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Effects of Bicarbonate on the Respiration and Glycolytic Activity of Boar Spermatozoa

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Abstract

The metabolism of washed boar spermatozoa was studied in the presence and absence of low levels of bicarbonate (6 mM) and carbon dioxide (2%). Bicarbonate stimulated the oxygen consumption of the spermatozoa but had no apparent effect on glycolysis. The stimulatory effect of bicarbonate on respiration depended on the presence of a utilizable exogenous energy source such as glucose, fructose, lactate, or pyruvate and no stimulation occurred when no substrate was added or when acetate was used as substrate. The response of the spermatozoa to bicarbonate also depended on the presence of adequate concentrations of potassium (5 mM) and to a lesser extent magnesium (1 mM).

Spermatozoa incubated with [^{14}C]bicarbonate failed to incorporate any significant amount of radioactivity. Furthermore, the addition of C4 compounds in the form of malate or oxaloacetate failed to influence the response of the spermatozoa to bicarbonate. These results suggest that the stimulating effects of bicarbonate on boar spermatozoa are not mediated by way of CO_2 fixation reactions which replenish the tricarboxylic acid cycle with C4 compounds. However, bicarbonate did stimulate respiration in the presence of various intermediates of the tricarboxylic acid cycle and it is possible, therefore, that the ion acts in boar spermatozoa to increase the activities of various enzymes of the tricarboxylic acid cycle, or to facilitate the transport of exogenous substrates into the cell, or both.

Introduction

It is now well established that the secretions of the female genital tract stimulate spermatozoal metabolism (see Stone *et al.* 1973 for references). This stimulatory effect of the female genital tract secretions has largely been attributed to their bicarbonate content (Hamner and Williams 1964; Foley and Williams 1967; Murdoch and White 1968, 1971), although it is recognized that other components in addition to bicarbonate may also act on spermatozoa to enhance their rate of metabolism (Foley and Williams 1967; Iritani *et al.* 1969; Stone *et al.* 1973).

In ram and rabbit spermatozoa the Embden–Meyerhof pathway of reactions appears to be a major site of action of bicarbonate in stimulating metabolism (Murdoch and White 1968, 1971; Wales and Murdoch 1971), a demonstrable effect being exerted at the pyruvate kinase (EC 2.7.1.40) step (Murdoch and White 1971). The glycolytic activity in the spermatozoa of these and many other species is high in relation to their oxidative capacity and relatively large amounts of lactate accumulate when they are incubated either under aerobic or anaerobic conditions with a glycolysable sugar such as glucose. Hence, since much of the energy demand of the sperm cell is apparently met by glycolysis in these species, it is not surprising that enhancing effects of bicarbonate on metabolism are directed towards increasing the rate of this

essential process. In contrast, the glycolytic rate of boar spermatozoa is low and the cells rapidly become immotile in the absence of oxygen (Polge 1956; Aalbers *et al.* 1961). Nevo *et al.* (1970) have suggested that 'in boar spermatozoa, the oxidative phase of sugar breakdown is probably the essential one'.

It is possible therefore that since the various metabolic pathways make different contributions to the energy status of the spermatozoa of different species, the metabolic site at which bicarbonate acts to stimulate spermatozoal metabolism may differ between the species. In the present paper an attempt has been made to investigate this possibility by studying the metabolism of boar spermatozoa in the presence and absence of bicarbonate and CO_2 and comparing the results with those obtained previously for the ram and rabbit.

Materials and Methods

Semen

Ejaculated spermatozoa were collected from boars by the staff at Steggles Pty Ltd, Benwerrin, N.S.W. Ejaculates were strained through cotton gauze to separate the gel particles but no attempt was made to fractionate the ejaculates into sperm-rich and sperm-poor portions. Metabolic studies were undertaken on the spermatozoa within 1·5 h of collection.

Diluent

Calcium-free Krebs–Ringer phosphate buffer (pH 7·4) (Umbreit *et al.* 1972) containing 123 mM NaCl, 1 mM $MgSO_4.7H_2O$, 5 mM KCl and 16 mM sodium phosphate was the basic diluent used in all studies. In experiments designed to examine interacting effects of cations and bicarbonate on sperm metabolism, KCl and $MgSO_4.7H_2O$ concentrations were adjusted at the expense of the NaCl concentration.

Substrates were added separately to the diluent to give the following amounts per Warburg flask: glucose, 10 μmol; acetate, 30 μmol; pyruvate, 20 μmol; lactate, 20 μmol, malate, 5 μmol; oxaloacetate, 5 μmol; succinate, 5 μmol; fumarate, 5 μmol; α-ketoglutarate, 5 μmol. All chemicals were of analytical reagent grade.

Preparation and Incubation of Sperm Suspensions

Samples of semen were centrifuged at 400 *g* for 5 min to remove seminal plasma. The spermatozoal plug was resuspended in a volume of diluent equal to that of the seminal plasma removed and centrifuged at 400 *g* for 5 min. After aspirating the supernatant, the spermatozoa were again washed and redispersed in a convenient volume of the washing diluent for use in manometric experiments.

Washed spermatozoal suspension ($2·1\times10^8$–$3·5\times10^8$ cells per flask) were incubated for 3 h in single side-arm Warburg flasks of approximately 6-ml capacity at 37°C and a shaking rate of 80 strokes/min. The total volume of reaction mixture in each flask was 1·0 ml. Oxygen uptake was measured in the absence of bicarbonate and CO_2 and also in the presence of 6 mM bicarbonate and 2% CO_2 by methods described previously (Murdoch and White 1968).

In some experiments aliquots of the spermatozoal suspension prior to incubation and of the flask contents after incubation were deproteinized by the addition of 1 volume of 5% (w/v) $ZnSO_4.7H_2O$ and 1 volume of 0·6 M $Ba(OH)_2$; glucose and lactate were then estimated in the neutral filtrates by enzymic methods (Barker and Britton 1957; Huggett and Nixon 1957). Counts of spermatozoa were made in duplicate using a haemocytometer and all values expressed on a 10^8 cell basis over the experimental period.

Fixation of CO_2

The extent to which boar spermatozoa are capable of the fixation of carbon dioxide was investigated using techniques described by O'Shea and Wales (1967). Aliquots (0·4 ml) of twice-washed spermatozoa ($4·2\times10^8$–$5·0\times10^8$ cells per flask) were incubated in Warburg flasks at 37°C for 1 h in Ca-free Krebs–Ringer phosphate buffer (pH 7·4) containing sodium [^{14}C]bicarbonate (1 μmol, 25 μCi), sodium pyruvate (10 μmol), penicillin (30 mg/100 ml), and streptomycin (50 mg/100 ml). The final volume of the reaction mixture was 1·0 ml. Following incubation, the

reaction mixture was acidified by adding 0·2 ml of 12 M H_2SO_4 and then incubated again for 3 h with 0·3 ml of 20% (w/v) KOH in the centre wells of the Warburg flasks. The acidified reaction mixture was neutralized with 6 M NaOH and centrifuged at 1000 *g* for 10 min. The supernatant and the plug were assayed for radioactivity in a toluene–Triton X-100 scintillation mixture (Patterson and Greene 1965). Aliquots of sperm-free diluent containing sodium [^{14}C]bicarbonate were treated in a similar manner and used as background for the radioactive counts. Spermatozoa immobilized by the addition of 4% (v/v) neutral formalin were used as controls to judge the extent of active incorporation of radioactive carbon in the motile cells. Ejaculates from four different boars were used.

Statistical Methods

Where necessary the results were subjected to standard analyses of variance which are presented in summary form giving only degrees of freedom and variance ratios for each source of variation. All non-significant second-order interactions were pooled with replicate interactions and used as error. The error value is given in italics at the base of the variance ratio columns. Student's *t*-test was employed to assess the significance of results in some experiments.

Results

Interacting Effects of Cations and Bicarbonate on the Metabolism of Glucose

In previous studies using ram spermatozoa it was shown that the stimulating effect of bicarbonate on metabolism depends on the presence of potassium (Murdoch and White 1971; Wales and Murdoch 1971). The results of an experiment designed to assess the metabolic response of boar spermatozoa to bicarbonate and cations (Mg^{2+} and K^+) are shown in Table 1.

Bicarbonate had no significant effect on the oxygen consumption of boar spermatozoa in the absence of added magnesium and potassium ions. However, a marked stimulation of oxygen uptake due to bicarbonate occurred when potassium was added to the incubation medium. Although bicarbonate stimulated oxygen uptake in the presence of either magnesium or potassium, both cations were required together to produce a maximal response. An interacting effect between magnesium and potassium ions on the oxygen uptake of the spermatozoa was also evident when incubations were conducted in the absence of bicarbonate. Under these conditions magnesium slightly depressed the rate of oxygen uptake of the spermatozoa, potassium had little apparent effect, whilst magnesium and potassium together significantly enhanced the oxygen consumption of the cells. The addition of bicarbonate effectively relieved the depressing effects of magnesium.

In contrast to the effects seen with oxygen uptake, bicarbonate had no significant effect on the glycolytic reactions in boar spermatozoa and did not influence the rate of glucose utilization or lactate production. The addition of magnesium and potassium ions also failed to significantly influence the amount of glucose utilized by the spermatozoa. In the absence of added potassium ions, the amount of lactate produced was almost negligible. Potassium, however, greatly stimulated the rate of lactate production but not to the extent of providing a stoichiometric relationship with the amount of glucose utilized. In general, the glycolytic activity of the spermatozoa was very low when compared with that in ram spermatozoa (Murdoch and White 1971).

Effects of Bicarbonate and Potassium on the Metabolism of Glucose, Acetate, and Pyruvate

Table 2 gives the results of an experiment conducted to assess whether bicarbonate and potassium act to affect the metabolism of substrates other than glucose by boar

spermatozoa. Magnesium ions (1 mM concentration) were included in all incubation diluents in this case but the potassium ion concentration was adjusted to give 0·0 or 5·0 mM KCl as required. Glucose, acetate or pyruvate were added as exogenous substrates.

Table 1. Interacting effects of cations (K^+ and Mg^{2+}) and bicarbonate on the aerobic metabolism of glucose by boar spermatozoa

Values are the means for three replicates

Bicarbonate concn (mM)	K^+ concn (mM)	Mg^{2+} concn (mM)	Oxygen uptake (μl)	Glucose utilized (μmol)	Lactate produced (μmol)
0	0	0	13·0	0·48	0·03
	0	1	9·0	0·53	0·03
	5	0	14·9	0·45	0·31
	5	1	22·4	0·49	0·42
6	0	0	13·7	0·52	0·03
	0	1	14·9	0·40	0·05
	5	0	32·4	0·63	0·23
	5	1	40·6	0·72	0·29

Summary of the analyses of variance

Source of variation	Degrees of freedom	Variance ratios		
		Oxygen uptake	Glucose utilized	Lactate produced
Effect of bicarbonate (*A*)	1	36·82**	0·62	1·92
Effect of cations (*B*)				
Effect of K^+	1	74·33**	0·76	64·44**
Effect of Mg^{2+}	1	3·44	0·03	1·92
$K^+ \times Mg^{2+}$ interaction	1	7·10*	0·22	1·21
Interaction $A \times B$	3	6·20**	0·46	1·71
Between replicates	2	0·50	0·62	0·85
Error	14	*18·09*	*0·065*	*0·0073*

* $P < 0·05$. ** $P < 0·01$.

Bicarbonate stimulated the oxygen uptake of the spermatozoa when glucose and pyruvate were used as substrates, the stimulatory effect being greater with glucose than with pyruvate. With acetate the oxygen consumption of the spermatozoa was less than with either of the other two substrates and bicarbonate slightly depressed rather than stimulated oxygen uptake in the presence of this substrate. Potassium significantly stimulated the oxygen uptake of the spermatozoa both in the presence and absence of bicarbonate particularly when glucose and acetate were used as substrates.

Effects of Bicarbonate on the Metabolism of Various Substrates

The results given in Table 3 show that bicarbonate acts to increase the oxygen consumption of boar spermatozoa only when an exogenous energy source is provided in the incubation diluent. All incubations in this case were conducted in the presence of magnesium and potassium ions.

The addition of glucose, fructose, pyruvate, lactate or acetate significantly ($P < 0{\cdot}01$) increased the oxygen uptake of the spermatozoa above that of the controls which contained no added exogenous substrate. Bicarbonate significantly ($P < 0{\cdot}01$) stimulated the rate of oxygen uptake only in the presence of glucose, fructose, pyruvate, and lactate and no stimulatory effect was obtained with acetate or when no exogenous substrate was added. The stimulation by bicarbonate was most evident with glucose and fructose.

Table 2. Effects of bicarbonate and potassium ions on the oxygen uptake of boar spermatozoa with glucose, acetate, and pyruvate as substrates

Values are expressed as microlitres of oxygen consumed and are the means for four replicates

Bicarbonate concn (mM)	K^+ concn (mM)	Substrate		
		Glucose	Acetate	Pyruvate
0	0	15·5	10·0	16·2
	5	18·1	14·5	16·8
6	0	32·8	8·6	20·9
	5	42·8	12·0	29·3

Summary of the analysis of variance

Source of variation	Degrees of freedom	Variance ratios
Effect of bicarbonate (A)	1	21·14**
Effect of potassium (B)	1	6·03*
Interaction $A \times B$	1	1·37
Between substrates (C)	2	21·45**
Interaction $A \times C$	2	10·95**
Interaction $B \times C$	2	0·12
Between replicates	3	9·90**
Error	35	*48·31*

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$.

Table 3. Effect of bicarbonate on the oxygen uptake of boar spermatozoa in the presence of various substrates

Values are expressed as microlitres of oxygen consumed and are the means ± standard errors for four replicates

Bicarbonate concn (mM)	Substrate added					
	None	Glucose	Fructose	Pyruvate	Lactate	Acetate
0	7·9±0·5	17·3±1·4	17·8±1·0	16·9±1·4	15·9±0·8	13·8±1·1
6	6·4±1·2	27·9±1·2	28·6±1·3	23·9±2·0	19·9±1·9	13·9±1·6

Effects of C4 Compounds on the Response of Boar Spermatozoa To Bicarbonate

Two experiments were conducted to assess whether the additions of C4 compounds are capable of influencing the response of boar spermatozoa to bicarbonate. In the first of these experiments malic acid was added as a C4 compound to sperm suspensions

in the presence or absence of bicarbonate and glucose. Magnesium and potassium were included in all incubation diluents. The results are shown in Table 4.

Malate caused no significant alteration in the oxygen consumption of the spermatozoa and failed to influence the response of the cells to bicarbonate when incubated in the presence of glucose. Bicarbonate was again only effective in stimulating the oxygen consumption of the spermatozoa when an exogenous energy source was provided in the incubation medium.

Table 4. Effect of malate on the response of boar spermatozoa to bicarbonate in the presence and absence of glucose

Values are expressed as microlitres of oxygen consumed and are means ± standard errors for three replicates

Bicarbonate concn (mM)	Malate concn (mM)	Substrate added	
		None	Glucose
0	0	10·5±2·5	24·2±3·1
	5	12·8±3·5	22·3±2·5
6	0	10·1±2·8	35·4±5·0
	5	14·5±4·0	34·0±5·1

In the second experiment, oxaloacetate was added as a C4 compound to sperm suspensions in the presence or absence of bicarbonate and pyruvate. Pyruvate rather than glucose was used in this case to provide a readily available C3 source for possible condensation with carbon dioxide. It was considered necessary to conduct this experiment as an additional check on the effects of adding C4 compounds since it is recognized that tricarboxylic acid (TCA) cycle intermediates differ greatly in their ability to permeate the plasma and mitochondrial membranes of spermatozoa (Flipse 1967). The results of preliminary experiments suggested that oxaloacetate may permeate the membranes in boar spermatozoa more readily than malate and may therefore be more available as an intracellular C4 source. As in the previous experiment, magnesium and potassium were included in all incubation diluents. The results are shown in Table 5.

Oxaloacetate significantly ($P < 0{\cdot}01$) stimulated the rate of oxygen consumption of the spermatozoa in the absence of added pyruvate. Pyruvate also stimulated the oxygen uptake of the spermatozoa but cooperative or additive effects of the two compounds were not apparent. Bicarbonate stimulated oxygen uptake in all cases when the spermatozoa were incubated separately with oxaloacetate or pyruvate and when the two compounds were added together. However, no stimulating effects of bicarbonate were observed in the absence of a utilizable exogenous energy source.

CO_2 Fixation Studies

The results of the previous experiments suggest that bicarbonate does not act to stimulate the respiration of boar spermatozoa by supplementing the TCA cycle with C4 compounds formed via CO_2 fixation reactions. These observations were supported by the results of a study conducted to assess directly whether boar spermatozoa are capable of the fixation of CO_2 when incubated in sodium [^{14}C]bicarbonate.

Following incubation the radioactivity in the supernatant and sperm plug of motile cell preparations did not differ significantly from that in the supernatant and plug of formalin-killed cells or sperm-free diluent incubated with the isotope.

Table 5. Effect of oxaloacetate on the oxygen uptake of boar spermatozoa in the presence and absence of bicarbonate and pyruvate

Values are expressed as microlitres of oxygen consumed and are the means ± standard errors for three replicates

Bicarbonate concn (mM)	Oxaloacetate concn (mM)	Substrate added	
		None	Pyruvate
0	0	14·4±2·8	21·2±3·3
	5	23·8±2·3	17·2±4·2
6	0	10·3±1·5	30·0±4·4
	5	42·4±5·5	37·2±5·5

Effects of Bicarbonate on the Respiration of TCA Cycle Intermediates

This experiment was conducted to assess the effects of bicarbonate on the oxygen uptake of boar spermatozoa incubated with intermediates of the TCA cycle. Glucose was included for comparison and all incubation diluents contained magnesium and potassium. The results are given in Table 6.

Table 6. Effect of bicarbonate on the oxygen uptake of boar spermatozoa in the presence and absence of glucose and various intermediates of the TCA cycle

Values are expressed as microlitres of oxygen consumed and are the means ± standard errors for four replicates

Substrate added	Bicarbonate concn (mM)	
	0	6
None	5·8±0·8	5·4±0·7
Glucose	13·5±2·3	22·2±3·3
α-Ketoglutarate	7·2±1·4	8·6±1·0
Fumarate	6·0±0·8	8·1±0·6
Oxaloacetate	14·5±2·1	24·8±3·7
Succinate	5·4±0·9	8·3±1·1

Oxaloacetate and glucose stimulated the oxygen uptake of the spermatozoa to about the same extent. α-Ketoglutarate was only slightly effective in increasing the oxygen consumption of the cells whilst fumarate and succinate were ineffective. Bicarbonate increased the oxygen uptake of the spermatozoa in all cases where substrate had been added but was most effective with glucose and oxaloacetate. No stimulation due to bicarbonate was observed in the absence of exogenous substrate.

Discussion

The results of the present investigations confirm the findings of Foley and Williams (1967) that bicarbonate, at a concentration consistent with its existence in the female

reproductive tract fluids (Vishwakarma 1962), stimulates the respiration of boar spermatozoa. The results also confirm the reports by Polge (1956), Aalbers *et al.* (1961), and Nevo *et al.* (1970) that the glycolytic rate of boar spermatozoa is low in comparison with that occurring in the spermatozoa of many other mammalian species.

It is possible to make four statements about the action of bicarbonate on the metabolism of boar spermatozoa based on the results of the present experiments. Firstly, bicarbonate acts on boar spermatozoa to increase respiration without appreciably altering the rate of glycolysis. Secondly, it requires the presence of magnesium and particularly potassium ions in order to maximally stimulate the respiration of the cells. Thirdly, bicarbonate is only effective in stimulating the respiration of the spermatozoa in the presence of a utilizable exogenous energy source. Finally, the bicarbonate ion does not appear to exert its stimulating effects on boar spermatozoa via CO_2 fixation reactions.

Garbers *et al.* (1973) have suggested that glycolysis normally proceeds maximally in boar spermatozoa and that oxidative pathways must function to provide additional energy in the form of ATP when needed. If this is true, the stimulating effect of bicarbonate on respiration and the lack of an effect on glycolysis observed in the present study are not surprising. However, this response of boar spermatozoa to bicarbonate differs from that observed with ram and rabbit spermatozoa. Thus, Murdoch and White (1971) found that bicarbonate increased glycolysis to a much greater extent than it increased oxygen uptake in ram spermatozoa, whilst in rabbit spermatozoa (Murdoch and White 1968) it stimulated glycolysis and respiration to almost the same extent. These observations suggest that the metabolic site(s) at which bicarbonate acts to stimulate spermatozoal metabolism differs between species. Since bicarbonate stimulated respiration of the spermatozoa in the presence of TCA cycle intermediates and was effective only when an exogenous energy source was added, it may act in boar spermatozoa to increase the activities of various enzymes of the TCA cycle, or to facilitate the transport of exogenous substrates into the cell, or both.

The results of Garbers *et al.* (1973) provide the basis for formulating an alternative mechanism for the action of bicarbonate. These workers found that phosphodiesterase inhibitors and cyclic nucleotide analogues stimulated the respiration of boar spermatozoa in the presence of pyruvate or fructose but not in the presence of acetate or in the absence of any exogenous substrate. These observations are very similar to the present effects obtained with bicarbonate and indicate the need for further experimental work to assess whether some of the bicarbonate effects on boar spermatozoal metabolism are mediated by cyclic AMP.

Although the spermatozoa of the ram and boar respond differently to bicarbonate and may have different metabolic sites preferentially influenced by the ion, the present results together with those obtained by Murdoch and White (1971) and Wales and Murdoch (1971) suggest that both cell types nevertheless require adequate concentrations of potassium for bicarbonate to exert maximal effects. Magnesium also plays an important role in boar spermatozoal metabolism and in determining the response of the cells to bicarbonate. Thus it would appear that the three ions act together in a cooperative fashion in boar spermatozoa to regulate metabolism.

Carbon dioxide fixation or anaplerotic reactions involving the condensation of C3 compounds with carbon dioxide are important in many cells for the replenishment of C4 compounds (Lachica 1968). Although such reactions may occur in ram and

bull spermatozoa (O'Shea and Wales 1967, 1970; Sexton and Flipse 1972), the present results suggest that they do not occur in boar spermatozoa. No significant amount of radioactivity was incorporated into cells incubated with [^{14}C]bicarbonate. Furthermore, the addition of C4 compounds in the form of malate or oxaloacetate failed to influence the response of the spermatozoa to bicarbonate when incubated in the presence of a utilizable exogenous energy source. Thus the results indicate that the stimulating effects of bicarbonate on boar spermatozoa are not mediated by way of CO_2 fixation reactions which supplement the TCA cycle with C4 compounds.

The ability of bicarbonate to alter cellular metabolism is not confined to spermatozoa but is also known to exert profound effects on other mammalian cells and on the activity of several enzymes *in vitro* (see McDaniel *et al.* 1971). The importance of such metabolic alterations to spermatozoa in the female tract is not known with any certainty, but adequate amounts of potassium and bicarbonate together with utilizable substrates such as pyruvate and lactate are present in the female genital tract secretions to cause a stimulation of spermatozoal metabolism *in utero* (Vishwakarma 1962; Restall and Wales 1968; Spilman *et al.* 1970; Iritani *et al.* 1971).

Rogers and Yanagimachi (1975) and Rogers *et al.* (1977) have suggested that the processes of capacitation and the acrosome reaction of spermatozoa are metabolically controlled and that pyruvate and lactate play important roles in this respect. It is possible that bicarbonate may have a role in the regulation of these processes through its effects on sperm metabolism. Further work, however, is required to evaluate this possibility.

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References

Aalbers, J. G., Mann, T., and Polge, C. (1961). Metabolism in boar semen in relation to sperm motility and survival. *J. Reprod. Fertil.* **2**, 42–53.

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

Flipse, R. J. (1967). Metabolism of bovine semen. XV. Rate-limiting reaction in the citric acid cycle. *J. Dairy Sci.* **50**, 381–4.

Foley, C. W., and Williams, W. L. (1967). Effect of bicarbonate and oviduct fluid on respiration of spermatozoa. *Proc. Soc. Exp. Biol. Med.* **126**, 634–7.

Garbers, D. L., First, N., Gorman, S. K., and Lardy, H. A. (1973). The effects of cyclic nucleotide phosphodiesterase inhibitors on ejaculated porcine spermatozoan metabolism. *Biol. Reprod.* **8**, 599–606.

Hamner, C. E., and Williams, W. L. (1964). Identification of sperm stimulating factor of rabbit oviduct fluid. *Proc. Soc. Exp. Biol. Med.* **117**, 240–3.

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

Iritani, A., Gomes, W. R., and Van Demark, N. L. (1969). The effect of whole, dialyzed and heated female genital tract fluids on respiration of rabbit and ram spermatozoa. *Biol. Reprod.* **1**, 77–82.

Iritani, A., Nishikawa, N., Gomes, W. R., and Van Demark, N. L. (1971). Secretion rates and chemical composition of oviduct and uterine fluids in rabbits. *J. Anim. Sci.* **33**, 829–35.

Lachica, R. V. F. (1968). Carbon dioxide fixation. *Enzymologia* **34**, 281–98.
McDaniel, M. L., Liang, A. M., and Longmore, W. J. (1971). Effects of carbon dioxide concentration on intermediates of the Embden–Meyerhof pathway and the citric acid cycle in isolated perfused rat liver. *J. Biol. Chem.* **246**, 4813–17.
Murdoch, R. N., and White, I. G. (1968). The influence of the female genital tract on the metabolism of rabbit spermatozoa. I. Direct effect of tubal and uterine fluids, bicarbonate, and other factors. *Aust. J. Biol. Sci.* **21**, 961–72.
Murdoch, R. N., and White, I. G. (1971). Studies of the stimulating effect of bicarbonate on the metabolism of ram spermatozoa. *J. Reprod. Fertil.* **25**, 231–42.
Nevo, A. C., Polge, C., and Frederick, G. (1970). Aerobic and anaerobic metabolism of boar spermatozoa in relation to their motility. *J. Reprod. Fertil.* **22**, 109–18.
O'Shea, T., and Wales, R. G. (1967). Fixation of carbon dioxide by ram spermatozoa. *J. Reprod. Fertil.* **14**, 333–4.
O'Shea, T., and Wales, R. G. (1970). Studies of the fixation of carbon dioxide by washed ram spermatozoa. *Aust. J. Biol. Sci.* **23**, 889–901.
Patterson, M. W., and Greene, R. C. (1965). Measurement of low energy beta-emitters in aqueous solution by liquid scintillation counting of emulsions. *Anal. Chem.* **37**, 854–7.
Polge, C. (1956). Artificial insemination in pigs. *Vet. Rec.* **68**, 62–6.
Restall, B. J., and Wales, R. G. (1968). The fallopian tube of the sheep. V. Secretion from the ampulla and isthmus. *Aust. J. Biol. Sci.* **21**, 491–8.
Rogers, B. J., Ueno, M., and Yanagimachi, R. (1977). Inhibition of hamster sperm acrosome reaction and fertilization by oligomycin, antimycin A, and rotenone. *J. Exp. Zool.* **199**, 129–36.
Rogers, B. J., and Yanagimachi, R. (1975). Retardation of guinea pig sperm acrosome reaction by glucose: the possible importance of pyruvate and lactate metabolism in capacitation and the acrosome reaction. *Biol. Reprod.* **13**, 568–75.
Sexton, T. J., and Flipse, R. J. (1972). Carbon dioxide fixation in bovine semen, washed spermatozoa and seminal plasma. *J. Dairy Sci.* **54**, 417–22.
Spilman, C. H., Duby, R. T., and Black, D. L. (1970). Effect of an intrauterine device on sheep oviduct fluids: chemical composition and stimulation of spermatozoan respiration *in vitro*. *Biol. Reprod.* **3**, 76–81.
Stone, R. T., Foley, C. W., Thorne, J. G., and Huber, T. L. (1973). Effect of oviductal fluids on oxidative phosphorylation in spermatozoa. *Proc. Soc. Exp. Biol. Med.* **142**, 64–7.
Umbreit, W. W., Burris, R. H., and Stauffer, J. F. (1972). 'Manometric and Biochemical Techniques'. 5th edn. p. 146. (Burgess Publ. Co.: Minneapolis.)
Vishwakarma, P. (1962). The pH and bicarbonate ion concentration of the oviduct and uterine fluids. *Fertil. Steril.* **13**, 481–6.
Wales, R. G., and Murdoch, R. N. (1971). Factors influencing the response of ram spermatozoa to bicarbonate and carbon dioxide. *Aust. J. Biol. Sci.* **24**, 345–54.

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