THE INFLUENCE OF THE FLUIDS FROM THE GENITAL TRACT OF THE EWE ON THE SURVIVAL OF RAM SPERMATOZOA

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Summary

The survival and viability of ram spermatozoa in the presence of fluids from the genital tract of the ewe was studied under various incubation conditions for 6 hr at 37°C.

Cervicovaginal fluid maintained spermatozoa at a relatively high level of activity for the 6-hr period. Uterine and tubal fluid did not maintain the spermatozoa as well, and after 6 hr the percentage of motile sperm was very low.

Tris buffer favoured survival of spermatozoa in the saline controls, whilst a bicarbonate buffer enhanced the survival of spermatozoa incubated in uterine or tubal fluid. The effects of the addition of lactate as a substrate were superior to those of glucose, and favoured the survival and viability of spermatozoa in uterine or tubal fluids. Spermatozoa incubated in the presence of cervocivaginal fluid were relatively immune to changes in the incubation conditions. Despite these interactions, in all experiments cervicovaginal fluid was superior to uterine and tubal fluid in the ability to maintain the viability of spermatozoa.

The results are discussed in relation to the known physiology of spermatozoa in the female genital tract.

I. INTRODUCTION

Several recent studies have examined the effect of fluids obtained from the female genital tract on the metabolism of spermatozoa (Olds and Vandemark 1957; Hamner and Williams 1963, 1964; Kirton and Hafs 1965; Restall and Wales 1966*a*; Wales and Restall 1966; Foley and Williams 1967; Murdoch and White 1968). Most of these studies have been aimed at defining the metabolic changes in the spermatozoa resulting from their incubation with genital tract fluids. Another possible important function of these fluids, their effect on the survival and viability of spermatozoa, has received scant attention.

This paper reports several experiments examining the survival and viability of spermatozoa incubated with genital fluids under various conditions.

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II. Materials and Methods

(a) *Genital Fluids*

Tubal and uterine fluids were collected from ewes with cannulae in either the fallopian tubes or a horn of the uterus (Restall 1966). Cervicovaginal fluid was obtained by aspiration from the vaginae of ewes in oestrus. Follicular fluid was obtained by aspiration of follicles post mortem. In all experiments the fluid samples were pooled from at least four ewes, although the pooled samples were not necessarily the same in all experiments.

(b) *Semen*

Ram semen was collected by electrical stimulation (Blackshaw 1954) and only ejaculates of good initial motility were used. After collection, the spermatozoa were twice washed in the appropriate buffered saline diluent and resuspended before being added to the incubation mixture.

(c) *Incubation of Semen*

The incubation of spermatozoa was carried out in small test tubes at 37°C for 6 hr, in the presence of one of the four types of genital fluid. The survival and viability of the spermatozoa were compared with that recorded when the genital fluid was replaced by an equal volume of 0·9% (w/v) sodium chloride.

Where bicarbonate buffer was used, the bicarbonate content of the genital fluids was determined manometrically and the final concentration of bicarbonate in the incubation mixture adjusted to 15 mM. Carbon dioxide (5%) in air was reticulated over these mixtures. Phosphate and Tris buffers were used at a final concentration of 20 mM per tube (pH 7·3). Except where specified, each tube contained 15 μmoles of glucose and had a final volume of 1·0 ml, tonicity being maintained by adding the appropriate amount of sodium chloride.

(d) *Observations*

The activity of the spermatozoa was scored from samples in thin films under glass coverslips. A microscope with the stage warmed to 37°C to heat the samples was used. A motility score of 0–40 was used to describe the velocity of progression of the spermatozoa and a visual estimation of the percentage of motile spermatozoa was made. These scores were recorded for samples taken from the incubation tubes at 1·5, 3·0, and 6·0 hr.

(e) *Statistical Analyses*

Analyses of variance were made on the sets of data for each experiment using a CDC 3200 computer. Each experiment was treated as a split-plot design (Snedecor 1957) and the appropriate error variances used for significance testing. Each ejaculate in these experiments was used for a complete replication of the experimental design so that the term "ejaculates" in the analyses represents replicates and no individual ram gave more than one ejaculate for each experiment.

III. Experimental and Results

(a) *Experiment 1: Effect of Phosphate and Tris Buffers on the Survival of Spermatozoa in the Presence of Genital Fluids*

Four ejaculates of ram semen were divided and washed in either phosphate or Tris buffer in sodium chloride. The spermatozoal suspension ($1·2 \times 10^8$ cells per tube) was then added to incubation tubes containing either saline (control), cervicovaginal, uterine, tubal, or follicular fluid.

The effects of the treatments (Table 1) was essentially similar for both motility and percentage motile spermatozoa. Tris buffer favoured the survival and viability

of spermatozoa in the saline controls, while having no effect on the spermatozoa incubated with genital fluids. Both motility and percentage motile spermatozoa declined over time, but the genital fluids had markedly different effects. Cervico-

TABLE 1

SURVIVAL AND VIABILITY OF RAM SPERMATOZOA IN THE PRESENCE OF FLUIDS FROM THE GENITAL TRACT OF THE EWE, IN EITHER PHOSPHATE OR TRIS BUFFER (EXPERIMENT 1)

Data are means for four ejaculates

Genital Tract Fluid	Incubation Period 1·5 hr		Incubation Period 3·0 hr		Incubation Period 6·0 hr	
	Motility	% Motile	Motility	% Motile	Motility	% Motile
Phosphate buffer						
Saline	17·5	33·8	16·3	25·0	3·8	7·5
Cervico-vaginal	32·5	55·0	32·5	47·5	22·5	37·5
Uterine	22·5	50·0	8·8	30·0	0·0	0·0
Tubal	23·8	50·0	16·3	35·0	2·5	5·0
Follicular	27·5	50·0	22·5	45·0	13·8	27·5
Tris buffer						
Saline	28·8	52·5	26·3	47·5	16·3	30·0
Cervico-vaginal	32·5	60·0	31·5	50·0	10·0	20·0
Uterine	20·0	47·5	8·8	17·5	0·0	0·0
Tubal	26·3	60·0	13·8	30·0	1·3	2·5
Follicular	30·0	57·5	23·8	50·0	18·8	35·0

Summary of the Analyses of Variance

Source of Variation	Degrees of Freedom	Variance Ratios	
		Motility	% Motile
Between treatments:			
Effect of buffer (A)	1	2·82	3·28
Effect of fluids (B)	4	33·27**	27·20**
Ejaculate differences (C)	3	3·63*	3·51*
$A \times B$	4	6·70**	6·30**
$A \times C$	3	0·40	1·46
$B \times C$	12	0·60	0·38
$A \times B \times C$ (mean square)	12	*32·58*	*50·89*
Effect of time (D)	2	160·25**	208·15**
$D \times A$	2	0·89	0·98
$D \times B$	8	4·81**	11·98**
$D \times C$	6	1·40	1·95
Residual (mean square)	62	*19·63*	*36·09*

* $P < 0·05$. ** $P < 0·01$.

vaginal and follicular fluids maintained the spermatozoa better than did uterine and tubal fluids. The decline in motility and percentage motile spermatozoa was quite rapid in tubal and uterine fluids.

(*b*) *Experiment 2: Effect of Phosphate and Bicarbonate Buffers on the Survival and Viability of Spermatozoa Incubated with Genital Fluids*

Four ejaculates were used in the manner described in experiment 1 with the exception that bicarbonate and phosphate buffers were used, and follicular fluid was omitted.

TABLE 2

SURVIVAL AND VIABILITY OF RAM SPERMATOZOA IN THE PRESENCE OF FLUIDS FROM THE GENTIAL TRACT OF THE EWE, IN EITHER PHOSPHATE OR BICARBONATE BUFFER (EXPERIMENT 2)

Data are means for four ejaculates

Genital Tract Fluid	Incubation Period 1·5 hr		Incubation Period 3·0 hr		Incubation Period 6·0 hr	
	Motility	% Motile	Motility	% Motile	Motility	% Motile
Phosphate buffer						
Saline	25·0	37·5	22·5	30·0	7·5	7·5
Cervico-vaginal	36·3	55·0	30·0	45·0	22·5	40·0
Uterine	22·5	52·5	13·8	35·0	0·0	0·0
Tubal	27·5	50·0	11·3	27·5	1·3	2·5
Bicarbonate buffer						
Saline	27·5	40·0	25·0	37·5	22·5	35·0
Cervico-vaginal	32·5	50·0	27·5	42·5	31·3	45·0
Uterine	25·0	45·0	22·5	40·0	12·5	12·5
Tubal	27·5	52·5	25·0	47·5	8·8	12·5

Summary of the Analyses of Variance

Source of Variation	Degrees of Freedom	Variance Ratios	
		Motility	% Motile
Between treatments:			
Effect of buffer (*A*)	1	40·76**	19·74**
Effect of fluids (*B*)	3	52·80**	14·05**
Ejaculate differences (*C*)	3	7·55**	0·39
$A \times B$	3	3·37	2·86
$A \times C$	3	0·32	0·59
$B \times C$	9	4·45*	1·89
$A \times B \times C$ (mean square)	9	*18·56*	*54·83*
Between times:			
Effect of time (*D*)	2	93·43**	122·20**
$D \times A$	2	12·04**	17·81**
$D \times B$	6	5·50**	13·87**
$D \times C$	6	0·37	0·92
Residual (mean square)	48	*18·75*	*33·64*

* $P < 0·05$. ** $P < 0·01$.

Similar results were obtained for motility and the percentage of motile spermatozoa (Table 2). Survival and viability of the spermatozoa were much greater when bicarbonate buffer was used, with the exception that in cervicovaginal fluid

the results were similar irrespective of the buffer used. The fluids again showed marked effects with spermatozoa surviving well in cervicovaginal fluid but performing poorly in uterine and tubal fluids. The decline in the parameters measured over time was greatest in phosphate buffer and in uterine and tubal fluids.

TABLE 3

SURVIVAL AND VIABILITY OF RAM SPERMATOZOA IN THE PRESENCE OF FLUIDS FROM THE GENITAL TRACT OF THE EWE WITH EITHER GLUCOSE OR LACTATE AS AN ADDED SUBSTRATE AND WITH BICARBONATE BUFFER

Data are means of three experiments each with three replicates

Genital Tract Fluid and Substrate	Incubation Period 1·5 hr		Incubation Period 3·0 hr		Incubation Period 6·0 hr		Means	
	Motility	% Motile	Motility	% Motile	Motility	% Motile	Motility	% Motile
Saline (control)								
Glucose	39·4	47·0	37·8	45·0	29·4	39·7	35·6	43·9
Lactate	37·2	44·3	33·3	44·3	27·2	41·7	32·6	43·0
Cervicovaginal								
Glucose	39·4	46·3	38·3	46·3	35·6	42·3	37·5	45·0
Lactate	36·1	45·7	37·2	43·7	35·0	41·7	36·1	43·7
Uterine								
Glucose	32·2	45·0	25·0	35·7	12·2	21·3	23·7	34·0
Lactate	33·9	45·7	30·6	41·0	21·7	34·0	28·7	40·2
Tubal								
Glucose	37·2	50·3	25·0	39·7	11·7	21·3	24·6	37·1
Lactate	36·7	49·0	36·7	45·0	26·1	35·7	33·2	43·2

Summary of the Analyses of Variance

Source of Variation	Degrees of Freedom	Variance Ratios	
		Motility	% Motile
Between treatments			
Effect of substrate (A)	1	3·96	12·76*
Effect of fluids (B)	3	41·66**	12·93**
Difference between experiments (C)	2	15·31**	13·82**
Ejaculate differences within experiments (D)	6	1·40	1·58
$A \times B$	3	15·81**	91·90**
$B \times C$	6	4·51**	1·39
Other interactions	32	1·39	1·55
$A \times B \times D$ (mean square)	18	*26·38*	*26·03*
Between times			
Effect of time (E)	2	33·65**	93·55**
$E \times C$	4	5·88**	1·40
$E \times D$	12	0·64	1·28
$E \times A$	2	9·28**	12·12**
$E \times B$	6	11·09**	14·20**
Other interactions	22	3·61	2·18
Residual (mean square)	96	*20·25*	*22·83*

* $P < 0·05$. ** $P < 0·01$.

(c) *Experiment 3: Effect of Glucose and Lactate on the Survival and Viability of Spermatozoa Incubated with Genital Fluids*

Three identical experiments were performed, each using three ejaculates. The incubation of spermatozoa with either saline, cervicovaginal, uterine, or tubal fluid in bicarbonate buffer (pH 7·3) was as described previously. The incubation mixtures contained either 15 μmoles of glucose or 10 μmoles of lactate as the added substrate

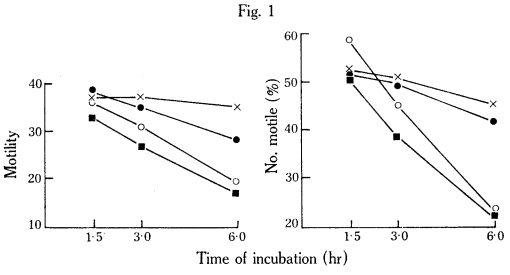
Essentially similar results were observed for both motility and the percentage of motile spermatozoa (Table 3). The pattern of the results was also similar for the three experiments with the following exceptions:

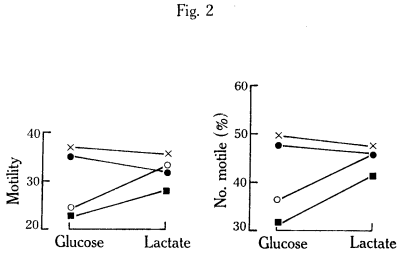
(1) The overall magnitude of the parameters varied between the experiments.

(2) In one experiment, viability and survival of spermatozoa in uterine fluid were observed to be much lower than in the other two experiments. However, the ranking of the fluids was not affected.

(3) In one experiment the decline in the parameters was much sharper over the time period than in the other two experiments.

The results confirmed the earlier observations. Survival and viability declined with time, but both declined less in cervicovaginal fluid and in the saline controls than in uterine and oviduct fluids. This decline with time was also much more rapid when uterine or tubal fluid was present (Fig. 1).





Figs. 1 and 2.—Interaction of time of incubation and type of genital tract fluid (Fig. 1) and of type of substrate and type of genital tract fluid (Fig. 2) on the viability and survival of ram spermatozoa incubated with bicarbonate buffer. All data are means from three experiments, each with three ejaculates. ● Saline controls. × Cervicovaginal fluid. ■ Uterine fluid. ○ Tubal fluid.

The addition of lactate to the incubation mixture enhanced the survival and viability of sperm in uterine and tubal fluids, while having no effect in the presence of cervicovaginal fluid or the saline controls (Fig. 2). Lactate also prevented the rapid decline in viability over time that was observed when glucose was present (Fig. 3).

IV. Discussion

Most of the reports cited in the introduction have been concerned with the biochemical changes in spermatozoa resulting from contact with secretions of the

female genital tract. In this way it was hoped to shed light on the mechanism of "capacitation" or the attainment of fertilizing ability, a property imparted to the spermatozoa by the female genital tract (Austin 1951; Chang 1951). It is difficult to correlate biochemical changes with capacitation because there are no techniques available for determining, *in vitro*, if spermatozoa have become capacitated. While much effort has been put into solving these problems, other possible physiological effects of the female genital fluids have been largely neglected. However, the results of the experiments reported here indicate that the genital fluids have a significant role in the maintenance of spermatozoa in the female tract.

In all the experiments, cervicovaginal fluid was able to maintain the survival rate and viability of the spermatozoa at a relatively high level with little decline over the 6-hr incubation period. On the other hand, spermatozoa incubated in tubal or uterine fluid showed a rapid decline during the same period. This ranking was unaffected by changes in the incubation conditions, which mainly affected the magnitude of the responses observed in spermatozoa incubated with saline or with uterine and tubal fluid. Spermatozoa in cervicovaginal fluid were relatively immune to changes in the buffer system or the substrate added.

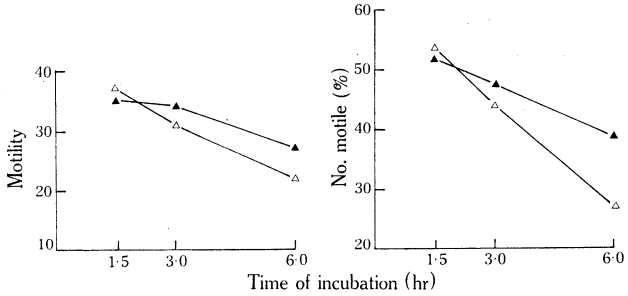


Fig. 3.—Interaction of time of incubation and type of substrate on the viability and survival of ram spermatozoa incubated in bicarbonate buffer with fluids from the genital tract of the ewe. Data are means from three experiments, each with three ejaculates. △ Glucose substrate. ▲ Lactate substrate.

The provision of a bicarbonate buffer system and lactate as a substrate was beneficial to spermatozoa in contact with uterine or tubal fluid. Both bicarbonate and lactate are present in the reproductive tract fluids of the ewe (Restall and Wales 1966*b*; Perkins and Goode 1966), and so appear to provide optimum conditions for the survival of spermatozoa in these parts of the tract.

The results from the *in vitro* system used here correlate well with reports of the viability of spermatozoa in the female reproductive tract. Quinlan, Maré, and Roux (1932, 1933) concluded that the cervix of the ewe acts as a reservoir for spermatozoa and that motility is still maximal 24 hr after insemination. They further found that only 5% of spermatozoa retained motility after 9 hr in the fallopian tubes. These conclusions have been confirmed by later workers (Warbritton, *et al.* 1937; Starke 1949; Edgar and Asdell 1960; Mattner 1963). The agreement between the results *in vitro* and the observations in the live animal suggests that the secretions of the various parts of the tract have a significant role in the maintenance of the spermatozoa.

The significance of the cervical population of spermatozoa is well illustrated by Lightfoot and Salamon (personal communication) who showed that with deep-frozen ram semen, fertilization will only result if an adequate cervical population of live spermatozoa is established. Mattner (1966) has shown that it is the particular rheological properties of cervical mucus that are responsible for the retention of spermatozoa in the cervix and their subsequent slow release to other parts of the tract. The results reported here suggests that this mucus is also responsible, at least in part, for the survival of spermatozoa in this region. At this time the factors in cervical mucus responsible for this survival are unknown, but Gibbons and Mattner (1966) found abundant galactose and amino sugars in the epithelial glycoprotein fraction of mucus and these may be involved. Further speculation is provided by Terner (1965) who found that human spermatozoa can incorporate glucosamine carbon into their lipids and suggests that the spermatozoa may be able to replenish their reserves in this way while they are in the female tract.

Spermatozoa die relatively rapidly in the uterus and tubes (Quinlan, Maré, and Roux 1933; Edgar and Asdell 1960) and the results reported here again indicate that the secretions of these parts are significantly involved. Aged spermatozoa are detrimental to early embryonic development (see review by Salisbury 1965) and their early elimination in the uterus and tubes would be an advantage. The continued progression of viable spermatozoa from the cervical reservoir over a long period of time and their rapid death in the uterus and tubes would tend to maximize the possibility of successful fertilization and subsequent development.

V. Acknowledgments

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