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THE EFFECTS OF POTASSIUM AND CALCIUM SALTS ON THE MOTILITY OF RAM, RABBIT AND BULL SPERMATOZOA

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The effects of pH, osmotic pressure and sodium chloride concentrations on the motility of rabbit, ram, bull and human spermatozoa have been reported (Emmens, 1947; Blackshaw & Emmens, 1951). It was demonstrated that variations in the sodium chloride content of isotonic media had little influence on spermatozoal motility, except at a pH above 9.

The effects of potassium and calcium on ciliary activity have been reviewed by Gray (1928), but their importance for the activity and metabolism of spermatozoa has not been fully demonstrated. Many successful semen diluents contain neither potassium nor calcium, and some contain isotonic sodium citrate which might be expected to convert the seminal calcium into the unionized state. Despite this Winters, Comstock, Cole, Green & Bulik (1938) and Milovanov (1934) appear to have found calcium necessary in diluents for ram spermatozoa, the concentration in Milovanov's diluent being 0·006 M.

However, Lardy, Winchester & Phillips (1945) found that 0·004 M-calcium depressed the motility of ram spermatozoa. Lardy & Phillips (1943) found that calcium depressed the motility of bull spermatozoa, but that potassium was necessary for optimal motility.

In view of the uncertainty concerning the need for calcium and the lack of knowledge about the effects of potassium a study has been made of the effects of these ions, alone and in combination, on the motility of ram, bull and rabbit spermatozoa.

MATERIALS AND METHODS

Ejaculates of high initial motility were used in tests. This was necessary as the washing and dilution procedures considerably reduced the number of active spermatozoa. Ram semen was collected by electrical stimulation as described by Gunn (1936) and bull and rabbit semen by the artificial vagina.

The spermatozoa were freed of seminal plasma by dilution 1 in 5 in buffered glucose saline and then centrifuged for 10 min at 1500 rev/min. In the preliminary experiments with ram and rabbit

semen which involved only potassium ions the supernatant was removed and sufficient diluent added to restore the original semen volume.

When potassium and calcium were both used the centrifuging and washing procedure was repeated.

In the preliminary experiments 0·1 M-sodium phosphate buffer was used and in those concerned with both potassium and calcium, 0·1 M-veronal–hydrochloric acid was used. Both were added at the rate of 20 ml. buffer per 100 ml. total diluent. The nominal pH was 7·0. The basic diluent in all tests contained 2 % (w/v) glucose and 0·46 % (w/v) sodium chloride and buffer. Potassium and calcium chlorides in 0·154 M solution were added to the diluent to give the desired cation concentrations. The resulting solutions were approximately isotonic with 0·9 % (w/v) NaCl.

In each tube the washed ejaculate was diluted 1 in 10 to 1 in 20.

Observations of motility were made at ½, 1½, 2½, 4 and 5 hr with ram and rabbit semen, at ½, 1½, 2½ and 4½ hr with bull semen. Motility was scored as described by Emmens (1947); maximum motility as 4, complete immotility as 0, and the added scores for each tube constitute the motility index which was used as unit observation in the analyses of variance. For publication, however, the tables have been modified by the omission of the results for individual ejaculates and the use of scaling-up factors to give a maximum score of 100 for a single treatment, whatever the number of ejaculates, or observations on each ejaculate. Nevertheless, the figures for each species are not directly comparable.

RESULTS

Veronal–hydrochloric as a buffer

To prevent precipitation of calcium in the higher concentrations used, a veronal–hydrochloric acid buffer was used. The motility of ram, rabbit and bull spermatozoa in this buffer system did not differ significantly from that in phosphate buffers. Three ejaculates were used in each test at three pH levels. The results for each treatment are shown in Table 1.

TABLE 1. Treatment motility scores (maximum 100) for the test of the suitability of veronal–hydrochloric acid as compared with phosphate buffers with ram, rabbit and bull spermatozoa

	Ram			
Diluent	6·5–7·0	7·2–7·4	7·8–8·0	Mean score
Veronal–HCl	82	79	70	77
Phosphate	82	75	67	67
	Rabbit			
	7·0–7·5	7·7–7·8	8·0–8·1	Mean score
Veronal–HCl	63	67	62	64
Phosphate	59	60	59	59
	Bull			
	6·8–7·1	7·3–7·7	7·6–8·1	Mean score
Veronal–HCl	75	68	63	69
Phosphate	70	65	61	65

Effects of cations

Experiments with spermatozoa washed once indicate that neither ram nor rabbit spermatozoa are markedly affected by variations in potassium-ion concentration (Table 2) and that the type of response of those of the rabbit is not altered by incubation at 37° C.

In the second series of tests, concentrations of potassium chloride at 0, 0·005, 0·01, 0·02, 0·04 M and of calcium chloride at 0, 0·0015, 0·003, 0·006 M were used in a factorial design.

Four replications, each with a different ejaculate, were made with ram and bull semen and six with rabbit semen. Tables 3–5 list the motility scores (maximum 100) for each treatment, with the ram, rabbit and bull respectively.

TABLE 2. Treatment motility scores (maximum 100) for the tests of the influence of potassium ion, concentration on the motility of once-washed ram and rabbit spermatozoa

Potassium chloride concn. (M)	Ram	Rabbit		
	Room temp.	Room temp.	37° C	Mean score
0·000	82	66	79	72
0·0025	81	69	81	75
0·005	82	70	82	76
0·01	79	71	81	76
0·02	76	69	77	73

TABLE 3. Treatment motility scores (maximum 100) for the test of the influence of potassium and calcium salts on the motility of ram spermatozoa *in vitro* (4 ejaculates)

Potassium chloride concn. (M)	Calcium chloride concn. (M)				
	0·000	0·0015	0·003	0·006	Mean score
0·00	79	66	75	66	71
0·005	85	85	78	74	80
0·01	89	86	83	80	85
0·02	80	79	78	76	78
0·04	80	77	75	74	77
Mean score	83	79	78	74	—

TABLE 4. Treatment motility scores (maximum 100) for the test of the influence of potassium and calcium salts on the motility of rabbit spermatozoa *in vitro* (6 ejaculates)

Potassium chloride concn. (M)	Calcium chloride concn. (M)				
	0·000	0·0015	0·003	0·006	Mean score
0·00	52	44	44	44	46
0·005	47	47	54	50	50
0·01	48	47	49	45	47
0·02	46	46	46	44	45
0·04	46	43	46	43	44
Mean score	48	45	47	45	—

Ram (*Table* 3). All the main effects and the ejaculate–potassium interaction were highly significant ($P < 0·01$). The potassium main effect was also tested against the ejaculate–potassium interaction and gave $F = 3·48$, D.F. = 4 and 12, $P < 0·05$. Likewise the calcium main effect tested against the ejaculate–calcium interaction gave $F = 5·88$, D.F. = 3 and 9, $P < 0·05$.

It therefore appears that the optimum level of potassium ions for ram spermatozoa is about 0·01 M, and that calcium ions at all concentrations are somewhat deleterious. Also there is no interaction between potassium and calcium on the motility of ram spermatozoa.

Rabbit (*Table* 4). Rabbit spermatozoa were adversely affected by the washing and dilution procedures; head agglutination occurred in all tubes with some ejaculates and motility was rather low throughout.

Rabbit spermatozoa show no significant sensitivity to any particular level of potassium and are not adversely affected by calcium levels up to 0·006 M.

Bull (*Table* 5). Agglutination of spermatozoa was slight after washing and dilution.

The main effects for ejaculates and potassium levels and the ejaculate–potassium interaction are all significant at the 0·001 level. Again the potassium main effect was tested against the ejaculate–potassium interaction; $F = 72·2/22·6 = 3·20$, D.F. = 4 and 12, $P > 0·05$. The 0·05 level of significance for D.F. = 4 and 12 gives $F = 3·26$.

TABLE 5. Treatment motility scores (maximum 100) for the test of the influence of potassium and calcium salts on the motility of bull spermatozoa *in vitro* (4 ejaculates)

Potassium chloride concn. (M)	Calcium chloride concn. (M)				
	0·000	0·0015	0·003	0·006	Mean score
0·00	53	52	53	53	53
0·005	59	62	60	60	60
0·01	62	63	61	58	62
0·02	60	59	58	57	59
0·04	55	57	55	55	56
Mean score	58	59	57	56	—

Although the potassium level probably does influence the motility of bull spermatozoa, this action is not so firmly established as is the case with ram spermatozoa. As with the ram, motility is best at the 0·01 M level of potassium, calcium having no significant effect. Again there was no significant K/Ca interaction.

DISCUSSION

The results presented show that potassium and calcium salts do significantly influence the motility of ram and bull spermatozoa. In the case of ram spermatozoa comparison of the potassium and calcium main effects with the corresponding interactions establishes more conclusively the significance of the effects. It may also be noted that in none of the species is there a significant K/Ca interaction which means that the effect of either ion on motility is not dependent on the concentration of the other.

Although the effects of these ions are not striking, calcium is clearly undesirable in diluents for ram and bull spermatozoa, and unnecessary for rabbit spermatozoa. On the other hand, potassium appears to be necessary only for washed spermatozoa.

The increased sensitivity of twice-washed spermatozoa to potassium lack, suggests that intracellular potassium may be lost during washing. Other

observations (Blackshaw, 1953) have shown that repeated washing of ram spermatozoa causes great loss in motility which may be partially restored by the addition of potassium after the last washing. White (1953) has also found that the motility of ram and bull spermatozoa washed four times at 200 million cells per ml., and incubated at 37° C, rapidly decreases unless potassium chloride is present in at least 0·004 M concentration. Both these reports indicate that potassium may be lost from the cell by washing and later taken up again, when resuspended in fresh diluent containing potassium.

SUMMARY

1. The motility of ram, rabbit and bull spermatozoa has been studied at various concentrations of potassium and calcium salts.

Sodium phosphate buffers were used when potassium alone was investigated and veronal–hydrochloric acid buffers when both potassium and calcium were used.

2. Potassium had little effect on the motility of ram and rabbit spermatozoa washed once, and, in the case of rabbit spermatozoa, incubation at 37° C caused no change in response.

3. The motility of twice-washed ram spermatozoa is significantly affected by potassium and calcium salts. A concentration of 0·01 M-KCl gives best motility and all levels of calcium chloride are deleterious to motility.

4. Twice-washed rabbit spermatozoa are not significantly influenced by variations in potassium and calcium chloride concentrations.

5. Potassium chloride influences the motility of twice-washed bull spermatozoa, the optimum level being about 0·01 M, while calcium chloride has no significant effect on motility.

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