COMPOSITION OF OVIDUCT FLUID KEPT AT TWO TEMPERATURES DURING COLLECTION FROM SHEEP

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It has never been established that fluid collected from the oviducts of rabbits (Clewe & Mastroianni, 1960), ewes (Black, Duby & Riesen, 1963; Restall, 1966; Perkins, Goode, Wilder & Hensen, 1965; Iritani, Gomes & VanDemark, 1969), cows (Carlson, Black & Howe, unpublished data) and monkeys (Mastroianni, Shah & Abdul-Karim, 1961) is comparable with the fluid normally present in the oviduct. The results of Holmdahl & Mastroianni (1965) suggest that it may not be comparable since they found that fluid held at low temperatures during collection from rabbits contained more glucose and less calcium than fluid collected at room temperature. A similar experiment was therefore carried out in sheep.

Fourteen ewes of the Dorset and Shropshire breeds were used in the experiment. They were placed in individual stalls, given 1 lb of grain, hay or silage, and unrestricted water. Oestrus was detected with the aid of a ram.

Animals to be cannulated for the collection of oviduct fluid were anaesthetized with Equithesin (Jen-Sal Labs) and the reproductive tract was exposed through a ventral incision anterior to the mammary glands. A silicone rubber (Silastic-Dow Corning) cannula (0.217 cm outer diameter, 0.102 cm inner diameter, 61 cm long) was inserted through the infundibulum of one oviduct and into the ampulla for about 1 cm. The cannula was retained within the oviduct by two ligatures of synthetic suture material, care being taken not to occlude the lumen. The position and patency of the cannula were determined by forcing a small amount of air into the oviduct. After ligating the uterine end of the cannulated oviduct to prevent passage of oviduct fluid into the uterus, the distal end of the cannula was brought out through the right flank and attached to a plastic test-tube cap which had an opening for air. The cap fitted tightly on the sterile plastic test-tubes in which the oviduct fluid was collected. The wound was closed in the usual manner and the animal allowed to regain full consciousness before being returned to its stall. Each ewe was fitted with a canvas blanket in which there was a pocket in the right side to hold the test-tube and container.

The temperature within the collection vials was controlled by circulating

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fluid through a double-walled test-tube container made from copper tubing. The container was insulated in polystyrene and the entire apparatus inserted into the pocket in the canvas blanket covering the animal. The temperature was measured with thermocouples and controlled by circulating either alcohol cooled to -20°C , or water at 37°C , through the collection device.

Fluid from the oviducts of one group of seven ewes was held at -10°C or lower during collection ('cold' collection) and that from a second group of

TABLE 1
OVIDUCT SECRETION IN THE EWE

Days of sample	'Cold' collection, (ml/24 hr)		'Warm' collection (ml/24 hr)	
	No. of samples	Mean $\pm$ S.D.	No. of samples	Mean $\pm$ S.D.
6 and 7 Pro-oestrus	8	0.39 ± 0.32	7	0.34 ± 0.30
4 and 5 Pro-oestrus	10	0.25 ± 0.18	6	0.29 ± 0.15
2 and 3 Pro-oestrus	11	0.47 ± 0.36	12	0.61 ± 0.52
Oestrus and 1 Pro-oestrus	13	0.75 ± 0.40	12	0.88 ± 0.51
1 and 2 Post-oestrus	13	0.47 ± 0.25	16	0.72 ± 0.46
3 and 4 Post-oestrus	12	0.27 ± 0.23	14	0.40 ± 0.33
5 and 6 Post-oestrus	11	0.25 ± 0.20	11	0.23 ± 0.27

TABLE 2
CHEMICAL COMPOSITION OF SHEEP OVIDUCT FLUID AND BLOOD SERUM*

Constituent	Oviduct fluid		Blood serum	
	'Warm'	'Cold'	'Warm'	'Cold'
Dry matter (g/100 ml)	2.66 ± 0.90	2.72 ± 0.71	8.86 ± 0.40	9.08 ± 0.45
Calcium (mg/100 ml)	3.97 ± 0.33	4.02 ± 0.45	12.48 ± 0.35	11.86 ± 0.99
Magnesium (mg/100 ml)	1.20 ± 0.07	1.22 ± 0.10	2.11 ± 0.14	2.04 ± 0.14
Sodium (mg/100 ml)	264.43 ± 18.00	235.00 ± 8.29	221.71 ± 2.90	207.86 ± 11.32
Potassium (mg/100 ml)	31.28 ± 2.25	28.09 ± 1.66	30.93 ± 1.58	27.14 ± 3.88
Chloride (mg/100 ml)	570.86 ± 72.12	422.83 ± 65.23	393.49 ± 34.04	392.75 ± 30.94
Protein (g/100 ml)	1.94 ± 0.65	1.66 ± 0.56	6.92 ± 0.41	6.81 ± 0.84
Glucose (mg/100 ml)	1.37 ± 0.24	1.07 ± 0.25	Not determined	Not determined

* Average of all samples collected during periods listed in Table 1.

seven ewes was held at 25° to 40°C ('warm' collection) under aseptic conditions. The samples were placed on ice until they reached the laboratory (30 min) and kept frozen until they were analysed.

Dry matter was determined by placing 200 μl of oviduct fluid on pre-weighed aluminum planchets with a microlitre pipette. The pipette was washed twice with doubly distilled water and the washings were added to the planchet. The samples were dried at 65°C for 48 hr, transferred to a desiccator, and allowed to cool before weighing.

Sodium, potassium, calcium and magnesium were analysed by atomic absorption spectrophotometry. Chloride was determined by the mercuric chloranilate method of Barney & Bertolacini (1957) and Hodge & Gerarde

(1963). Protein was determined by the biuret method of Kingsley (1939) and glucose was determined enzymatically (Bergmeyer, 1963).

The effect of oviduct fluid on the oxygen uptake by spermatozoa was measured as previously described (Black, Crowley, Duby & Spilman, 1968).

There were no significant differences between the two groups of ewes in the rates of secretion of fluid by the oviduct. Both groups showed the highest rate of secretion at oestrus and the lowest at 5 to 6 days after oestrus (Table 1), as had been previously observed by Black *et al.* (1968). The composition of the fluid was not affected by keeping it cold during collection (Table 2) and the values were similar to those already reported for dry matter (Iritani *et al.*, 1969) and magnesium, potassium and chloride (Restall & Wales, 1966) in the ewe. Our values were lower than those previously reported for protein (Restall & Wales, 1966) and glucose (Iritani *et al.*, 1969).

TABLE 3
EFFECT OF OVIDUCT FLUID ON RESPIRATION OF RAM SPERMATOZOA

Days sample pooled	Percentage increase (+) or decrease (−) of oxygen consumption of spermatozoa in oviduct fluid			
	'Warm' collection		'Cold' collection	
	1st hr (%)	2nd hr (%)	1st hr (%)	2nd hr (%)
6 and 7 Pro-oestrus	+106.43	+7.41	+89.03	+18.41
4 and 5 Pro-oestrus	+150.60	+23.21	+115.66	+33.85
2 and 3 Pro-oestrus	+75.23	+11.21	+91.17	+26.20
Oestrus and 1 pro-oestrus	+24.78	+9.08	−0.77	−26.92
1 and 2 Post-oestrus	+42.26	+37.26	+41.09	+44.71
3 and 4 Post-oestrus	+52.97	+47.97	+40.47	+35.47
5 and 6 Post-oestrus	+92.60	+18.48	+23.19	+28.19

Values in the ewe for blood serum composition (Table 2) were similar to those reported for calcium, magnesium, chloride and protein but higher for potassium and glucose (Restall & Wales, 1966).

Although oviduct fluid generally increased sperm respiration (Table 3), the 'cold' sample collected on the day of pro-oestrus plus the day of oestrus caused practically no increase in the respiration of spermatozoa during the 1st hr and then decreased oxygen consumption by 27% during the 2nd hr of incubation. With the corresponding 'warm' sample, there was little increase in oxygen consumption during the 1st hr of incubation but it increased slightly during the 2nd hr of incubation. These data are different from those reported earlier (Black *et al.*, 1968).

In general, data on the chemical composition of oviduct fluid and blood serum obtained in this study agree well with those obtained by other workers. The discrepancies which were observed may have resulted from different nutritional and environmental conditions. The important fact is that there were no changes evident in the concentrations of substances measured in oviduct fluid collected at the two different temperatures. The possibility remains that oviduct fluid

may change chemically before it enters the collection device. The cannula from the oviduct to the collection device in the sheep, unlike that in the rabbit, must be long to accommodate the size of the animal. Since the volume of fluid produced is essentially the same in the two animals, the time required for passage through the cannula is increased and, during this interval, chemical change may occur.

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