

DILUTION AND SHORT-TERM STORAGE OF COCK SPERMATOZOA BY INHIBITION OF MOTILITY WITH FRUCTOSE AT AMBIENT TEMPERATURE

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Summary

A simplified dilutor for cock spermatozoa at ambient temperature was achieved by adjusting the 5% concentration of fructose in isotonic saline. Motility of cock spermatozoa was arrested completely for maximum 6 hrs without affecting the survivability of spermatozoa by employing this sugar. To study the effect of high concentration of fructose on fertility, sperm were inseminated with or without fructose at different hrs. Fructose from semen samples was removed by centrifugation. High fertility obtained in the hens inseminated with fructose free sperm (washed). In addition, washed sperm maintained the 85.00% fertility for 6 hrs in winter season (17-21°C) and 82.67% fertility for 3 hrs in summer season (31-35°C). Whereas control groups showed 47.33 and 25.33% fertility in winter and summer season respectively. No significant difference was found in percent motility and live counts between the control and washed experimental groups during winter season. However, these measures differed significantly in summer. Washing of cock spermatozoa more than once, high speed centrifugation and more duration for centrifugation proved harmful to fertility. It may be concluded that fructose (5%) can be used as a motility or metabolic inhibitor of spermatozoa for short-term storage of cock semen at ambient temperatures.

(Key Words : Cock, Spermatozoa, Dilutor, Fructose, Storage, Fertility)

Introduction

Carbohydrate metabolism has been studied most extensively as the source of energy for maintaining the activities of spermatozoa under *in vitro* conditions. In diluents, sugars may perform various functions, they may provide an energy substrate for promoting sperm motility or may be used to maintain the osmotic pressure of the diluent. Lapwood and Martin (1966) reported the inclusion of fructose in a extender and advocated that this sugar is most beneficial for the storage of ram spermatozoa at 37°C as compared to 5°C. Shaffner (1942) obtained 25% fertility from the fowl spermatozoa that were partially dehydrated with a sugar solution. Sexton (1977) stored fowl semen in a fructose based dilutor for 24 hrs at 5°C with little loss of fertilizing ability of spermatozoa. However, this ability of cock spermatozoa is more difficult to retained *in vitro* at ambient temperature (5-20°C) beyond 30 to 45 min (Lake and Stewart, 1978). A rapid loss in fertilizing power of spermatozoa was reported

when incubated at 41°C (Schindler et al., 1955; and Ashizawa et al., 1976). Control of the metabolic, motility and fertility functions of the spermatozoa is not solely dependent on the temperature and can be regulated by other chemical means including carbondioxide (Salisbury et al., 1985).

Diluent and short-term storage of cock semen at ambient temperature is still in the experimental phase and is not yet sufficiently advanced for practical application in the poultry. Besides, the composition of chicken semen diluents available so far is very complex and required many ingredients in their composition (Sexton, 1977; Lake and Ravie, 1979). Therefore, the objective of the present study was to develop a very simple dilutor with minimum ingredients at ambient temperature for short-term preservation of cock semen. Fructose is selected as the main component of this dilutor since, chicken spermatozoa utilize fructose more efficiently than other sugars (Sexton, 1974).

Materials and Methods

The diluent was prepared aseptically by autoclaving all glassware and distilled water. Subsequently, the 5%

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concentration of fructose (Kemphasol Company, Bombay) solution was freshly prepared in isotonic saline. Semen was collected from adult healthy broiler cocks by the abdominal massage method (Burrows and Quinn, 1937) and care was taken to avoid contamination of semen with transparent fluid, urates and feces. Pooled semen was equally divided into three groups. First group of semen served as the control and diluted with 3 parts (1:3) of saline and maintained at ambient temperature either 17-21 °C during winter or 31-35 °C during summer season for 0, 3 and 6 hrs. The second and third groups termed as the experimental groups and extended with fructose based dilutor in the similar manner. At different hrs (0, 3 and 6), the semen samples of third group were centrifuged at 2,500 rpm for 6 min to remove the fructose from the spermatozoa. Pallet of sperm dissolved in saline and centrifuged and finally sperm pallet divided into two equal portions. Portion A pallet reconstituted to its original volume with 25% seminal plasma in saline whereas portion B pallet reconstituted in saline alone. Under the present study, the experiments were conducted with only 5 % concentration of fructose. We selected this as an optimum dose level based upon our preliminary tests carried out using 1, 2, 3, 4, 6 and 7% concentration of fructose.

Motility of spermatozoa was examined microscopically (Wheeler and Andrews, 1943). The percentage of live spermatozoa were calculated after preparing smears and staining them with eosin and nigrosin according to the method described by Lake and Stewart (1978). To estimate the fertilizing ability of spermatozoa, hens were inseminated with a dose containing 60×10^6 spermatozoa obtained from control as well as experimental groups. The

fertility of semen samples was examined by incubating the eggs laid by the hens 2-10 days after the intravaginal insemination of diluted semen. The eggs were assessed by candling after 6 days of incubation to determine fertilization and questionable eggs were broken out and observed macroscopically to confirm the same.

Significant differences of the means of experimental groups were compared statistically with the means of controls by using student's t-test.

Results

In the present study, various concentrations of fructose were employed to arrest the motility and metabolism of chicken spermatozoa. Only 5 percent concentration of fructose in saline revealed the best results. This concentration of fructose maintained the spermatozoa immotile throughout the study period until it was washed or removed by centrifugation. No significant difference was found in motility between control and washed sperm (fructose free) group during winter at different hrs whereas it was significantly ($p < 0.01$) higher exhibited by washed sperm group in summer season (table 1). Percent sperm survival was found maximal ($p < 0.01$) at 6 hrs storage of semen at high ambient temperature in experimental group as compared to control. However, no significant difference in survivability of spermatozoa was noticed between the groups of washed and unwashed sperm (table 2).

A declining trend of fertility was observed in control as well as in unwashed group of sperm throughout the investigation period in comparison to the washed sperm. Fertilizing ability of washed sperm was recorded significantly ($p < 0.05$) higher than other groups in both

TABLE 1. EFFECT OF FRUCTOSE (5%) ON MOTILITY (MEANS \pm STANDARD DEVIATION OF 9 REPLICATION) AT AMBIENT TEMPERATURES AND DIFFERENT HRS

Season	Ambient temperature	Storage period (hrs)	% Motility		
			Control group	Experimental group	
				Washed sperm	Unwashed sperm
Winter	17-21 °C	0	89.33 \pm 2.37	86.40 \pm 2.71	0
		3	85.11 \pm 2.77	79.88 \pm 2.71	0
		6	84.80 \pm 2.61	80.09 \pm 3.13	0
Summer	31-35 °C	0	91.30 \pm 2.10	88.10 \pm 3.45	0
		3	68.65 \pm 2.60	83.72 \pm 3.05**	0
		6	44.68 \pm 2.85	72.60 \pm 2.79**	0

Significant differences of the means of fructose free sperm (washed) were compared statistically with controls by using Student's t-test (** $p < 0.01$).

TABLE 2. EFFECT OF FRUCTOSE (5%) ON PERCENT LIVE COUNTS (MEANS \pm STANDARD DEVIATION OF 9 REPLICATION) AT AMBIENT TEMPERATURES AND DIFFERENT HRS

Season	Ambient temperature	Storage period (hrs)	% Live counts		
			Control group	Experimental group	
				Washed sperm	Unwashed sperm
Winter	17-21°C	0	94.10 \pm 3.45	88.20 \pm 4.70	92.39 \pm 5.30
		3	90.07 \pm 3.30	83.20 \pm 4.50	86.77 \pm 4.95
		6	90.27 \pm 2.77	81.95 \pm 4.36	83.65 \pm 5.10
Summer	31-35°C	0	95.17 \pm 3.85	89.05 \pm 5.75	90.33 \pm 4.70
		3	74.66 \pm 3.95	85.10 \pm 5.34	84.55 \pm 4.45
		6	49.85 \pm 3.80	75.66 \pm 4.90**	77.17 \pm 6.70**

Means of both the experimental groups compared statistically with the means of control group by using of Student's t-test (** p < 0.01).

TABLE 3. EFFECT OF FRUCTOSE (5%) ON FERTILITY (MEANS \pm STANDARD DEVIATION OF 3 REPLICATION) AT AMBIENT TEMPERATURES AND DIFFERENT HRS

Season	Ambient temperature	Storage period (hrs)	% Fertility		
			Control group	Experimental group	
				Washed sperm	Unwashed sperm
Winter	17-21°C	0	91.33 \pm 2.34	86.67 \pm 2.41	59.33 \pm 4.06*
		3	65.00 \pm 4.20	87.33 \pm 2.19*	52.67 \pm 5.49
		6	47.33 \pm 4.10	85.00 \pm 2.89*	37.00 \pm 3.47
Summer	31-35°C	0	89.00 \pm 1.53	88.05 \pm 3.06	42.11 \pm 3.79**
		3	25.33 \pm 4.06	82.67 \pm 3.18**	30.33 \pm 2.91
		6	10.67 \pm 3.48	64.00 \pm 5.20*	18.00 \pm 2.65

Significant differences of the means of experimental groups were compared statistically with controls by using Student's t-test (* p < 0.05, ** p < 0.01).

the seasons (table 3). Overall, the washed spermatozoa proved to possess better fertilizing capacity than unwashed and control groups irrespective of storage period and season. It has also been observed that artificial insemination with centrifuged sperm (washed) reconstituted in 25% seminal plasma have better fertilizing ability (table 3) than those reconstituted in saline alone (data not shown). The average fertility was found high when sperm were subjected to single washing by employing centrifugation at 2,500 rpm for 6 min.

Discussion

In the present study, we used a high concentration of fructose (5%) for the complete inhibition of the motility (table 1) and metabolism of cock spermatozoa and

followed reversal of these traits after different hrs at ambient temperature. We failed to achieve these results by employing the other concentration of fructose. This may be due to the changes in tonicity or osmolarity beyond the limits. Actually spermatozoa are capable of extensive changes in size depending upon the tonicity of the medium in which they exist (Drevius and Eriksson, 1966; Bredderman and Foote, 1969). Chicken spermatozoa retained their full fertilizing ability in media having wide ranges both osmolarity and pH levels (Yamane et al., 1962; Hobbs and Harris, 1963; Bogdonoff and Shaffner, 1954). Even the cock spermatozoa were noted as resistant to cold shock unlike that of mammals (Mohan et al., 1991, 1992a). Keeping in view such structural resistance of cock spermatozoa, the tonicity or osmolarity may not be having the adverse effects relatively within a wide

range on the survivability and fertilizing ability of spermatozoa (table 2 and 3).

The exact biochemical mechanisms for the inhibition of the motility of spermatozoa with the treatment of fructose are not clear but probably involve the glycolytic pathways with the ultimate formation of CO_2 from the fructose utilization by the spermatozoa (White et al., 1954). It is well established that CO_2 markedly caused drop in sperm metabolism and subsequently arrest the motility of spermatozoa (Salisbury et al., 1985). Considering this principle, external gassing of some specific dilutor with CO_2 is widely used and it serves a biochemical means to control the spermatozoa metabolism and thereby extend the life of spermatozoa several days at ambient temperature (Salisbury et al., 1985). Cornell University Extender (CUE) contained glycine and does not require external gassing with CO_2 have given consistently good sperm survival at 25°C . In this context, glycine may promote the desirable production of CO_2 as a result of sperm metabolism (Salisbury et al., 1985). On the basis of foregoing discussion, it may be believed that the ability of fructose based extender to maintain high fertility over a period of several hrs (table 3) may be due to the CO_2 production from the fructose which in turn suppress the metabolism of spermatozoa and preserves sperm survival at ambient temperature (table 2) similar to the CUE extender. Besides, Sexton (1974) also reported that chicken spermatozoa depends primarily on glycolytic pathways for survival under *in vitro* storage.

The present study indicated the better fertilizing ability of washed spermatozoa contained 25% seminal plasma in saline as compared to the washed sperm inseminated with saline alone. This could be due to the existence of low molecular weight sperm motility stimulating factors present in seminal plasma (Ashizawa and Wishart, 1987). The present finding received the support from the report of Lake and Ravie (1988).

As compared to washed sperm, poor fertility was observed in unwashed sperm (table 3). However, apparently no significant difference was recorded in the survivability of spermatozoa between these two groups (table 2). This may be due to the fact that higher concentration of fructose may causes the partially contraceptive action on spermatozoa at ambient temperature. This view was supported by removing or washing the fructose from spermatozoa and which in turn yielded the higher fertility (table 3).

Semen was centrifuged at 2,500 rpm for 6 min for washig or removing the fructose from spermatozoa. An increase in the number of washing times, high speed centrifugation as well as more duration of centrifugation

were found harmful to the fertilizing ability of cock spermatozoa which is in agreement with the previous studies on motility of fowl psermatozoa (Wales and White, 1958; Mohan et al., 1992b). It is therefore suggested to be careful at this front to get the optimum results.

The most important finding in these studies was that fertilizing ability of spermatozoa could be obtained by inhibiting the motility of chicken sperm with high concentration (5%) of fructose. There were indications that the effect of high concentration of fructose under the conditions of the experiment was to regulate the metabolism and thereby influence the maintenance of the fertilizing ability of spermatozoa. The adaptability of this technique may have wide practical application for short-term preservation of chicken spermatozoa. Considering these results, higher fertility after storage and further extension of the storage period at ambient temperature could be possible with various improvements in the technique. Further research on these aspect would be very helpful to unravel many new facts of the basic metabolism of spermatozoa.

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