THE FALLOPIAN TUBE OF THE SHEEP

V.* SECRETION FROM THE AMPULLA AND ISTHMUS

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Summary

The fallopian tubes of four ewes were cannulated so that secretions collected from the isthmus and ampulla of one side could be compared with the secretion from the other entire fallopian tube. Maximum fluid output from all sites occurred at oestrus and was least during the luteal phase of the cycle. Secretion from the ampulla was generally twice that from the isthmus.

In general, fluids obtained from the ampulla and isthmus were similar in composition, sodium being the major cation and chloride the major anion. Bicarbonate concentration was higher in the isthmus than in the ampulla and was also higher during dioestrus. Lactate was present in all fluids and was highest during oestrus. No glucose was detected.

Oxygen uptake by spermatozoa was significantly lower in all the tubal fluids when compared with control incubations. However, spermatozoa in fluid from the isthmus had a higher oxygen uptake than spermatozoa in fluid from the ampulla. In all cases, incubation in tubal fluids enhanced the glycolysis of spermatozoa. All metabolic parameters were highest in fluids from the dioestrous phase of the cycle.

I. INTRODUCTION

Fertilization is generally considered to occur in the ampulla or upper portion of the tube, and the zygotes reside in the isthmus or lower portion for up to 3 days before passing to the uterus (Austin 1961). During these 3 days the zygote undergoes several cell divisions and shows some significant changes in its requirements for exogenous substrates (Brinster 1965; Brinster and Thomson 1966). Further, Zamboni, Hongsanand, and Mastroianni (1965) have shown that a tubal factor is required for the removal of the corona radiata, and Whittingham and Biggers (1967) demonstrated that the cultured fertilized mouse ovum requires a period in the tube before cleavage will occur.

The fallopian tube also shows morphological differences along its length, the secretory cells being mainly contained in the ampulla region (Hadek 1955; Restall 1966*c*). The epithelia of the isthmus and ampulla also appear to respond differently to oestrogen (Restall 1966*b*).

These considerations have lead us to investigate the secretions from the ampulla and isthmus of the fallopian tube of sheep.

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II. Materials and Methods

(*a*) *Secretion Studies*

Siliconized rubber cannulae were inserted in the infundibulum and the region of the ampulla–isthmic junction of one fallopian tube, and in the infundibulum of the other fallopian tube in four mature Border Leicester × Merino ewes. This procedure enabled secretions collected from the isthmus and ampulla of one fallopian tube to be compared with the secretion obtained from the other entire fallopian tube. The general surgical procedures and details of the collecting devices have been described previously (Restall 1966*a*).

The daily secretion rate of tubal fluid was recorded for two complete oestrous cycles and, in some cases, for three oestrous periods. The oestrous cycle was divided into three stages: stage 1 corresponding to oestrus, stage 2 to metoestrus, and stage 3 to dioestrus (see Restall 1966*b*), and the mean daily secretion rate was calculated for each stage. During the course of the experiment some cannulae suffered temporary blockage, particularly the smaller ones draining the isthmus. In assessing the results only those stages in which no interruption of flow occurred have been considered.

(*b*) *Chemical Analyses*

For each ewe, tubal fluid from each stage of the cycle was pooled and deep frozen at −30°C until chemical analyses could be performed.

Sodium, potassium, calcium, and magnesium were estimated with an atomic absorption spectrophotometer (Willis 1963), chloride was determined colorimetrically (Schoenfeld and Lewellan 1964), and bicarbonate by adding a known excess of hydrochloric acid and back-titrating with sodium hydroxide (Van Slyke 1922). Glucose and lactate were determined enzymatically by the methods of Huggett and Nixon (1957) and Barker and Britton (1957).

(*c*) *Metabolic Studies*

In order to assess the influence of the tubal secretions on the metabolism of ram spermatozoa, fluids from the various tubal sites of three ewes were pooled according to stage of the oestrous cycle. Incubation was carried out at 37°C in 5-ml Warburg flasks containing 0·3 ml washed spermatozoal suspension ($1–2 \times 10^8$ cells per flask), 0·3 ml tubal fluid, 0·2 ml substrate diluent [40 mM phosphate buffer (pH 7·0), 75 ml sodium chloride, 50 mM glucose], and 0·1 mM of either [U-^{14}C]glucose or [1-^{14}C]lactate in 0·9% sodium chloride. Control flasks contained 0·3 ml 0·9% sodium chloride instead of tubal fluid. Owing to the small amounts of tubal fluid available, only two ejaculates were used. Total oxygen uptake, μg-atoms of carbon oxidized from glucose and lactate, total glucose utilized, and total lactate accumulated were calculated for the 3-hr incubation period. Details of the estimation of these parameters have been given previously (Restall and Wales 1966*b*).

(*d*) *Statistical Analyses*

All results were subjected to analyses of variance using the method of unweighted means for disproportionate subclass numbers (Snedecor 1957) where necessary. In the analysis of the metabolic data, ejaculate interactions were used as error.

III. Results

(*a*) *Secretion Studies*

The mean daily secretion rate from each tubal site within stages of the oestrous cycle is shown in Table 1 together with a summary of the analysis of variance. Maximum fluid output from all tubal sites occurred at oestrus and was least during

the luteal phase of the cycle. In contrast with the entire tube and the ampulla, there was no difference in the amount of fluid secreted from the isthmus during metoestrus and dioestrus.

Secretion from the ampulla was generally twice that from the isthmus during all stages of the cycle. The sum of the amounts of fluid collected from the ampulla and isthmus agreed with the total secretion from the intact fallopian tube except during metoestrus, when the total amount of fluid from the divided tube exceeded that from the other entire tube.

TABLE 1

MEAN DAILY SECRETION OF FLUIDS FROM THE ENTIRE FALLOPIAN TUBE AND FROM THE AMPULLA OR ISTHMUS OF THE TUBE

Data are derived from four ewes over two complete oestrous cycles. Only those periods of the cycle in which there was no interruption of flow from the tubal cannulae have been considered in assessing the results. Number of observations given in parenthesis

Stage of Cycle	Volume of Secretion (ml)		
	Entire Tube	Ampulla	Isthmus
Oestrus	1·31 (9)	0·83 (8)	0·41 (6)
Metoestrus	0·66 (9)	0·61 (7)	0·25 (4)
Dioestrus	0·42 (6)	0·35 (6)	0·25 (3)

Summary of the Analysis of Variance

Source of Variation	D.F.	Mean Square	Variance Ratio	P
Stage of cycle (A)	2	0·2145	33·00	$<0·001$
Origin of secretion (B)	2	0·1981	30·48	$<0·001$
$A \times B$	4	0·0337	5·18	$<0·010$
Error	49	0·0065		

(*b*) *Chemical Analyses*

The data from chemical analyses are presented in Table 2 together with a summary of the analyses of variance. In all tubal fluids sodium was found to be the major cation and chloride the major anion. Fluids from the ampulla and isthmus were similar in composition except that bicarbonate concentration was higher in the isthmus (30·5 *v.* 27·3 m-equiv/l). Bicarbonate levels also were significantly higher during dioestrus.

The concentration of lactate, estimated on fluid pooled from all ewes, was elevated during oestrus and minimal during dioestrus in all fluid samples. The other chemical constituents examined showed no significant differences from one stage of the cycle to another. The amount of sodium, chloride, and bicarbonate showed a

small significant increase during the second cycle of observation. No glucose was detected in any of the fluids examined.

TABLE 2

ELECTROLYTE CONCENTRATION OF TUBAL FLUID FROM AN ENTIRE FALLOPIAN TUBE, AND FROM THE AMPULLA OR ISTHMUS OF THE OTHER TUBE

Data are derived from fluid secreted by four ewes over two complete oestrous cycles. Electrolyte concentrations are in milliequivalents per litre and lactate concentration is in μmoles per millilitre

Origin of Fluid	Stage of Cycle	$[Na^+]$	$[K^+]$	$[Ca^{2+}]$	$[Mg^{2+}]$	$[Cl^-]$	$[HCO_3^-]$	[Lactate]
Entire tube	1	137	7·52	3·59	0·46	126	27·0	3·1
	2	142	8·10	3·90	0·53	121	28·3	2·5
	3	144	7·86	3·35	0·65	127	29·4	1·7
Mean		141	7·83	3·61	0·54	125	28·2	2·4
Ampulla	1	135	8·12	3·80	0·59	122	23·7	5·6
	2	134	7·76	3·34	0·51	128	26·5	3·3
	3	148	7·85	2·76	0·50	131	31·7	0·6
Mean		139	7·91	3·29	0·54	127	27·3	3·2
Isthmus	1	141	6·90	2·98	0·54	120	27·3	3·4
	2	143	8·23	3·08	0·60	125	29·3	2·5
	3	148	8·70	2·94	0·54	123	34·8	0·3
Mean		144	7·94	2·98	0·56	123	30·5	2·1

Summary of Analyses of Variance

Source of Variation	D.F.	Variance Ratios Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-
Origin of fluid (A)							
Intact tube *v.* others	1	0·00	0·08	5·56*	0·02	0·01	0·41
Ampulla *v.* isthmus	1	1·10	0·01	1·81	0·26	3·65	7·39*
Stage of cycle (B)							
Linear	1	3·32	2·37	3·32	0·61	3·25	26·50**
Quadratic	1	0·37	0·34	1·14	0·00	0·00	0·93
Oestrous cycle (C)	1	4·40*	1·20	0·92	0·02	31·96**	5·03**
Interactions:							
$A \times B$	4	0·29	1·76	1·09	1·90	1·37	1·34
$A \times C$	2	0·05	0·52	0·26	0·59	4·13*	1·29
$B \times C$	2	0·86	0·88	1·79	0·02	0·54	0·27
$A \times B \times C$	4	2·56*	0·29	0·29	1·46	0·75	0·80
Error mean square between estimations†		26·47 (60)	0·4890 (61)	0·1608 (61)	0·0061 (58)	14·17 (54)	4·03 (57)

* $P < 0·05$. ** $P < 0·01$. † No. of degrees of freedom given in parenthesis.

(c) *Metabolic Studies*

The metabolism of spermatozoa incubated in the various tubal fluids is shown in Table 3 and the analyses of variance in Table 4. Oxygen uptake of spermatozoa was significantly lower in all fluid samples compared with controls. However, spermatozoa in fluid from the isthmus had a higher oxygen uptake than those in

TABLE 3

METABOLISM OF SPERMATOZOA INCUBATED IN TUBAL FLUID FROM THE AMPULLA, ISTHMUS, OR ENTIRE TUBE

Each value is the mean of two ejaculates incubated in fluids pooled from four ewes at three stages of the oestrous cycle. All measurements except carbon oxidized are expressed as μmoles per 10^8 spermatozoa over the 3-hr incubation period. Carbon oxidized expressed as μg-atoms

Origin of Fluid	Stage of Oestrus	Total Oxygen Uptake	Carbon Oxidized	Other Oxygen Uptake	Total Glucose Utilized	Total Lactate Accumulated
Saline	Control	1·24	0·963	0·28	0·56	0·78
Entire tube	1	0·52	0·335	0·19	1·79	3·80
	2	0·72	0·488	0·24	2·31	4·36
	3	1·19	0·851	0·34	2·58	4·61
Mean		0·81	0·558	0·25	2·23	4·25
Ampulla	1	0·50	0·497	0·05	1·26	3·84
	2	0·75	0·572	0·19	2·03	3·95
	3	0·73	0·530	0·21	2·50	4·66
Mean		0·66	0·533	0·15	2·10	4·15
Isthmus	1	0·64	0·491	0·16	1·97	3·97
	2	0·81	0·599	0·20	2·26	4·57
	3	1·37	0·947	0·42	2·35	4·04
Mean		0·94	0·679	0·26	2·19	4·19
Means, all fluids	1	0·55	0·441	0·13	1·84	3·87
	2	0·76	0·553	0·21	2·20	4·29
	3	1·10	0·776	0·32	2·48	4·43

fluid from the ampulla. These oxidative changes were also reflected in the μg-atoms of carbon oxidized, representing the contribution of glucose and lactate oxidation to the total oxygen uptake.

On the other hand, glycolysis (total glucose utilized and total lactate accumulated) was significantly enhanced in spermatozoa incubated in tubal fluids compared with the controls. No differences were observed between fluid samples, but all metabolic parameters were highest in fluids from the dioestrous phase of the cycle.

IV. Discussion

The results of the secretion studies demonstrate a distinct cyclic pattern of secretion of fluid from both the ampulla and the isthmus. The levels of fluid secreted from the entire tube are slightly higher than those previously reported (Restall 1966*b*) but this may be due to a breed difference, as the earlier studies were carried out on pure Merino ewes.

Table 4

SUMMARY OF THE ANALYSES OF VARIANCE FOR VALUES GIVEN IN TABLE 3

Source of Variation	D.F.	Variance Ratios				
		Total Oxygen Uptake	Carbon Oxidized	Other Oxygen Uptake	Total Glucose Utilized	Total Lactate Accumulated
Treatment effects	9					
Saline *v.* tubal fluids	(1)	22·36**	16·29**	2·24	101·28**	122·59**
Between tubal fluids						
Entire tube *v.* others	(1)	0·02	0·60	5·60*	0·62	0·16
Ampulla *v.* isthmus	(1)	15·05**	4·15	14·08**	0·61	0·03
Stage of oestrus						
Linear (*L*)	(1)	58·23**	21·86**	44·84**	26·61**	5·59*
Quadratic (*Q*)	(1)	0·98	0·80	0·70	0·16	0·45
Origin of fluid × stage						
Entire tube *v.* others (*L*)	(1)	2·09	3·20	0·88	0·74	0·54
(*Q*)	(1)	0·55	0·31	0·04	0·27	0·01
Ampulla *v.* isthmus (*L*)	(1)	7·98*	5·81*	2·20	1·43	1·65
(*Q*)	(1)	4·95	1·38	5·80*	0·65	2·94
Ejaculate differences	1	3·13	3·90	0·40	12·29**	31·94**
Treatment × ejaculate	9	0·0307†	0·0154†	0·0025†	0·0920†	0·3427†
Duplicates	20	0·0030†			0·0167†	0·0299†

* $P < 0·05$. ** $P < 0·01$. † Error mean squares.

The present study also shows that secretion from the ampulla is twice that from the isthmus. This is not surprising as previous reports have shown that the ampulla has many more secretory cells than the isthmus (Hadek 1955; Restall 1966*c*). This large volume of secretion from the ampulla may be necessary to provide a fluid vehicle to assist the entry of the egg into the fallopian tube.

The levels of electrolytes found in the tubal secretions are also in general agreement with those reported previously (Restall and Wales 1966*a*). However, in contrast to the previous report, the concentration of bicarbonate, as well as being generally higher, was found to be highest during the dioestrous phase of the cycle. The only significant difference between the fluids from the ampulla and isthmus was in the level of bicarbonate. However, chemical differences between regions of the tube are unlikely to be of physiological importance if the conclusion of Black and Asdell (1958), that tubal fluid is distributed uniformly along the entire tube, is true.

The depression of oxygen uptake and the elevation of glycolysis in spermatozoa incubated in tubal fluids confirms earlier reports (Restall and Wales 1966*b*) but is again at variance with reports for other species (Olds and VanDemark 1957; Hamner and Williams 1963). Species differences probably exist but direct comparisons must be made with caution because of the differences in technique in the various studies.

Fluids from the ampulla and isthmus were similar in their effect on the metabolism of spermatozoa except that oxygen uptake was elevated in fluid from the isthmus. However, in no incubation did the oxygen uptake exceed that of the controls. Hamner and Williams (1963) ascribe the increase in respiration of rabbit spermatozoa in tubal fluid to the bicarbonate present, and in the present study the isthmic fluid had a higher bicarbonate concentration. Respiration was also enhanced in fluid from the dioestrous phase of the cycle, in which bicarbonate levels were elevated. In contrast is the report of Wales and Restall (1966) that the accumulation of metabolic carbon dioxide had no effect on the metabolism of spermatozoa in tubal fluid, and it would appear that the influence of bicarbonate needs further clarification.

The physiological significance of tubal fluid as far as the spermatozoa are concerned is not at all clear. In the ewe, spermatozoa continually migrate through the reproductive tract in relatively small numbers over a long period of time (Mattner 1963) and thus are likely to have already developed the capacity to fertilize ("capacitation") by the time they arrive in the fallopian tubes. However, the increase observed in metabolism of spermatozoa incubated in tubal fluid may mitigate against their survival. The rapid elimination of aging spermatozoa would appear to be an advantage as ovulation approaches (Boyd 1965; Salisbury 1965) and this aspect warrants investigation.

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