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BRIEF COMMUNICATION

The effect of inorganic ions on ram sperm motility using Computer Assisted Sperm Analysis (CASA)

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ABSTRACT

The role of specific inorganic ions in the maintenance of ram sperm motility in a chemically defined ram semen diluent (RSD-1) was examined. A preliminary experiment identified that magnesium was required to maintain motility. Significant treatment and incubation effects were observed for all motility parameters (P<.05). RSD-1 (treatment T1) was modified to provide a series of treatments. Effects measured were the substitution of $MgCl_2$ (T2) or $MgSO_4$ (T3) and the elimination of KH_2PO_4 (T4) or $NaHCO_3$ (T5). A total of 10 ejaculates were analysed using the Hobson Sperm Tracker (HST) over a period of 7 days. Semen was cooled to 15°C after dilution with treatment diluents and reheated to 38°C after 1 hr and 5 days storage. Motility was measured after incubation at 38°C for 0, 24, and 48 hours. Treatments had a significant (P<0.001) effect on the velocity parameters and the percentage of motile sperm. Treatments T4 and T5 had greatest depression in motility parameters with fresh semen while T5 also had an effect with stored sperm. Substitution of the $MgSO_4$ (T3) reduced the velocity parameters in the fresh semen e.g. mean Curvilinear velocity (VCL) values (µm/sec) for fresh semen were T1 = 188 ± 10.6, T2 = 186 ± 10.6, T3 = 164 ± 10.6, T5 = 143 ± 10.6. Both storage and incubation had effects (P<0.001) on the motility parameters with a reduction both on storage and with incubation. There was a storage incubation interaction (P<0.001) with stored sperm showing a more rapid decline with incubation e.g. VCL values were Fresh 0h = 169 ± 5.3, 24h = 143 ± 5.3, 48h = 103 ±5.3, and stored 0h = 154 ± 5.3, 24h = 77 ± 5.3, 48h = 74 ± 6.7. These results show that various anions influence sperm motility.

Keywords: spermatozoal motility; anions; bicarbonate; phosphate; sulphate.

INTRODUCTION

A chemically defined ram semen diluent RSD-1 (Upreti *et al.,* 1995) modified from Krebs Ringer (KR) buffer (Dawson *et al.,* 1979) was used to examine the role of specific inorganic ions in the maintenance of sperm motility and viability. Organic components of RSD-1, calcium and bicarbonate were optimised for motility when developing the diluent however, other inorganic ions were maintained to the levels of KR buffer. In this study a computer assisted sperm analyser, Hobson Sperm Tracker (HST) was used to measure the path movement and speed distribution of sperm by videomicrography. The analyser can simultaneously track many moving objects in the field of view of a microscope continuously in real time. HST has the capability of measuring 10 motility parameters, percentage motile and motile concentration.

A preliminary experiment (data not shown) identified that magnesium salts were required to maintain motility. Replacing $MgSO_4$ with $MgCl_2$ or omitting both components was detrimental to motility parameters. Previous studies have shown bicarbonate is a useful nutrient (Upreti *et al.,* 1995).

METHODS

RSD-1 (Treatment 1; T1) was modified to provide a series of treatments. Effects measured were the substitution of $MgCl_2$ for $MgSO_4$ (T2), substitution of $MgSO_4$ for $MgCl_2$ (T3) and the elimination of KH_2PO_4 (T4) or $NaHCO_3$ (T5). A total of 10 ejaculates were collected by artificial vagina. Each ejaculate was diluted to 100 x 106 ml using test diluents. Semen was cooled to 15°C after dilution and reheated to 38°C after 1 hr and 5 days of storage at 15°C. Motility was measured after incubation at 38°C for 0, 24, and 48 hours using the Hobson Sperm Tracker (HST) (Smith *et al.,* 1995). Data was analysed using REML (restricted maximum likelihood) techniques of the Genstat statistical package.

RESULTS

Treatments had a significant (P<0.001) effect on the velocity parameters (Table 1) and the percentage of motile sperm. Treatments T4 and T5 had greatest depression in motility parameters with fresh semen while T5 also had an effect with stored sperm. Substitution of the $MgSO_4$ (T3) with $MgCl_2$ reduced the velocity parameters in the fresh semen. Sperm head movement was significantly reduced e.g. Beat Cross Frequency values in fresh semen were T1= 7.4 ± 0.49, T2=7.0 ± 0.49, T3 = 6.5 ± 0.49, T4 = 6.15 ± 0.49, T5 = 5.96 ± 0.49

Both storage and incubation had effects (P<0.001) on the motility parameters with a reduction both on storage and with incubation. There was a storage x incubation interaction (P<0.001) with stored sperm showing a more

TABLE 1: Overall treatment mean values for Velocity parameters.

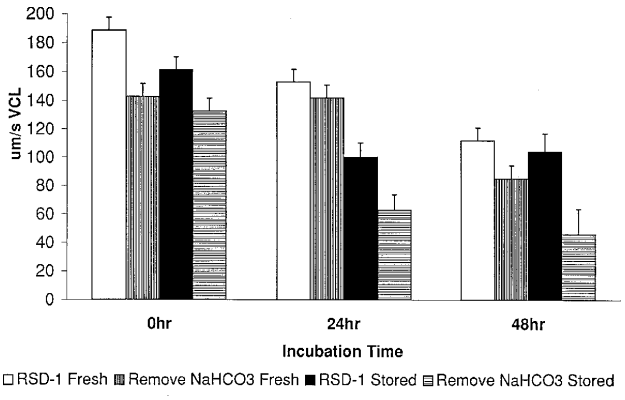
Treatment[1] Velocity Parameter[2]	T1	T2	T3	T4	T5	SEM
VCL µm/sec	131.9	132.0	122.6	112.3	101.5	6.826
VAP µm/sec	100.21	100.02	91.79	84.68	73.76	5.609
VSL µm/sec	92.54	92.43	84.73	78.54	68.81	5.144

[1] T1 = RSD-1, T2 = substitution of $MgCl_2$ for $MgSO_4$, T3 = substitution of $MgSO_4$ for $MgCl_2$, T4 = elimination of KH_2PO_4, T5 = elimination of $NaHCO_3$.

[2] Curvilinear velocity (VCL), Average path Velocity (VAP) and Straight Line Velocity (VSL).

rapid decline with incubation. Overall mean Curvilinear Velocity (VCL) values (µm/sec) VCL values were; Fresh 0h = 169 ± 5.3, 24h = 143 ± 5.3, 48h = 103 ± 5.3, and stored 0h = 154 ± 5.3, 24h = 77 ± 5.3, 48h = 74 ± 6.7. Figure 1 shows the storage by incubation interaction for RSD-1 with and without bicarbonate on VCL.

FIGURE 1: Effect of storage and incubation on the curvilinear velocity of Ram sperm in RSD-1 with and without Bicarbonate.



DISCUSSION

These results indicate that various anions (SO_4, PO_4, and HCO_3) have positive effects on sperm motility. The biochemical mechanism(s) involved for each anion would vary depending on the physiological status of the spermatozoa and the concentration of the anion. HCO_3 (³20 mM) is required for the capacitation leading to the reduction of linear velocity. Lower concentration of HCO_3 have shown its ability to support cellular functions (Chagnon and Corbeil, 1973). Depression of motility with elimination of 3.6 mM bicarbonate suggests a role as a nutrient as well as for capacitation. It has been found that bicarbonate stimulates the synthesis of cAMP from ATP via the activation of adenylate cyclase (Okamura *et al.*, 1985). This bicarbonate-sensitive adenylate cyclase plays an important role in controlling sperm through modulation of intracellular cAMP concentration. It has been reported (Tajima *et al.*, 1987: Tajima *et al.*, 1990) that most extracellularly added HCO_3 does not act directly on the adenylate cyclase inside the plasma membrane but exogenous HCO_3 may act to suppress the diffusion of metabolic CO_2 by a simple gas equilibrium. Under these conditions endogenous HCO_3 would be generated from metabolic CO_2. Increased HCO_3 concentration may stimulate adenylate cyclase leading to elevation of cAMP concentration and enhancement of progressive motility (Okamura *et al.*, 1988). Potassium is well known for its effect on maintaining the membranal potential. In RSD-1, potassium was added as KCl (4.7 mM) and KH_2PO_4 (1.1 mM). Therefore the elimination of KH_2PO_4 from RSD-1 focuses on the effects of the PO_4 anion on the spermatozoal motility. Elimination of PO_4, reduced velocity parameters, which indicates its involvement in the synthesis and degradation of ATP. The effect of SO_4 could be via two biochemical mechanisms. First, that SO_4 and HCO_3 share same transport mechanisms (Okamura *et al.*, 1988) and removal of SO_4 might allow increased HCO_3 transport. Secondly free SO_4 ions may block the ability of cholesterol-SO_4 to regulate membrane fluidity. Sulphated sterols may influence the alteration on the surface charges, ion permeability and modulation of the lipid distribution in the plane of lipid bilayer of sperm membrane (Go *et al.*, 1983).

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