THE METABOLISM OF RAM SPERMATOZOA IN THE PRESENCE OF GENITAL FLUIDS OF THE EWE

By R. G. Wales* and B. J. Restall†

[*Manuscript received August* 9, 1965]

Summary

The metabolism of washed ram spermatozoa has been studied in the presence of fluids collected from the genital tract of the ewe. The presence of cervicovaginal, uterine, tubal, and follicular fluids increased oxygen uptake by spermatozoa when compared with their oxygen uptake in saline alone. Stimulation of oxygen uptake above controls did not occur when glucose was added to the incubation medium, indicating that the increase observed was due to the presence of substrate in the fluids. In the presence of added glucose, however, vaginal, tubal, and follicular fluid stimulated aerobic and anaerobic glycolysis. The effect was abolished by dialysis.

The metabolism of spermatozoa incubated in the presence of the genital fluids was not significantly affected by the presence or absence of carbon dioxide. Neither oxygen uptake nor substrate oxidation were affected. There was a slight reduction in the utilization of glucose in the presence of carbon dioxide but this occurred whether or not the fluids were added to the incubation medium.

I. Introduction

At the present time, the role of the fluids of the female genital tract in the metabolism of spermatozoa is largely unknown. Most experiments to elucidate their importance have been carried out in the rabbit and cow (Bishop 1956, 1957; Olds and Van Demark 1957; Clewe and Mastroianni 1959; Mastroianni and Wallach 1961; Mastroianni *et al.* 1961). This paper reports some preliminary observations on the effect of the genital fluids of the ewe on the metabolism of ram spermatozoa.

II. Materials and Methods

(*a*) *Genital Fluids*

Cervicovaginal fluid was obtained by aspiration from the vaginae of ewes in oestrus. Uterine fluid was aspirated from reproductive tracts obtained from slaughtered sheep. Fluid secreted by the fallopian tubes was collected from ewes with cannulated oviducts (Restall 1966*a*). Follicular fluid was obtained by aspiration of follicles *post-mortem*. Where required, all fluid samples were dialysed for 24 hr in Visking dialysis tubing against 100 vol. of the diluent used in the particular experiment. The diluent was then replaced by 100 vol. of fresh diluent and dialysis continued for a further 24 hr.

* Department of Veterinary Physiology, University of Sydney.

† School of Wool Technology, University of New South Wales, Kensington, N.S.W.; present address: Department of Veterinary Physiology, University of Sydney.

(*b*) *Semen*

Ram semen was collected by electrical stimulation and only ejaculates containing spermatozoa with good initial motility were used. One volume of semen was diluted to 10 vol. with a diluent composed of 20 mM mono- and disodium phosphate buffer (pH 7·0), 119 mM sodium chloride, 5 mM potassium chloride, and 2 mM magnesium chloride, and centrifuged at 200 *g* for 7 min. The supernatant was removed and spermatozoa resuspended and recentrifuged at 200 *g* for a further 7 min. After removing the second supernatant, the spermatozoa were made up to an appropriate volume with diluent. In experiments 3, 4, and 5, potassium and magnesium chlorides were omitted from the diluent, and tonicity was maintained by increasing the sodium chloride content.

In all experiments direct spermatozoal counts were made in a haemocytometer.

(*c*) *Methods of Incubation*

Five experiments were undertaken. In each, spermatozoa were incubated at 37°C in standard Warburg flasks in the presence of one of the four types of genital fluid collected. The metabolism of the spermatozoa was compared with that obtained when the genital fluid was replaced by an equal volume of 0·9% (w/v) sodium chloride. Preliminary tests with genital fluid alone indicated that it was unnecessary to make any correction for the oxygen consumption of the fluids themselves. Incubations were also carried out in the presence of uniformly labelled [^{14}C]glucose and 1-[^{14}C]sodium lactate under both aerobic and anaerobic conditions and in the presence or absence of carbon dioxide. Precise experimental details are given in Section III.

(*d*) *Analytical Methods*

After incubation, protein-free extracts were prepared by precipitation with equal volumes of 0·3N barium hydroxide and 5% (w/v) zinc sulphate. Glucose and lactate content of the extracts were estimated by enzymic methods (Huggett and Nixon 1957; Barker and Britton 1957). The radioactivity of the trapped carbon dioxide was measured by precipitation as $Ba^{14}CO_3$ and counting under an end-window Geiger-Müller tube (see Wallace and Wales 1964).

(*e*) *Statistical Analysis*

All results have been subjected to standard analyses of variance, which are presented in summary form in the tables, giving only the number of degrees of freedom and variance ratios for each source of variation. Where the comparisons to be made between fluids could not be decided in advance of performing the experiment, the multiple-range test (Duncan 1955) was used to compare treatment means. In experiments 4 and 5, predetermined treatment contrasts were partitioned by making use of polynomial coefficients.

III. Experimental and Results

In all experiments, two separate collections of each fluid were made at different times. With the exception of experiment 2, sample 1 of all fluids was tested with a

single ejaculate and sample 2 with another ejaculate. In experiment 2, each sample of fluid was tested with two ejaculates. Thus, differences between ejaculates are confounded to some extent with differences between fluid samples. However, as only limited volumes of fluid were available, this confounding helped to give a more general indication of the effects of the fluids.

(*a*) *Experiment 1: Effect of the Genital Fluids on Oxygen Uptake*

The oxygen uptake of ram spermatozoa which had been incubated for 90 min in the presence of cervicovaginal, uterine, tubal, and follicular fluids was measured (see Fig. 1). Washed spermatozoal suspension (0·5 ml, 4–5$\times 10^8$ cells per flask) was added to a Warburg flask of approximately 15 ml capacity containing 0·4 ml

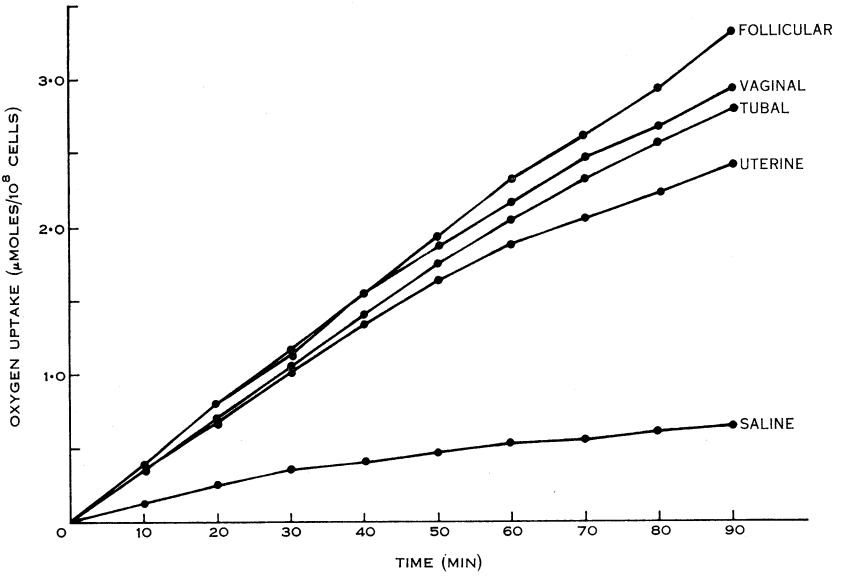

Fig. 1.—Oxygen uptake of washed ram spermatozoa incubated in fluids obtained from the reproductive tract of the ewe. The multiple-range test gave the following ranking: follicular = vaginal = tubal = uterine fluid > control ($P<0·05$).

of genital fluid and 0·4 ml saline. For controls, spermatozoal suspensions were incubated with 0·8 ml saline only. Statistical analysis of the total oxygen uptake gave an error variance of 0·22 (4 degrees of freedom) and this was used for gauging significant differences. The spermatozoa incubated in the presence of fluids had a higher oxygen uptake than in the controls. There was no statistical evidence of any difference between the effects of the different fluids.

(*b*) *Experiment 2: Effect of Follicular Fluid on Oxygen Uptake*

Follicular fluid was obtained from ovaries classified as being oestrous, metoestrous, or dioestrous (Restall 1964). The oxygen uptake of washed spermatozoal suspensions incubated together with these fluids was measured as described in experiment 1. Results are given in Figure 2. The comparison between mean oxygen

uptakes (based on an error variance for total oxygen uptake of 0·08, 6 degrees of freedom) showed that follicular fluid from ewes in oestrus and metoestrus stimulated oxygen uptake to a similar extent. Follicular fluid from ewes in dioestrus was not as effective, only doubling oxygen uptake in comparison with the controls, whereas in the former case oxygen uptake was trebled.

(*a*) *Experiment 3: Effect of Aerobic and Anaerobic Conditions on Spermatozoal Metabolism in the Presence of Genital Fluids*

Aliquots of washed spermatozoal suspensions were incubated for 3 hr under aerobic and anaerobic conditions in the presence of samples of dialysed and non-dialysed cervicovaginal, uterine, tubal, and follicular fluid. Two samples of tubal fluid were used. One was collected from an entire ewe during oestrus, while the other was collected over 3 days from a spayed ewe which had been given 30 μg of oestradiol on the day preceding the commencement of collection.

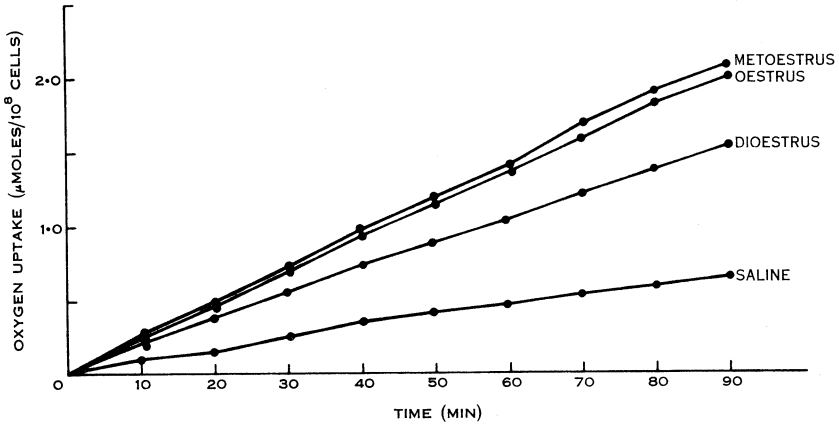

Fig. 2.—Oxygen uptake of washed ram spermatozoa incubated in follicular fluid obtained from ewes in various stages of the oestrous cycle. The multiple-range test gave the following ranking: metoestrus = oestrus > dioestrus fluid > control ($P<0·05$).

Aerobic incubations were made with air as gas phase. For anaerobic conditions, flasks were flushed with dry nitrogen for 10 min at the start of incubation. Warburg flasks of 5 ml capacity containing 0·3 ml of washed spermatozoal suspension (1–2 $\times$ 10^8 cells per flask), 0·3 ml of genital fluid (0·3 ml of 0·9% w/v saline in the controls), and 0·2 ml of diluent (40 mM mono- and disodium phosphate buffer, pH 7·0; 75 mM sodium chloride; 50mM glucose) were incubated at 37°C for 3 hr. Duplicate Warburg flasks were prepared, 0·1 ml of uniformly labelled [^{14}C]glucose being added to one and 0·1 ml of 1-[^{14}C]sodium lactate as carrier-free isotope in 0·9% sodium chloride to the other. By measurement of the initial specific activities of the glucose and lactate, the oxidation of these substrates was calculated by assay of the trapped carbon dioxide from the two flasks. Wales and O'Shea (personal communication) have found that the use of 1-[^{14}C]lactate overestimates lactate oxidation to

TABLE 1

AEROBIC AND ANAEROBIC METABOLISM OF WASHED RAM SPERMATOZOA IN THE PRESENCE OF DIALYSED AND NON-DIALYSED GENITAL FLUIDS OF THE EWE

All values are expressed as μmoles/10^8 spermatozoa over the experimental period (3 hr)

Fluid	Dialysis	Respiration			Aerobic Glycolysis		Anaerobic Glycolysis	
		Oxygen Uptake	Glucose Oxidized	Lactate Oxidized	Glucose Utilized	Lactate Accumulated*	Glucose Utilized	Lactate Accumulated*
Nil (control)	−	2·15	0·285	—	1·22	1·89	2·60	4·34
	−	2·26	0·386	—	1·69	2·13	2·40	4·24
Cervicovaginal	−	2·23	0·281	0·063	2·08	2·69	3·04	5·17
	+	1·97	0·288	—	1·53	1·95	2·58	4·50
Uterine	−	0·33	0·016	0·095	1·31	2·63	1·84	2·55
	+	1·26	0·164	—	1·59	2·46	1·72	2·98
Tubal (entire)	−	1·39	0·139	0·099	2·45	4·00	2·52	4·82
	+	2·35	0·284	—	1·36	1·36	1·56	3·47
Tubal (spayed)	−	1·94	0·148	0·085	2·17	3·50	3·11	5·63
	+	2·02	0·248	—	1·62	2·26	2·09	4·09
Follicular	−	2·36	0·154	0·252	1·88	3·28	3·12	5·61
	+	1·88	0·279	—	1·61	2·21	1·62	5·01

*Refers to the difference between final and initial lactate levels, and total lactate formed under aerobic conditions will equal the "lactate accumulated" plus that oxidized.

some extent, but under the present conditions of incubation it would not be expected to introduce any appreciable error.

The detailed results of these experiments are given in Table 1, and a summary of the analysis of variance of the data is given in Table 2. Preliminary analyses showed that the lactate and glucose concentration varied considerably between fluids. Thus tubal fluids contained approximately 3·0 μmoles/ml lactate, cervico-vaginal fluid 0·3 μmole/ml, follicular fluid 12·0 μmoles/ml, and uterine fluid 18·0 μmoles/ml. Glucose was present in significant amounts (1 μmole/ml) in follicular and uterine fluids only. Thus, for the non-dialysed fluids, differences in the amount of glucose or lactate oxidized probably only reflect variations in the relative concentrations of the two substrates in the flask. However, the oxygen uptake due

TABLE 2

SUMMARY OF THE ANALYSES OF VARIANCE FOR THE DATA OF TABLE 1

Source of Variation	Degrees of Freedom	Variance Ratios		Degrees of Freedom	Variance Ratios	
		Total Oxygen Uptake	Glucose plus Lactate Oxidized		Glucose Utilized	Lactate Accumulated
Between treatments (*A*)	(11)			(11)		
Between controls	1	0·02	0·69	1	0·21	0·30
Controls *v.* fluids	1	0·92	2·54	1	0·48	0·39
Between fluids	4	1·86	1·43	4	4·21*	4·20*
Effect of dialysis	1	0·44	0·72	1	16·09**	16·92**
Dialysis × fluid interaction	4	0·64	0·26	4	2·05	2·38
Effect of gas phase (*B*)	—	—	—	1	37·76**	75·60**
A × *B* interaction	—	—	—	11	1·06	1·55
Between replicates	1	7·02*	33·59**	1	168·25**	221·47**
Replicate interactions (error)	11	0·67	0·54	23	0·17	0·54

* $P < 0·05$. ** $P < 0·01$.

to oxidation of the combined glucose plus lactate pool has a greater physiological significance and such oxygen uptake per 10^8 cells has been used as the unit of observation in the statistical analysis of substrate oxidation.

In general there was little effect of the fluids on both oxygen uptake and substrate oxidation when compared with the controls. On the other hand, incubation of spermatozoa with the genital fluids under both aerobic and anaerobic conditions had marked effects on the utilization of glucose and the accumulation of lactate. Comparison of the means showed that, except for uterine fluid, glucose breakdown by spermatozoa was stimulated by the presence of non-dialysed genital fluids. However, glucose breakdown in the presence of dialysed fluids was no greater than in the controls. Under anaerobic conditions glucose was utilized at a rate greater

than under aerobic conditions, but a change in the gas phase did not influence the response of spermatozoa to the addition of fluids. Unlike the other fluids examined, uterine fluid did not stimulate glycolysis above the controls and dialysis did not modify its effect.

(*d*) *Experiments 4 and 5: Metabolism in the Presence or Absence of Carbon Dioxide*

The effects of the presence or absence of respired carbon dioxide on the aerobic metabolism of spermatozoa incubated in cervicovaginal or follicular fluid (expt. 4) and of its presence on the aerobic metabolism of spermatozoa incubated with tubal fluid (expt. 5) were examined. In experiment 4, two samples of unclassified follicular fluid were used, and volumes of fluid, numbers of sperm, diluents used, and methods are as given for experiment 3. In experiment 5, two samples of tubal fluid were used, one from an entire ewe collected at the time of oestrus and one from a spayed ewe given progesterone and oestrogen (as described by Restall 1966*b*) and collected during the time of oestrus. In both experiments buffered saline controls containing glucose were used.

For the determination of the aerobic metabolism of spermatozoa incubated in the presence of respired carbon dioxide, the method described by Wales and O'Shea (1966) was modified to enable incubations to be carried out in small, single side-arm flasks of 6 ml capacity. At the completion of incubation, 0·1 ml of 2N HCl was injected through the serum cap into the side-arm and then tipped into the semen suspension to terminate metabolism. This was followed by the injection of 20% (w/v) potassium hydroxide into the side-arm to absorb liberated carbon dioxide. Carbon dioxide output and oxygen consumption were then calculated after correction for the volume of fluid injected and the initial levels of carbon dioxide in the flasks. Results are set out in Tables 3 (expt. 4) and 4 (expt. 5). Analyses of the flasks containing carbon dioxide showed that, in addition to the carbon dioxide formed during metabolism of the spermatozoa, there was bicarbonate present in the fluids. The cervicovaginal fluid contained 1·3 μmoles/ml bicarbonate, follicular fluid 1·3 μmoles/ml, and tubal fluid 10·5 μmoles/ml, which at 37°C equilibrated with the carbon dioxide in the gas phase of the flask during incubation.

Summaries of the analyses of variance for both experiments are given in Tables 3 and 4. Separate statistical analyses were made of data for glucose and lactate oxidized in order to establish if there was any difference in the effect of carbon dioxide on the oxidation of the two substrates. In these tests, cervicovaginal and follicular fluid caused a significant increase in respiration, while tubal fluids slightly depressed respiration in comparison with their controls. As in the previous test, however, there was a large and highly significant increase in glucose utilization and lactate accumulation when the fluids were included in the incubation medium. However, there was no effect of carbon dioxide on respiration, either in the presence or the absence of the fluids. In experiment 4, there was no effect of carbon dioxide on glucose utilization or lactate accumulation. In experiment 5, there was a slight depression in glucose utilization in the presence of carbon dioxide, but there was no corresponding change in lactate accumulation.

TABLE 3

METABOLISM OF SPERMATOZOA INCUBATED WITH CERVICOVAGINAL OR FOLLICULAR FLUIDS IN THE PRESENCE OR ABSENCE OF CARBON DIOXIDE

Values are expressed as μmoles/10^8 spermatozoa over the experimental period (3 hr) and are the means for two replications

Gas Phase	Fluid Added	Total Oxygen Uptake	Glucose Oxidized	Lactate Oxidized	Glucose Utilized	Lactate Accumulated
$-CO_2$	Nil (control)	1·57	0·258	0·000	0·79	0·86
	Cervicovaginal	2·64	0·213	0·483	2·22	2·60
	Follicular	2·38	0·089	0·671	1·31	2·49
	Follicular	2·62	0·113	0·603	1·62	2·81
$+CO_2$	Nil (control)	1·50	0·301	0·000	0·53	0·95
	Cervicovaginal	2·50	0·254	0·491	2·08	2·77
	Follicular	2·54	0·124	0·729	1·16	2·88
	Follicular	2·51	0·117	0·756	1·37	3·18

Summary of the Analyses of Variance

Source of Variation	Degrees of Freedom	Variance Ratios				
		Total Oxygen Uptake	Glucose Oxidized	Lactate Oxidized	Glucose Utilized	Lactate Accumulated
Effect of carbon dioxide (A)	1	0·61	1·94	0·52	4·75	2·29
Differences between fluids (B)						
Control *v.* cervicovaginal	1	214·3**	2·23	—	134·54**	55·09**
Control *v.* follicular	1	254·8**	39·96**	—	40·17**	86·83**
Between follicular	1	2·2	0·74	0·34	4·09	1·67
Cervicovaginal *v.* follicular	(1)	—	—	3·24	—	—
Replicate differences (C)	1	58·11**	6·56*	7·08*	51·30**	79·97**
Interactions						
$A \times B$	3†	1·86	0·18	0·17	0·13	0·18
$A \times C$	1	0·10	—	—	12·78*	0·20
$B \times C$	3	42·94**	—	—	7·12*	21·22**
$A \times B \times C$	3	0·21*	—	—	2·52	0·48
Replicate interactions	7‡		0·002	0·030		
Between duplicates	16	0·02	—	—	0·066	0·23

* $P < 0·05$. ** $P < 0·01$.

† 2 degrees of freedom for lactate oxidized.

‡ 5 degrees of freedom for lactate oxidized.

IV. Discussion

All the genital fluids tested were found to stimulate the respiration of ram spermatozoa when compared with saline. In general, follicular and cervicovaginal fluids sustain respiration at the highest rate, while tubal fluids are not quite as effective. The response to the addition of uterine fluid, however, was somewhat

Table 4

METABOLISM OF SPERMATOZOA INCUBATED WITH TUBAL FLUIDS IN THE PRESENCE OR ABSENCE OF CARBON DIOXIDE

Values are expressed as μmoles/10^8 spermatozoa over the experimental period (3 hr) and are the means for two replications

Gas Phase	Fluid Added	Total Oxygen Uptake	Glucose Oxidized	Lactate Oxidized	Glucose Utilized	Lactate Accumulated
$-CO_2$	Nil (control)	1·74	0·202	0·000	0·99	0·86
	Tubal (entire)	1·55	0·133	0·055	1·77	2·12
	Tubal (spayed)	1·57	0·135	0·062	1·47	1·57
$+CO_2$	Nil (control)	1·70	0·213	0·000	0·80	0·80
	Tubal (entire)	1·35	0·117	0·064	1·39	1·98
	Tubal (spayed)	1·60	0·125	0·065	1·25	1·71

Summary of the Analyses of Variance

Source of Variation	Degrees of Freedom	Variance Ratios				
		Total Oxygen Uptake	Glucose Oxidized	Lactate Oxidized	Glucose Utilized	Lactate Accumulated
Effect of carbon dioxide (A)	1	1·92	0·28	7·92	14·08**	0·02
Fluid differences (B)						
Control *v.* tubal	1	14·32**	143·50**	—	53·77**	134·34**
Entire *v.* spayed	1	4·52	4·17	3·36	6·47*	16·39**
Replicate differences (C)	1	50·49**	23·96**	71·40**	25·38**	18·58**
$A \times B$ interactions	2†	2·04	2·27	2·64	0·48	0·55
Replicate interactions	5‡	0·62	0·0002	0·000008	2·25	1·17
Between duplicates	12	0·015	—	—	0·03	0·04

* $P < 0·05$. ** $P < 0·01$.

† 1 degree of freedom for lactate oxidized.

‡ 3 degrees of freedom for lactate oxidized.

variable. The fluid obtained by aspiration of the uterus varies in appearance and quantity. Fluid collected by this method may not always reflect the true uterine environment.

Olds and Van Demark (1957) found that bovine follicular and tubal fluids increased oxygen consumption above the saline control, while cervicovaginal mucus

and uterine fluid reduced respiration. However, their methods of collecting uterine and tubal fluids were very different to those used in the present study and therefore it is difficult to make valid comparisons between species. In both species, however, follicular fluid maintains respiration at a high rate. In experiments where glucose was added to both saline controls and flasks containing the fluids, respiration in the control flasks was better maintained and there were only slight effects of the fluids on oxygen uptake. The most consistent effect of these incubations in the presence of genital fluids, however, was the increase in glycolysis. The factor responsible is unlikely to be a macromolecule as it was removed by dialysis for 48 hr.

Lactate was present in all the fluids collected. Bishop (1956) has demonstrated the presence of lactate in the tubal fluid of the rabbit and found that the oxygen tension in the tube was sufficient to support aerobic metabolism. Yates and Olds (1959) found that the stimulation of respiration by bovine follicular fluid was due, at least in part, to the presence of substrate. These authors also suggested that substances of large molecular weight play some part in the stimulation, but in the present tests dialysed fluids had no significant effect on respiration. Thus, in these tests, substrate present in the fluids probably accounts for their stimulating effect when the comparison was made with controls incubated in saline only.

Hamner and Williams (1963, 1964) found that rabbit oviduct fluid stimulated respiration and that this effect was due to the bicarbonate content of the fluid. In the sheep, varying concentrations of bicarbonate were found in the fluids, but its absorption by alkali did not modify the reaction of the spermatozoa to the fluids. Reports on the effects of carbon dioxide on sperm metabolism are somewhat conflicting. Although Salisbury and Lodge (1963) found that carbon dioxide stimulated the respiration of bull spermatozoa, Wales and O'Shea (1966) could demonstrate no effect of low levels of carbon dioxide on either bull or ram spermatozoa. In the present experiments, bicarbonate absorption would not have caused any pH change to confuse the issue, as sufficient phosphate buffer was present in the medium to maintain the pH at approximately 7·0.

There is little doubt that the fluid environment in the female tract is important in the physiology of spermatozoa deposited in the female genital tract. Probably ram spermatozoa are in contact with all the fluids examined for varying periods while in the reproductive tract of the ewe. Austin (1951) and Chang (1951) have shown that rabbit and rat spermatozoa are unable to fertilize ova until after a period of incubation in the female reproductive tract. Austin (1952) has termed this phenomenon "capacitation" and Mattner (1963) estimates the time for capacitation to occur in the sheep to be 1·5 hr. The exact changes in the spermatozoa attributable to capacitation are unknown, but the fluids of the female genital tract, as well as providing a substrate diluent for spermatozoa, may be important in the process. It remains to be seen if the consistent stimulation of glycolysis by the fluids, found in the present study, plays any part in the process of capacitation.

V. Acknowledgments

The authors are indebted to Professor C. W. Emmens for the provision of facilities and for helpful criticism of the manuscript. The work was aided by grants

from the Rural Credits Development Fund of the Commonwealth Bank of Australia. One of us (B.J.R.) was supported by a General Motors Post-Graduate Fellowship and grants from the Rural Bank, Bank of New South Wales, and University of New South Wales.

VI. References

Austin, C. R. (1951).—Observations on the penetration of the sperm into the mammalian egg duct. *Aust. J. Sci. Res.* B **4**: 581.

Austin, C. R. (1952).—The capacitation of the mammalian sperm. *Nature, Lond.* **170**: 326.

Barker, J. N., and Britton, H. G. (1957).—The enzymatic estimtion of L(+)-lactic acid. *J. Physiol.* **138**: 3P.

Bishop, D. W. (1956).—Active secretion in the rabbit oviduct. *Am. J. Physiol.* **187**: 347.

Bishop, D. W. (1957).—Metabolic conditions within the oviduct of the rabbit. *Int. J. Fert.* **2**: 11.

Chang, M. C. (1951).—Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature, Lond.* **168**: 697.

Clewe, T. H., and Mastroianni, L. (1959).—A method for the prolonged collection of secretions from the oviducts of unanaesthetized rabbits. *Anat. Rec.* **133**: 261.

Duncan, D. B. (1955).—Multiple range and multiple *F* tests. *Biometrics* **11**: 1.

Hamner, C. E., and Williams, L. W. (1963).—Effect of the female reproductive tract on sperm metabolism in the rabbit and fowl. *J. Reprod. Fert.* **5**: 143.

Hamner, C. E., and Williams, L. W. (1964).—Identification of sperm stimulating factor of rabbit oviduct fluid. *Proc. Soc. Exp. Biol., N.Y.* **117**: 240.

Huggett, A. St. G., and Nixon, D. A. (1957).—Enzymatic determination of blood glucose. *Biochem. J.* **66**: 12P.

Mastroianni, L., and Wallach, R. C. (1961).—Effect of ovulation and early gestation on oviduct secretions in the rabbit. *Am. J. Physiol.* **200**: 815.

Mastroianni, L., Beer, F., Shah, U., and Clewe, T. H. (1961).—Endocrine regulation of oviduct secretion in the rabbit. *Endocrinology* **68**: 92.

Mattner, P. E. (1963).—Capacitation of ram spermatozoa and penetration of the ovine egg. *Nature, Lond.* **199**: 772.

Olds, D., and Van Demark, N. L. (1957).—The behaviour of spermatozoa in the lumenal fluids of bovine female genitalia. *Am. J. Vet. Res.* **18**: 603.

Restall, B. J. (1964).—The growth and retrogression of the corpus luteum in the ewe. *Aust. J. Exp. Agric. Anim. Husb.* **4**: 274.

Restall, B. J. (1966*a*).—The fallopian tube of the sheep. I. Cannulation of the fallopian tube. *Aust. J. Biol. Sci.* **19**: 181.

Restall, B. J. (1966*b*).—The fallopian tube of the sheep. II. The influence of progesterone and oestrogen on the secretory activities of the fallopian tube. *Aust. J. Biol. Sci.* **19**: 187.

Salisbury, G. W., and Lodge, J. R. (1963).—Respiration quotients of bovine spermatozoa. *J. Reprod. Fert.* **5**: 195.

Wales, R. G., and O'Shea, T. (1966).—Effect of low levels of carbon dioxide on the metabolism of ram and bull spermatozoa. *J. Reprod. Fert.* (In press.)

Wallace, J. C., and Wales, R. G. (1964).—Effect of ions on the metabolism of ejaculated and epididymal spermatozoa. *J. Reprod. Fert.* **8**: 187.

Yates, J. H., and Olds, D. (1959).—A study of bovine follicular fluid and its effect on oxygen consumption by bovine spermatozoa. *J. Dairy Sci.* **42**: 1223.