SELECTION OF STORAGE MEDIA FOR PIG A.I. – LABORATORY METHODS FOR REGULATING THE ACIDITY AND OSMOLARITY OF STORAGE MEDIA FOR BOAR SEMEN

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ABSTRACT

Simple laboratory methods were used to estimate optimal levels of acidity and osmolarity of four storage media for the boar semen. It was observed that above pH 7.0, less citric acid was needed for a unit change in pH of the sodium-citrate buffer, while above pH 8.0, more sodium carbonate and citric acid were needed for a unit change in the final Sodium-citrate buffered extender (Na-C), indicating high buffering capacity of this medium.

The Glucose-glycine buffered extender (G-G), also required more sodium carbonate for a unit change of pH at above pH 6.5, also indicating high buffering capacity of this medium. This was the same situation with the Tris-buffered extender (Tris), while the response with the Tissue – culture buffer extender (YCN) was always linear, indicating poor buffering capacity of the medium.

Key Words: Acidity, Osmolarity, Storage media, Buffering capacity, Buffer, Extender, Boar, Semen

INTRODUCTION

The concentration of hydrogen ion (pH) in an extender, has been shown to be important factor during semen storage (Norman *et al* 1956; Blackshaw, 1960). There was severe inhibition of bovine spermatozoan activity when the pH was low (pH 5.5 – 5.8) but could be reversed (Norman *et al* 1956) by the addition of an alkaline coconut solution.

The osmotic pressure (O.P) of the media of storage is also of major importance. While the O.P. has no effect on spermatozoal survival in some studies, in others, hypertonic diluents were less harmful to spermatozoal survival than hypotonic diluents (Stevermer *et al*, 1964, Steinbach and Foote; 1967; Igboeli, 1970). Pools *et al*, (1973) observed that differences existed for acidic, alkaline and total buffering capacity of boar semen. Igboeli (1970) showed that the boar spermatozoa like the bull spermatozoa (Steinbach, 1963) can tolerate a wide range of molarities. Dede (1983) also observed that the pH on the acidic side of

neutrality tended to be inferior in supporting spermatozoal survival compared to the pH on the alkaline side of neutrality. This study intends to use simple laboratory methods for estimating optimal levels of acidity and osmolarity of some storage media for the boar semen.

MATERIALS AND METHODS

Double strength solutions of four storage media, comprising of sodium-citrate buffer and extender (Na-C); Tris-buffer and extender (Tris); Glucose glycine buffer and extender (G-G) and Tissue-culture medium 199 buffer and extender (TCM), were prepared as shown in Table 1. Regression lines were established between the amount of citric acid and sodium carbonate and the resulting pH and the osmotic pressure (O.P) at different levels for the buffers and for the final extenders. The determination of the pH values was made with a Radiometer Type 25 Glass Electrode pH meter at a temperature range of 19°-21° while the determination of the osmotic pressure was by the use of the semi- micro Knauer Osmometers at the same temperature range of 19°-21° C. The osmotic pressure' of the seminal plasma was compared with the osmotic pressure of all the media. All the data were subjected to simple regression analysis (Little *et al.*, 1972).

RESULTS AND DISCUSSION

The amount of citric acid and sodium carbonate per 20ml of buffers and extenders, have been plotted against the pH values obtained with the sodium Citrate buffers (Fig. 1 A.B.) and extenders (Fig. 2A,B); with the Tris-buffers and extenders (Fig. 3A.B.); with the Glucose-glycine buffers (Fig. 4A.) and extenders (Fig. 4B.) and the Tissue-culture 199 buffers and extenders (Fig. 5). More citric acid and more sodium carbonate solution was needed per unit change in pH values of all the extenders when the pH was above pH 7.00. On the other hand, the TCM extender did not require more citric acid and more sodium carbonate solution per unit change in pH values. This is an indication of good buffering capacity of all the media except the TCM storage medium. The regression equations for calculating the citric acid requirements for the pH range from 3-15 (Na-C) from 4-9 (Tris); from 2-11 (G- G) and from 2-36 (TCM) are as indicated in Table 2.

Davies *et al* (1963) observed the highest motility rating for bovine spermatozoa with the 0.20M Tris buffered yolk glycerol at 5°C, with a pH of 6.75. Earlier on, Norman *et al* (1956) had observed that bovine spermatozoan activity was greatly inhibited at low levels (pH 5.5-5.8) but this inhibitory effect could be reversed by the addition of alkaline coconut milk. Steinbach (1963) observed that the alkaline conditions apparently beneficial during the processes of freezing and thawing were not tolerated by bull semen in the liquid state. Also Dede (1983) observed that the metabolic activities of boar spermatozoa were less marked in alkaline media than in acidic media.

The osmotic pressure (O.P.) of all the media tested, have been plotted against the pH values for the Na-C buffers and extenders; for the Tris buffers and extenders (Fig. 6). The relative O.P. of the seminal plasma of boar semen has been plotted in Fig. 6 to show deviations of the O.P. of the media from that of the seminal plasma (mean = 283 ± 8 mOsm) of boar semen.

Igboeli (1970) compared three molarities of Tris (1.50M, 0.20M and 0.25M) diluents with some other diluents and recommended 0.20M Tris diluent for storage of boar semen. It was observed (Dede 1983) that the O.P. of all media close to the O.P. of the sire's blood, tended to be superior for boar spermatozoan activity compared to lower and higher O.P. levels.

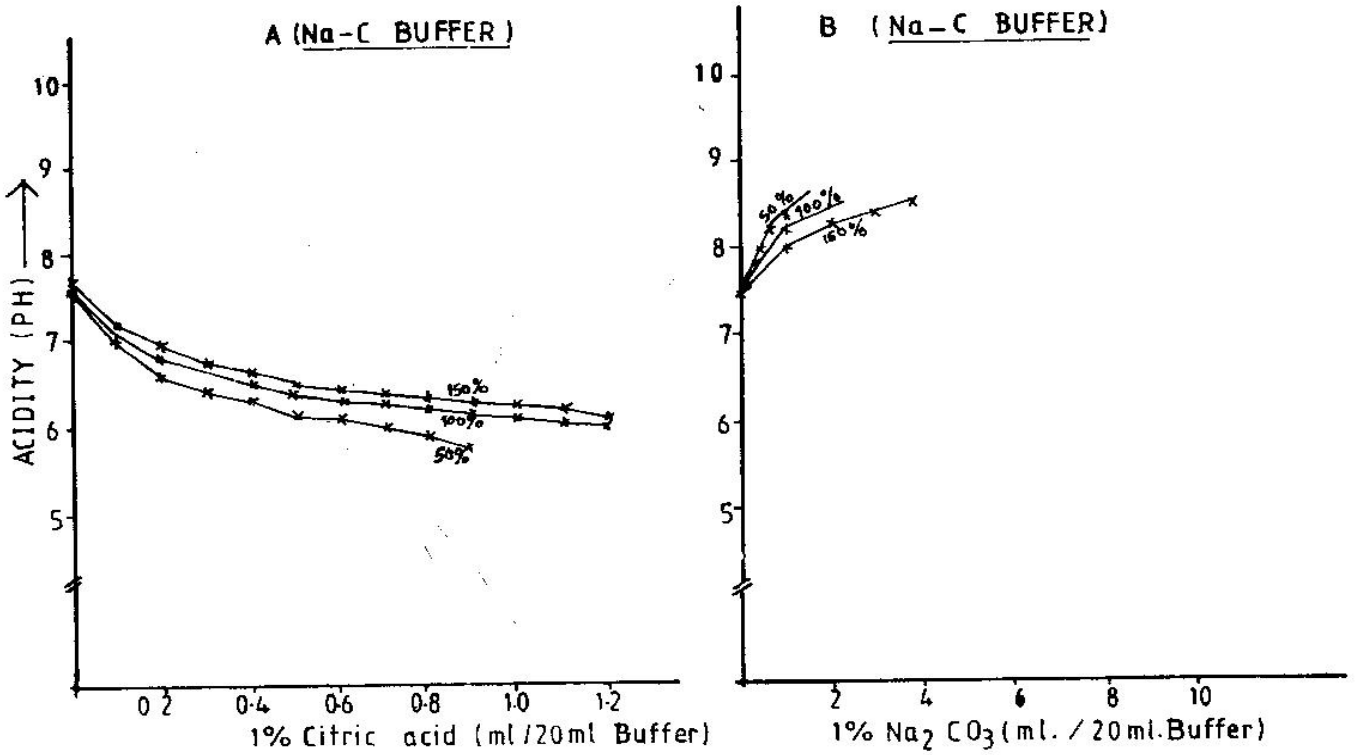

Fig. 1: The effect of adding one percent citric acid solution to 20ml. sodium-citrate-glycine buffer (A) and one percent sodium carbonate solution to 20ml. sodium citrate-clycine buffer (B) on the acidity of the buffers at different concentrations.

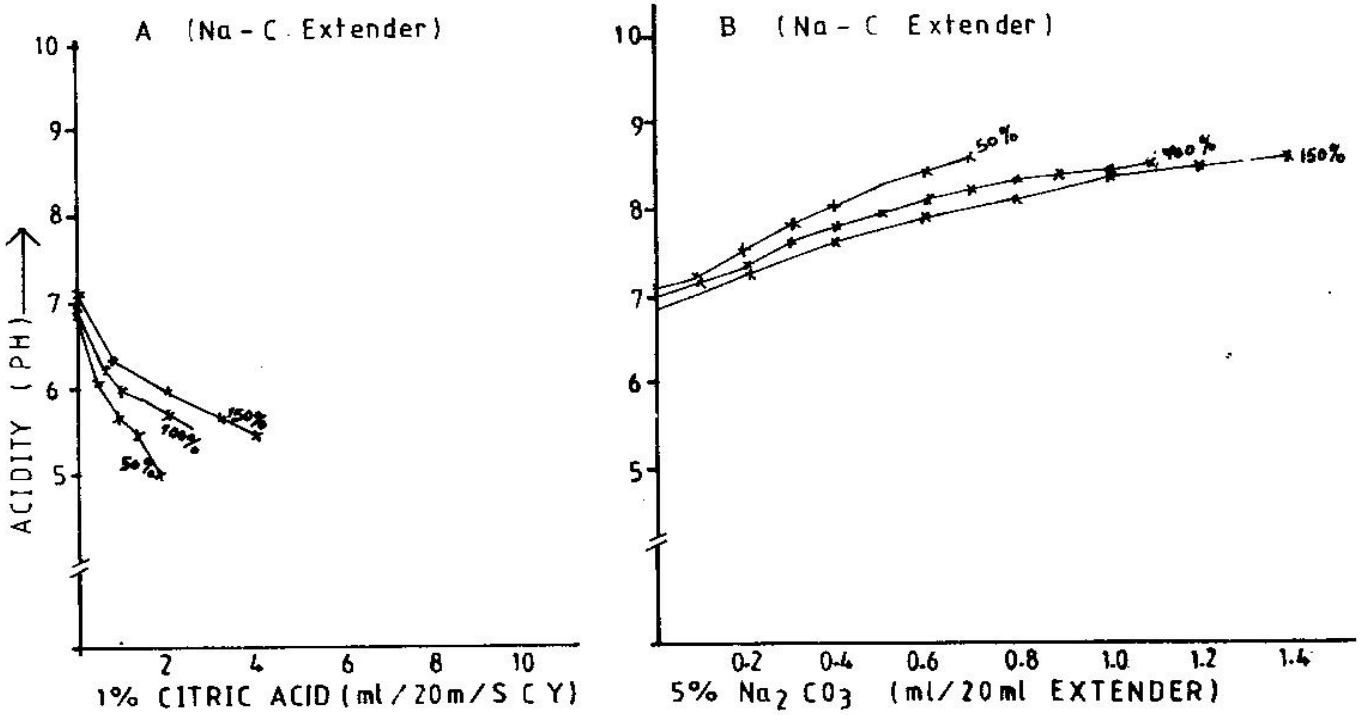

Fig. 2: The effect of adding one percent citric acid solution to 20ml. Sodium-citrate-glycine-yolk extender (A) and five percent sodium carbonate solution to 20ml. Sodium-citrate-glycine-yolk extender (B) on the acidity of the extender at different concentrations.

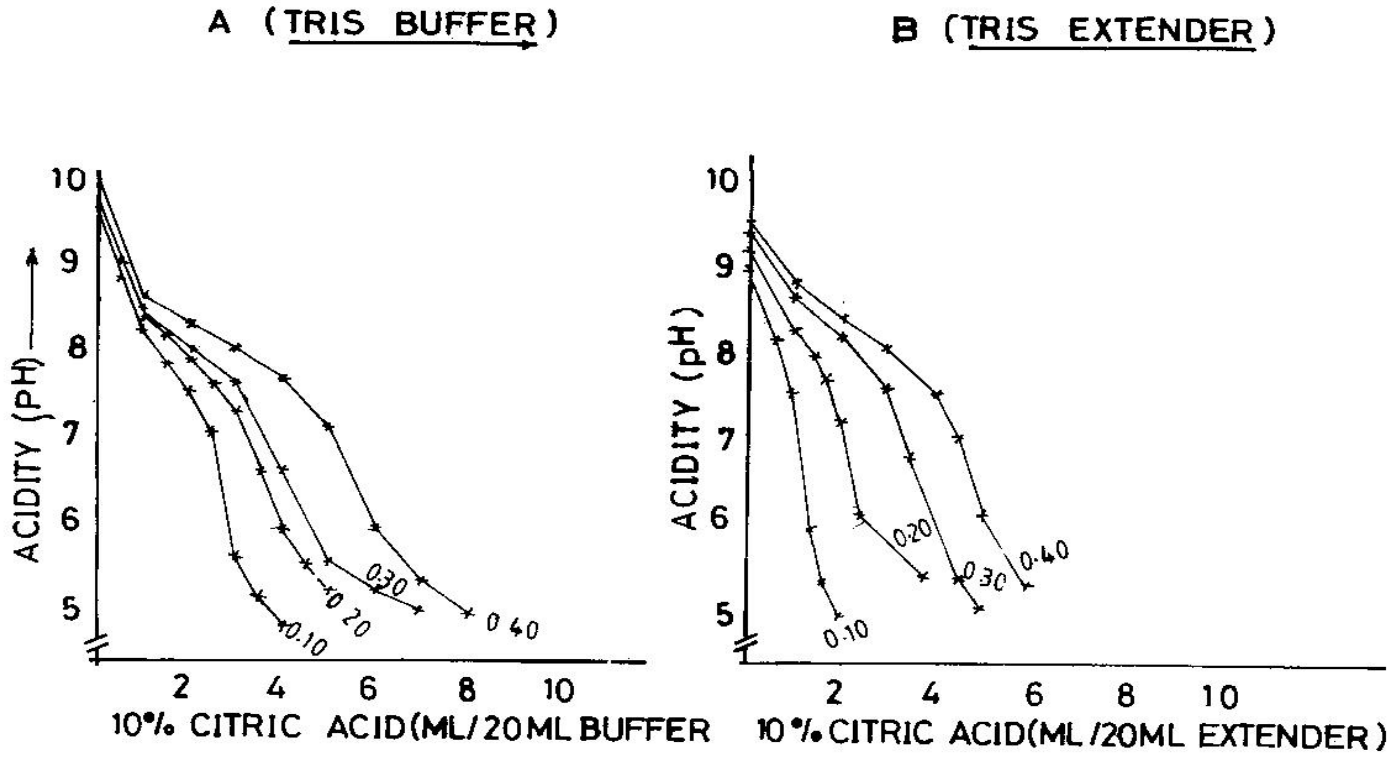

Fig. 3: The effect of adding ten percent citric acid to 20ml. Tris buffer (A)and ten percent citric acid to 20ml. Tris-yolk extender (B) on the acidity of the buffer and the extender at different concentrations.

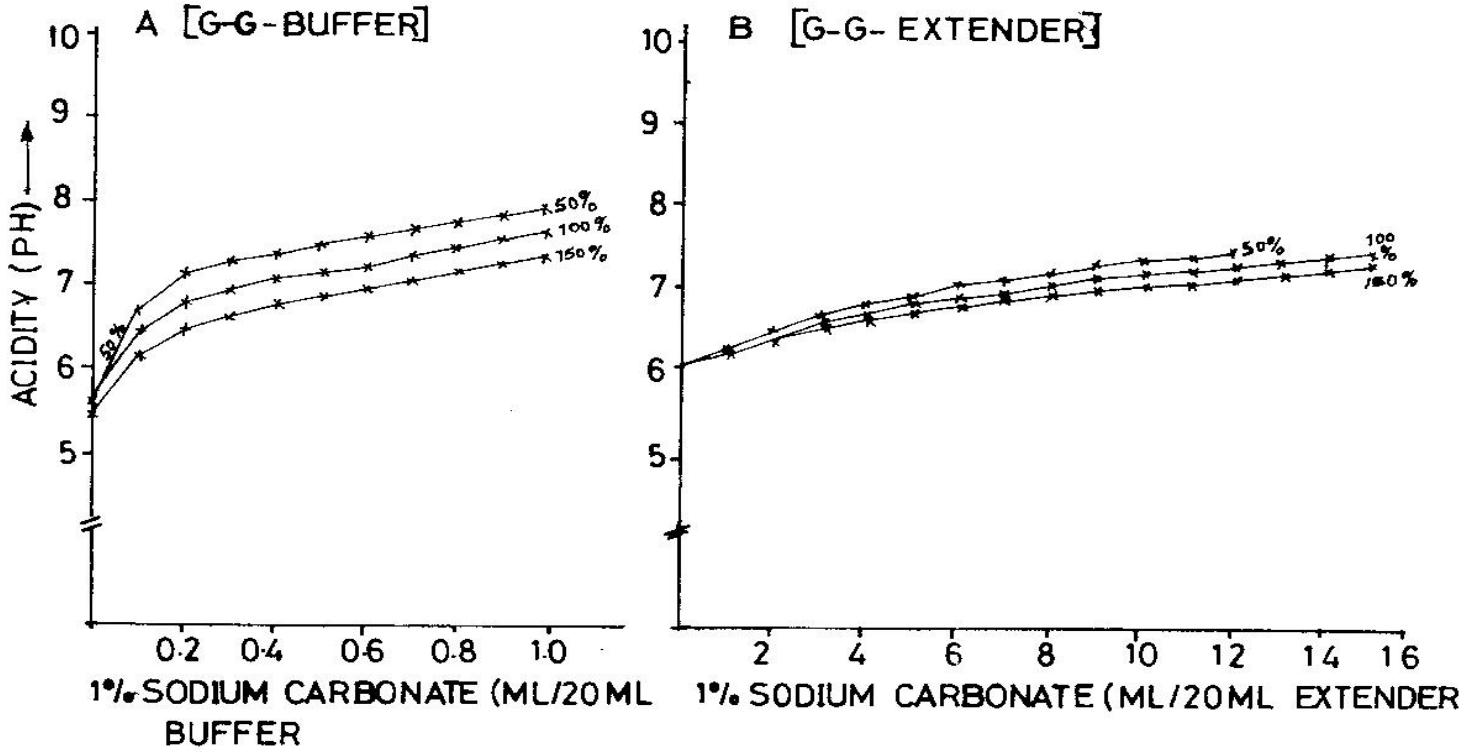

Fig. 4: The effect of adding one percent sodium carbonate solution to 20ml. Glucose-glycine buffer (A) and one percent sodium carbonate to 20ml. Glucose-glycine-yolk extender (B), on the acidity of buffer and extender at different concentrations.

(TCM BUFFER & EXTENDER)

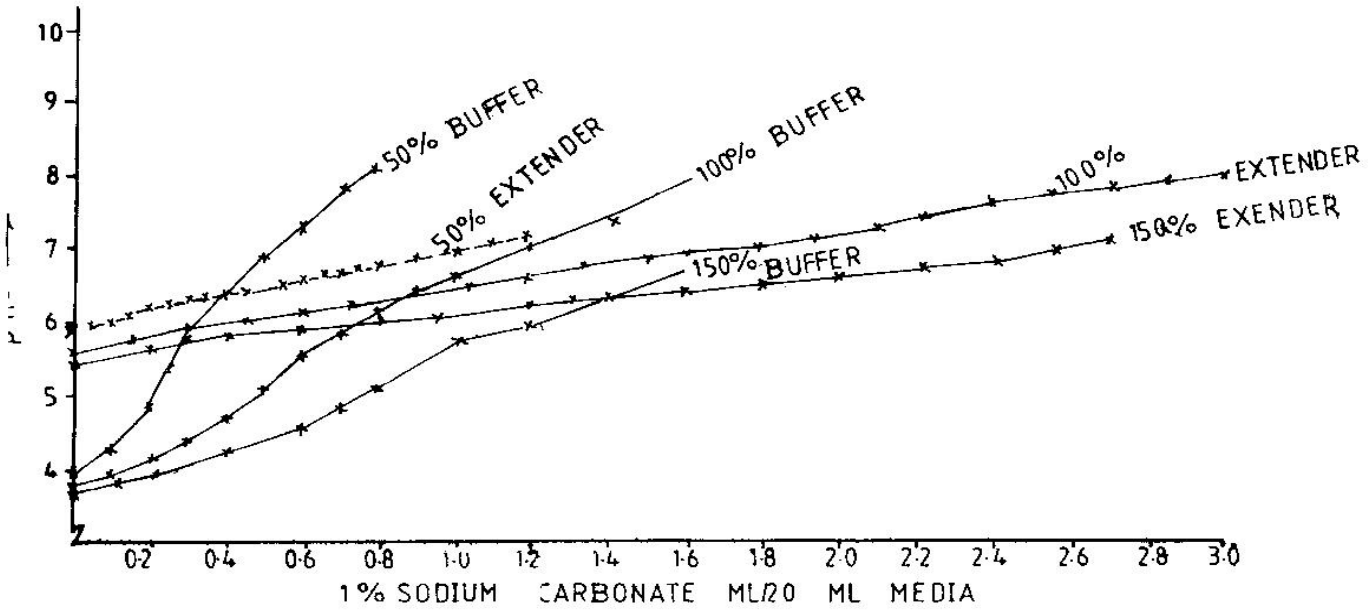

Fig. 5 : The effect of addling one percent sodium carbonate solution to 20ml. TCM Buffer and extender on the acidity of the buffer and extender at different concentrations.

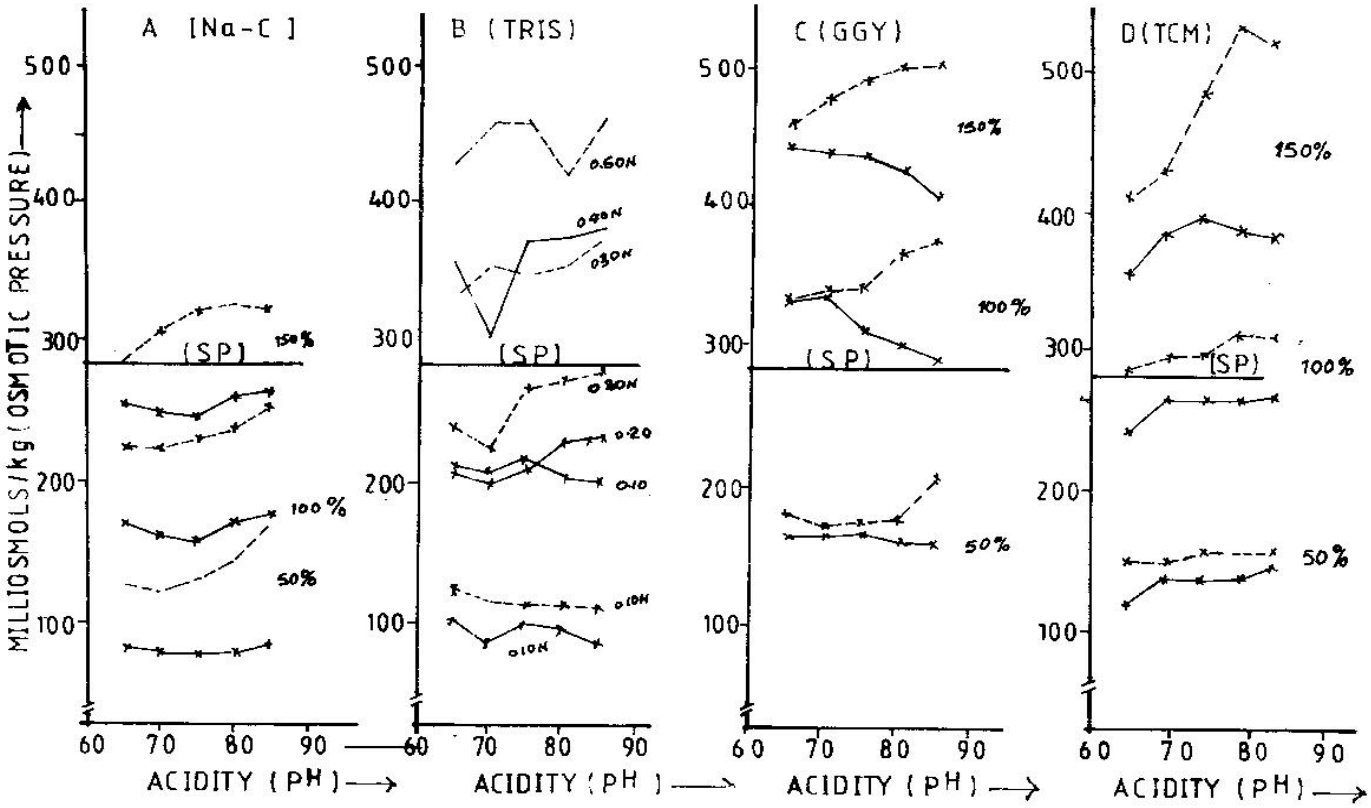

Fig 6 The osmotic pressure (mOsm/kg) of buffers _____ and extenders -------- at different acidity levels in relation to the mean osmotic pressure of the (S.P. Seminal Plasma (283±8mOsm) of boar semen.

Table 1
Composition of various buffers and extenders ingredients (per 100ml)

		Single Strength				Double Strength-			
Buffer		Na-C	Tris	B-G	TCM	Na = C	Tris	G-G	TCM
Sodium Dihydrate	(g)	10				20.0			
Tris (hydrogeymethyl) aminomethane	(g)		23.0				46.0		
Glycine	(g)	5.0		6.0				12.0	
Glucose	(g)			45.0		10.0		90.0	
TCM 199	(ml)				50.00				100.0
Citric Acid	(g)		12.0				24.0		
Fructose	(g)		13.0				26.0		
Auilfanilamide	(g)	3.0				6.0			
Boiling Distilled water	(ml)	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Extender 2									
Buffer (ml/100ml)									
Egg Yolk (ml/100m,l)		–	–	–	–	–	–	–	–
Peniciline (i.u./100ml)		–	–	–	–	–	–	–	–
Streptomycin (mg/100ml)		–	–	–	–	–	–	–	–
Boiling Distilled Water (ml)		–	–	–	–	–	–	–	–

Table 2
Mean Acidity and Osmoliarity and the regression equations of the extenders.*

Ingredients	Levels	*Acidity (ph)* Mean ± SEM	r	*Osmolarity* Y = a + b	Mean ± SEM	RSP (%)
Na-C	50%	2.6 ± 0.4	-0.96	7.19-1.08X	135.8 ± 20.0	47.9
	75%	5.7 ± 0.5	-0.92	7.11-1.03X	109.6 ± 21.2	38.7
	100%	8.01 ± 0.4	-0.93	12.48-1.85X	231.8 ± 24.4	81.9
	125%	10.17 ± 0.4	-0.96	15.72-2.33X	301.4 ± 14.3	106.5
	150%	15.52 ± 0.4	-0.95	17.41-2.55X	331.8 ± 9.9	117.2
Tris	0.10M	4.2 ± 1.48	-0.98	4.10-0.44X	114.8 ± 4.8	40.6
	0.15M	8.7 ± 1.42	-0.97	5.46-0.57X	195.0 ± 14.2	68.9
	0.20M	10.3 ± 1.17	-0.96	6.98-0.73X	224.2 ± 10.6	79.2
	0.25M	19.8 ± 1.55	-0.98	9.21-0.95X	253.09.2	89.4
	0.30M	26.0 ± 1.47	-0.97	8.67-0.96X	347.4 ± 18.4	123.5
	0.35M	19.9 ± 1.55	-0.95	10.63-1.09X	368.0 ± 16.4	130.0
					445.2 ± 15.9	157.3
G-G	50%	1.73 ± 0.45	0.97	-5.21 ± 0.83X	170.0 ± 7.9	60.9
	75%	2.13 ± 0.43	0.96	-5.85 ± 0.93X	164.1 ± 15.4	58.2
	100%	3.19 ± 0.43	0.96	-6.69 ± 1.07X	352.0 ± 19.0	124.4
	125%	4.55 ± 0.41	0.96	-7.84 ± 1.23X	437.0 ± 8.4	154.4
	150%	5.37 ± 0.42	0.97	-8.25 ± 1.31X	484.0 ± 8.2	171.0
	175%	11.68 ± 0.39	0.98	-10.96 ± 1.75X	525.4 ± 17.2	189.7
TCM	50%	1.8 ± 0.41	0.99	-5.63 ± 6.95X	154.2 ± 3.7	54.4
	75%	7.2 ± 0.75	0.99	-6.01 ± 1.23X	223.3 ± 18.6	75.8
	100%	9.8 ± 0.81	0.99	-6.89 ± 1.43DX	298.4 ± 9.0	105.3
	125%	13.1 ± 0.84	0.99	-8.04 ± 1.43X	417.8 ± 14.4	147.6
	150%	22.70 ± 0.87	0.99	-8.97 ± 1.64	471.2 ± 257	166.5
175%	36.3 ± 0.85	0.99	-10.59 ± 1.96X		517.3 ± 21.8	179.5

This study provided the simple laboratory procedures for estimating the acidic and the alkaline buffering capacities of various storage media for the boar semen. It provided regression equations for calculating the citric acid and the sodium carbonate requirements for the adjustment of the desirable hydrogen ion concentration and the optimal osmotic pressure for the storage media of boar semen, and it is hoped that these methods could be so adopted, for use when storage media are to be prepared for use in an A.I. programme.

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