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RABBIT SEMEN METABOLISM

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SUMMARY-

The present investigation aimed to assess the sperm metabolism in rabbits. Six mature NZW bucks were used . Ejaculates were extended in sodium citrate buffer and incubated at 37° C for 3hours. Significant decrease in the number of motile and viable sperm cells was noticed with the advancement of incubation. The time required for reduction of methylene blue was prolonged during the 2nd and 3rd hours of incubation. The lactate dehydrogenase (LDH) activity was significantly higher in the second than in the first ejaculate. LDH activity was significantly increased during the lst hour followed by a gradual decrease with the advancement of incubation. The rate of fructose utilization was greatly affected by the period of incubation and sequence of ejaculation. It decreased during the 2nd and 3rd hours of incubation and in the first ejaculate. The correlation coefficients between some of the parameters studied were also calculated.

Key words: Rabbits, Sperm metabolism, Methylene blue reduction time, Lactate dehydrogenase activity, Fructose utilization.

INTRODUCTION

There is abundant literature on the metabolic behaviour of spermatozoa of several mammalian species *(Wales and Wallace, 1964; Wallace and Wales, 1964; O'Shea and Wales, 1965; Murdoch and White, 1966 a & b and El-Menoufy, 1974).* However work on rabbit spermatozoa is rather scanty *(Wales and Wallace, 1964 and Murdoch and White, 1966a).*

Exposure of semen of farm animals to cold (0°c) resulted in a complete death of spermatozoa within few minutes *(Salisbury et al., 1985).* However, rabbit spermatozoa are highly resistant to cold shock *(Mann and Lutwack-Mann,1981 and Addel-Ghaffar et al., 1993).* The rabbit sperm resistance was attributed to its high content of cholesterol and phospholipids *(Abdel-Ghaffar et al., 1993).* These chemical compounds seemed to be essential to maintain the integral structure of the sperm cell membrane *(Mann and Lutwack-Mann,1981).* The great difference in resistance to cold between rabbit and other farm animals semen makes us very willing to study some metabolic activity of rabbit spermatozoa. Therefore the present work aimed to explore the effect of number of motile and viable spermatozoa on methylene blue reduction (M B R) time, LDH activity and fructolytic activity of rabbit semen.

MATERIALS AND METHODS

Six-healthy New Zealand. White bucks (8-10 months old and about 3.5kg body weight) were used in this work. Semen was collected once weekly by means of an artificial vagina for a total of six weeks . Two ejaculates were obtained at each collection and were assessed according to *Zemjanis (1962).* The semen samples were diluted 1:10 with sodium citrate buffer 2.9% and incubated at 37°C for 3 hours. The total number (No.) of motile and viable sperm cells and MBR time were recorded at 0, 1, 2 and 3hours of incubation. LDH activities *(McQueen, 1972)* and fructose utilization *(Mann, 1948)* were also estimated at the differnt times of incubation.

Statistical analysis of the data was carried out using the statistical analysis system *(SAS, 1987).*

RESULTS AND DISCUSSION

Motile and viable spermatozoa

There was a significant ($p < 0.01$) decrease in the number of motile and viable sperm cells in the first than the second ejaculate. This was appeared to be more pronounced with the advancement of incubation time (Table 1 and Fig. 1, 2).

This may be due to the fact that the second ejaculate was better than the first one *(El-Sherbiny, 1987; El-Sheikh, 1991 and Abdel-Ghaffar et al., 1993).* However, there was a relationship between the metabolic process and other sperm characteristics in particular sperm cell concentration, motility and survival *(Salisbury and Lodje, 1962; Mann, 1964 and Mann and Lutwack; Mann, 1981).*

Table (1):Effect of sequance of ejaculation and incubation period on the number of motile sperm and viable sperm; MBR-time; LDH -activity and fructose content of rabbit semen.

Item.	N	Motile sperm ($X10^6$/ml)	Viable sperm ($X10^6$/ml)	MBR-time (min.)	LDH-activity (U/100ml)	Fructos content (mg/100 ml)
Effect of ejaculation.						
a- First ejaculate.	144	245.9 ± 3.3^{b}	278.4 ± 3.7^{b}	13.3 ± 0.3^{a}	285.6 ± 6.3^{b}	240.9 ± 5.0^{a}
b- Second ejaculate.	144	273.0 ± 4.1^{a}	307.2 ± 4.3^{a}	11.7 ± 0.3^{b}	361.8 ± 7.4^{a}	220.5 ± 5.6^{b}
Effect of incubation period						
a- 0-hour.	72	288.8 ± 4.6^{a}	327.9 ± 5.1^{a}	9.5 ± 0.1^{d}	320.0 ± 5.1^{b}	309.6 ± 3.2^{a}
b- 1- hour.	72	272.2 ± 5.2^{b}	304.6 ± 5.3^{b}	10.7 ± 0.2^{c}	391.4 ± 5.9^{a}	252.0 ± 3.3^{b}
c- 2- hour.	72	247.0 ± 5.0^{c}	279.2 ± 5.1^{c}	13.3 ± 0.2^{b}	323.9 ± 6.7^{b}	202.0 ± 3.7^{c}
d- 3- hour.	72	229.7 ± 4.7^{d}	259.6 ± 4.8^{d}	16.6 ± 0.4^{a}	259.4 ± 6.4^{c}	159.2 ± 4.2^{d}
Ejaculate xincubation period Interaction						
a- Ist ejac. x o-hour.	36	272.6 ± 5.4^{bc}	312.0 ± 6.4^{bc}	9.7 ± 0.1^{e}	287.5 ± 3.5^{c}	315.7 ± 4.2^{a}
b- Ist ejac. x 1-hour.	36	257.7 ± 6.9^{cd}	289.1 ± 7.0^{de}	11.2 ± 0.2^{d}	352.8 ± 4.0^{b}	260.6 ± 4.2^{b}
c-Ist ejac. x 2-hour.	36	231.6 ± 5.6^{e}	$263..0 \pm 6.1^{fj}$	14.2 ± 0.2^{b}	281.4 ± 5.3^{c}	214.1 ± 4.5^{d}
d- Ist ejac. x 3- hour.	36	221.7 ± 5.1^{e}	249.6 ± 5.5^{j}	18.1 ± 0.4^{a}	220.6 ± 4.8^{d}	173.2 ± 5.1^{f}
e- 2nd ejac. x 0-hour.	36	305.0 ± 6.6^{a}	343.9 ± 7.3^{a}	9.2 ± 0.1^{f}	352.5 ± 5.6^{b}	303.6 ± 4.7^{a}
f- 2nd ejac. x 1-hour.	36	286.7 ± 7.0^{ab}	320.0 ± 7.1^{b}	10.1 ± 0.2^{e}	430.0 ± 7.6^{a}	243.4 ± 4.8^{c}
J- 2nd ejac. x 2- hour.	36	262.4 ± 7.4^{c}	295.5 ± 7.4^{cd}	12.4 ± 0.4^{c}	366.4 ± 7.6^{b}	189.8 ± 5.1^{e}
h- 2nd ejac. x 3-hour .	36	237.7 ± 7.6^{de}	269.6 ± 7.6^{ef}	15.1 ± 0.5^{b}	298.3 ± 7.7^{c}	145.2 ± 5.8^{j}

Means within the same category in each class, with different superscripts are significantly different at level ($p < 0.05$).

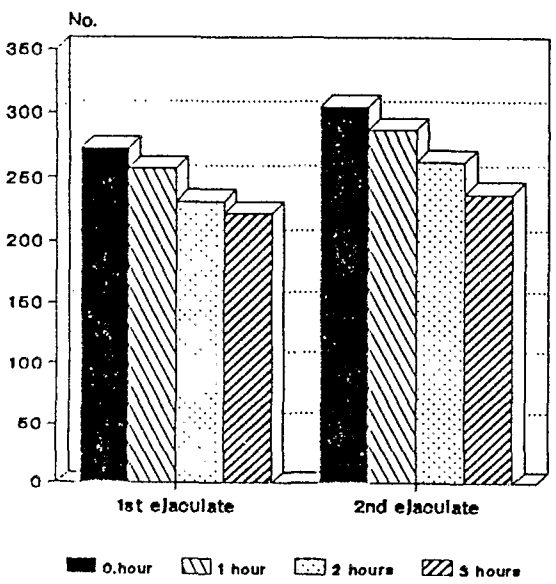


Fig.(1):Effect of sequence of ejaculation and incubation period on the motile sperm number (X 10^6/ml) of rabbit

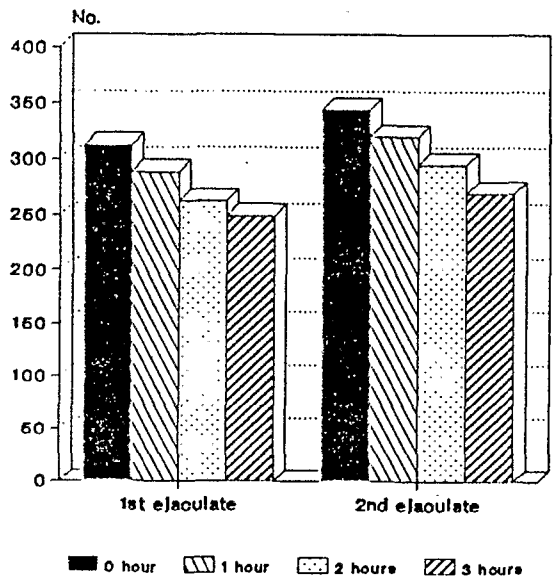


Fig. (2): Effect of sequence of ejaculation and incubation period on the viable sperm number (X 10^6/ml) of rabbit

MBR- time

Under the present experimental condition, the time needed for reduction of MB was significantly ($p < 0.01$) increased in the first than in the second ejaculates and with the advancement of incubation time (Table 1 and Fig 3). This may be attributed to the higher significant reduction in the number of motile and viable sperm cells in the first than in the second ejaculates. However, in our study the time needed for reduction of MB differed not to much from the time given by ***Pingel and Abo El-Ezz (1981) and El-Sherbiny (1987).*** The later author came in consistent with our finding, since he found a significant increase in MBR time in the first (14.6 ± 1.1 minutes) than in the second (12.4 ± 1.0 minutes) ejaculate of New Zealand white and Bouscat bucks.

LDH-activity

The present study indicated a significant ($p < 0.01$) increase of LDH activity in the second than in the first ejaculates. LDH activity increased during the first hour of incubation followed by a gradual significant decline in its activity during the second and third hours of incubation (Table 1 and Fig 4). This may be refferred to (1) the higher significant increase in the number of motile and viable sperm cells of the second than in the first ejaculates which gradually decrease with the advancement of incubation. (2) the gradual significant decline in the rate of fructose utilization with the advancement of incubation as presented herein (Table 2 and Fig. 6). Moreover, in anaerobic glycolysis, LDH is the terminative enzyme in the sequence of reaction that promote the breakdown of sugars to lactate ***(Tuli and Signh, 1980).*** However, as shown in (Table 1 and Fig. 4) LDH activities in rabbit semen for the first (287.5 U/100ml) and second (352.5 U/100ml) ejaculates appeared to be higher than for bovine (2.026 ± 0.151 U/ml, ***Stallcup and Hayden.,1960)*** and buffalo semen (144.6 ± 15.35 U/100ml,***Tuli and Singh., 1980)*** and lower than that observed (560.0 U/100ml, ***Macleod and Wroblewski, 1958)***) for human semen. These findings may be confirmed the high resistance of rabbit ***(Mann and Lutwack-Mann, 1981 and Abdel-Ghaffar et al., 1993)*** and human ***(Mann and lutwack-Mann, 1981)*** spermatozoa against cold shock.

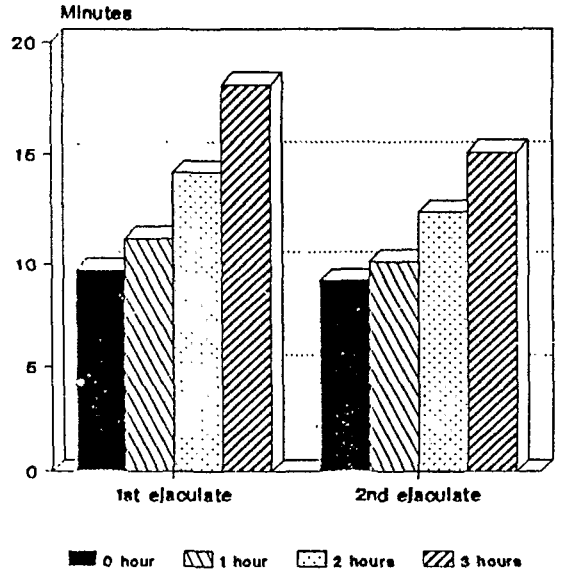


Fig. (3): Effect of sequence of ejaculation and incubation period on the MBR-time (minutes) of rabbit semen.

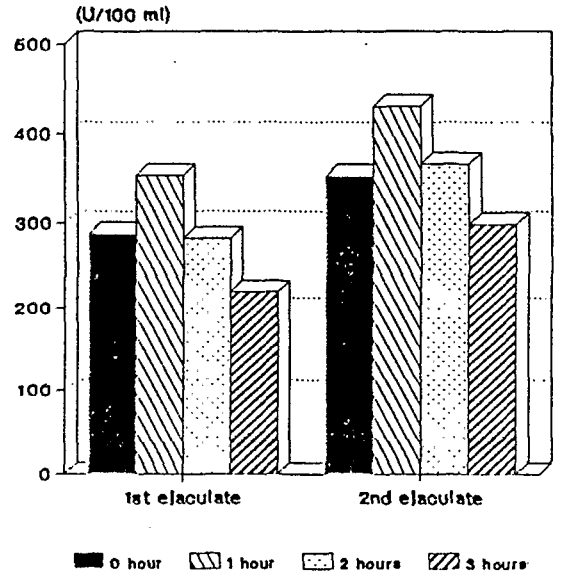


Fig. (4): Effect of sequence of ejaculation and incubation period on the LDH-activity (U/100 ml) of rabbit semen.

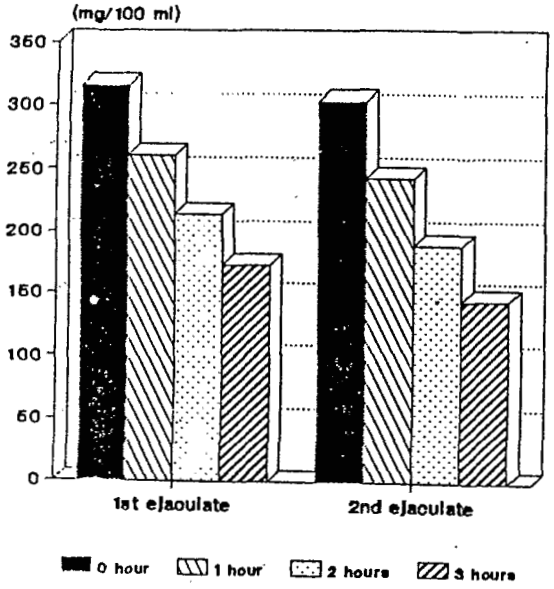
The rate of fructose utilization

Data presented in (Tables 1, 2 and Fig. 5,6 & 7) revealed a significant ($p < 0.01$) decline in the fructose content, fructose utilization and fructolysis index in the second than in the first ejaculates. This appeared to be more rapidly with the advancement of incubation. This may be returned to (1) The significant elevation in the number of motile and viable sperm cells in the second than in the first ejaculates which consumed a higher amount of fructose.(2) The higher concentration of fructose in the first ejaculate is depressing the viability of spermatozoa *(El-Sheikh and Mahmoud, 1967).* (3) The utilization of fructose increased by prolonging the incubation period *(Hopwood et al., 1956; Black-Shaw et al., 1957; Mixner et al., 1957; El-Sheikh and Mahmoud, 1967 and El-Sharabasy, 1974).* Moreover, there is evidence of relationship between seminal fructose and fertility *(Bishop et al., 1954 and Erb et al., 1955).* The rate of fructolysis is a satisfactory tool for prediction of fertility *(Hopwood et al., 1956)* and the fructolytic test provides an indication of testosteron activity of the bull *(Mann and Parsons, 1947).* On the other hand there was a thershold of sperm cell concentration, above which any increase can cause a reduction in fructose utilization *(Mann, 1964; El-Alamy, 1973 and El-Sharabasy, 1974).* Moreover, the higher level of initial fructose content in semen caused an increase in the rate of fructose utilization at all incubation periods in bovine *(Freund et al., 1957; Freund and Murphree, 1959 and Nashed et al., 1964),* human *(Macleod and Freund, 1958)* and rabbit *(El-Sharabasy, 1974).*

Table (2): Effect of sequence of ejaculation and incubation period on the rate of fructose consmption and fructolysis Index of rabbit semen.

Item	N	Fructose consumption (mg%)	Fructolysis index (mg)
Effect of ejaculation :			
a- First ejaculate	108	47.5 ± 1.0^{b}	1.26 ± 0.02^{b}
b- Second ejaculate	108	52.8 ± 0.9^{a}	1.36 ± 0.02^{a}
Effect of incubation period :			
a- 0 - 60 minutes	72	57.6 ± 1.2^{a}	1.49 ± 0.02^{a}
b- 60 - 120 minutes	72	50.0 ± 0.9^{b}	1.32 ± 0.02^{b}
c- 120-180 minutes	72	42.7 ± 0.8^{c}	1.12 ± 0.02^{c}
Ejaculate x incubation period interaction :			
a- 1st ejac. x 0-60 min.	36	55.1 ± 2.0^{b}	1.45 ± 0.02^{b}
b- 1st ejac. x 60-120 min.	36	46.5 ± 0.9^{c}	1.25 ± 0.02^{d}
c- 1st ejac. x 120-180 min.	36	40.9 ± 1.0^{d}	1.10 ± 0.02^{e}
d- 2nd ejac. x 0-60 min.	36	60.1 ± 1.3^{a}	1.54 ± 0.02^{a}
e- 2nd ejac. x 60-120 min	36	53.6 ± 1.2^{b}	1.39 ± 0.02^{c}
f- 2nd ejac. x 120-180 min.	36	44.6 ± 1.2^{c}	1.15 ± 0.02^{e}

Means within the same category in each class with differentsuperscripts are significantly different at level ($p < 0.05$).



Fig(5):Effect of sequence of ejaculation and incubation period on the fructose content (mg/100 ml) of rabbit semen.

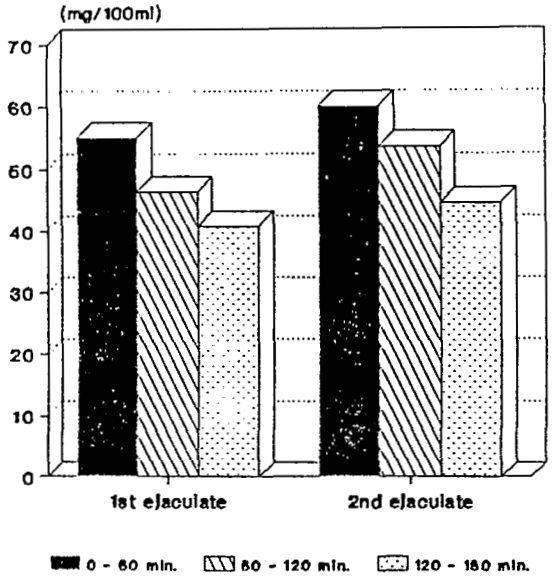


Fig.(6): Effect of sequence of ejaculation and incubation period on the rate of fructose consumption of rabbit semen.

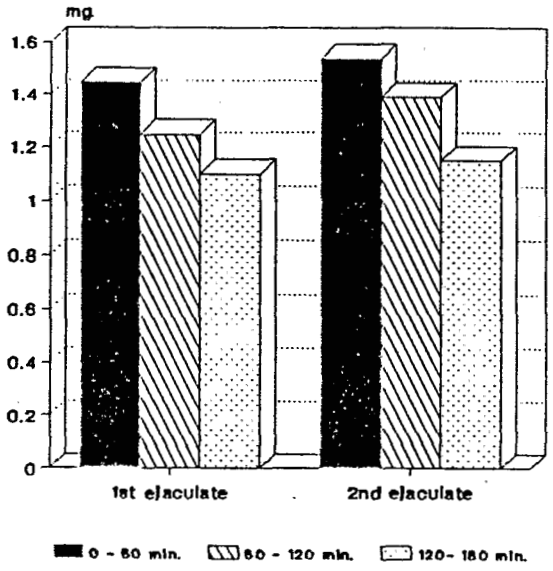


Fig.(7): Effect of sequence of ejaculation and incubation period on the fructolysis index of rabbit semen.

The phenotypic correlations

With respect to the phenotypic correlations, there was a highly significant positive correlation coefficient among the number of motile spermatozoa; viable spermatozoa; fructose content and LDH activity (Table, 3). A finding which came in accordance with the highly significant positive correlation coefficient between LDH activity associated with bull spermatozoa free of seminal plasma and with each of the sperm motility; concentration and percent live cells *(Roussal and Stallcup, 1965).* Likewise, the LDH activity of whole buffalo semen and seminal plasma was significantly positively correlated with mass activity and number of sperm cells *(Tuli and Singh, 1980).* In the same respect the rate of fructolysis was significantly correlated with both the concentration and motility of spermatozoa *(Anderson, 1946; and El-Sharabasy, 1974).* On the other hand, the

LDH activity of seminal plasm was negatively correlated with each of ejaculate volume; sperm motility, concentration and percent live cells ***(Roussal and Stallcup, 1965).*** Moreover, there was a non significant positive correlation between the LDH activity with the volume of semen and motility of spermatozoa ***(Stallcup and Hayden, 1960).*** In the mean time, there was a highly significant negative correlation between the time required for reduction of MB and the other traits studied (Table, 3) . This may be returned to the significant increase in the MBR time in the first than the second ejaculates and with the advancement of incubation. In contrast, a highly significant possitive correlation coefficient were obtained between MBR test and LDH activity in seminal plasma and spermatozoa free of seminal plasma ***(Roussal and Stallcup, 1965).*** However, this implies that the activity of LDH is directly associated with the metabolic activity of spermatozoa ***(Tuli and Singh, 1980).*** The present investigation seems to be the first to relate LDH in rabbit semen to varous traits studied.

Table (3): Phenotypic correlation among motile sperm (No.), Viable sperm (No.) MBR-time, LDH-activities and fructose content of rabbit semen.

Item	Motile sperm number	Viable sperm number	NBR-time	Fructose content
Viable-sperm number	0.984**	-----		
MBR-time	-0.676**	-0.674**	------	
Fructose content	0.229**	0.256**	-0.609**	----
LDH-activities	0.601**	0.572**	-0.684**	0.151**

N = 288

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