7.286 (19.3 million), respectively, at 25 hr. At each of these times, the difference between the means was significant (S.E. of difference = 0.073, d.f. = 8, $P < 0.05$). The mean percentages ($\pm$ S.E.) of the total numbers of polymorphs in the lumina of the spermatozoa-injected horns and the saline-injected horns were 25.8 ± 2.9 and $16.0 \pm 2.9\%$, respectively, at 15 hr, and 31.0 ± 4.4 and $16.6 \pm 3.6\%$, respectively, at 25 hr. Using analysis of variance, the difference between the means at 15 hr fell just short of significance at the 5% level but the difference between the means at 25 hr was significant (S.E. of difference = 4.5, d.f. = 8, $P < 0.05$).

DISCUSSION

Results obtained in the present study support the conclusion that, in ruminants, the majority of the polymorphs that are found in the lumen of the cervix during

oestrus have come from the lumen of the uterus (Mattner, 1968). Although similar numbers of polymorphs were present in the endometrial stroma of the ligated and unligated cervixes of ewes at about the end of oestrus, the number of luminal polymorphs in the cervixes of the ewes in which the cranial end of the cervix had been ligated was extremely small compared with the number in the cervixes of the control ewes. At the same time, the number of polymorphs in the uterine lumina was greater in the former than in the latter ewes and this could only be ascribed to the effect of the ligation of the cervix in preventing the escape of polymorphs from the lumen of the uterus to that of the cervix.

It was found, in ewes, that a stimulatory effect of spermatozoa on the migration of polymorphs into the lumen of the uterus only occurs, as in the hamster (Yanagimachi & Chang, 1963), when spermatozoa are in the uterus. The presence of spermatozoa in the cervix and vagina had no effect on the migration of polymorphs into the lumen of the uterus or the cervix. In ewes, the majority of spermatozoa that are retained in the genital tract after mating are located in the cervix, these forming the spermatozoan reserve (Quinlan, Maré & Roux, 1932; Mattner, 1963). Thus, the establishment of the spermatozoan reserve in the cervix in the ewe may help to reduce the incidence of phagocytosis of spermatozoa in the uterus by minimizing the stimulus to the migration of leucocytes into the uterus.

In small laboratory animals, the presence of spermatozoa in the uterine horns does not materially increase the number of leucocytes entering the lumen of the horns until late oestrus or metoestrus (McDonald *et al.*, 1952; Austin, 1957; Yanagimachi & Chang, 1963). However, the leucocytic response to spermatozoa in the uterine horns in sheep occurred during early oestrus and was evident within 5 hr of the introduction of spermatozoa. The leucocytic response observed in the present study may be greater, quantitatively, than would normally occur, for the number of spermatozoa introduced into the uterine horns was far greater than the numbers that are found in the uterus of the ewe at any time after mating (Mattner, 1963). Nevertheless, as polymorphs in mucus obtained from cervixes of cows will engulf motile spermatozoa *in vitro* (Mattner, 1966), the rapidity of the response should militate against the survival of spermatozoa in the uterus and reduce the number of spermatozoa entering the Fallopian tubes. However, it appears from the present and previous studies (Mattner, 1968) that the passage or drainage of polymorphs from the lumen of the uterus to that of the cervix is a normal phenomenon in ruminants which would tend to reduce spermatozoan mortality in the uterus.

The foregoing also re-emphasizes the physiological significance of the spatial separation of the polymorph and spermatozoan population which occurs in the cervix in ruminants (Mattner, 1968). Thus, drainage of polymorphs from the lumen of the uterus can be effected by way of the cervical lumen with little consequence to the reserve of spermatozoa established in the cervix.

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