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Effect of L-Carnitine on Semen Characteristics of Chilled Rabbit Semen

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Abstract: The present work aimed to study the effect of L-carnitine on preservability of rabbit semen with special emphasis on DNA integrity. 12 mature New Zealand bucks were divided into two equal groups (n=6). One group was kept as control and in the other group, each animal drenched 40 mg/kg body weight in solution. Semen was collected using an artificial vagina and then diluted in tris and galap. Visual motility (48.25 vs 47.92% in tris, 62.13 vs 64.13 in galap), percent of live sperm (65.04 vs 68.54 in tris, 69.22 vs 68.81 in galap), sperm morphology (19.50 vs 16.67 in tris, 19.00 vs 15.53 in galap), DNA integrity% (72.17 vs 73.83 in tris, 77.78 vs 75.41 in galap) were assessed for both control and carnitine treated group in tris- and galap extenders respectively. In conclusion the administration of L-carnitine has ameliorated the semen characters of bucks, especially when diluted with galap than using tris-extender due to its long duration in preservation of good semen characters.

Key words: Rabbit · Carnitine · Semen · Extender(s) · DNA

INTRODUCTION

Developing and improving methods for semen preservation to provide adequate fertility rates that maintain the high production rates demanded by rabbit industry would be economically beneficial. Several studies on preservation protocols and extender composition have been carried out [1].

L-carnitine, which is an essential cofactor for fatty acid metabolism, is present in epididymal plasma and spermatozoa at a concentration of 1-63mM [2-5], while the blood plasma concentration is about 50mM. L-carnitine provides valuable support for the male reproductive system and plays an important role in sperm energy metabolism and support semen quality [6].

The most common, sperm chromatin structure assay (SCSA), uses flow cytometry to evaluate the fluorescence of sperm stained with acridine orange [7-10]. Gledhill [11] identified for the first time spermatozoa with chromatin alteration in subfertile bulls. Preservation buffalo spermatozoa causes considerable damage to DNA, motility apparatus, plasma membrane and acrosomal cap [12], leakage of intracellular enzymes [13] and thus, reduced fertility. Also, it results in sperm DNA fragmentation, DNA over condensation and apoptosis (e.g. phosphatidylserine externalization and DNA fragmentation) a s evidenced in human [14], boar [15], bull [16-17] and buffalo bulls [18]. The integrity of sperm DNA, whose stability largely depends on the integrity of the chromatin, was very important for the success of fertilization and the development of fetus and offspring [19].

It is therefore recommended that chromatin integrity should be studied as an independent complementary parameter for the better assessment of sperm quality [10]. Furthermore, changes in sperm chromatin structure and DNA integrity have been widely related to infertility in several mammalian species (boar [20]; humans [10]; bull [21]; stallion [22]. Sperm DNA integrity is important for the success of natural or assisted fertilization, including normal development of the embryo, fetus or offspring [19]. Jian-hong Hu *et al*. [23] suggested that evaluation of sperm DNA integrity, coupled with correlative and basic characteristics such as motility, acrosome integrity and membrane integrity, may aid in determining the quality of frozen boar semen.

The current work was carried out to study the effect of L-carnitine on preservability of rabbit semen with special emphasis on DNA integrity.

MATERIALS AND METHODS

Experimental Design: A total number of 12 mature white New Zealand bucks, with an average weigh of 2.50 kg, caged in metal boxes and fed commercial equilibrated ration. The water system is an automated system.

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The animals were divided into two comparable groups: control (n=6) and experimental (n=6). The treated group was drenched L-carnitine solution (40 mg/kg body weight) [24]) daily for two months under the same environmental conditions and management.

Semen Collection: Semen was collected in artificial vagina at 42°C at weekly intervals for a period of 8 weeks. Visual motility of each ejaculate was assessed at 37°C by using microscope. Sperm concentration was assessed by sperm concentration was determined in a hemocytometer in a 1:200 dilution.

Ejaculates possessing more than 60% visual motility were used individually.

Each ejaculate of individual buck was processed for cooling into Tris [25] and galap® (IMV, France) extenders.

After dilution, semen was cooled to 4°C in 2 h. The cooled semen was thawed at 37°C for 60 s before evaluation.

Sperm Functional Assays: These assays were conducted in fresh and chilled rabbit spermatozoa.

Visual Motility: A drop of thawed semen sample was placed on a pre-warmed glass slide and cover slipped. Sperm motility was evaluated at 400× magnification based on the visual estimation of the percentage of sperm possessing progressive motility and the percentage was rounded to nearest 5%.

Sperm Morphology: Total sperm morphological abnormalities (head, mid-piece and tail) of buffalo bull spermatozoa was determined using Eosin-Nigrosin.

Percent Live Sperm Cells: were evaluated using Eosin–Nigrosin staining method [26].

DNA Fragmentation Using Acridine Orange Staining: Acridine orange staining was performed according to the method of Katayose *et al*. [27]. A stock solution of 0.1% acridine orange (3, 6-bis [dimethylamino] acridine, hemi[zinc chloride]salt, Sigma Chemical Co., St. Louis, MO) was made and stored in the dark at 4°C until use. At the time of staining, a working solution was prepared by mixing 4 parts acridine orange stock solution with 16 parts 0.1 M citrate and 1 part 0.3 M Na_2HPO_4• $7H_2O$. The acridine orange final concentration of the resultant phosphate-citrate buffered solution was adjusted to 0.019% (pH 2.5). First, spermatozoa were smeared on the glass slide. After being air dried, the samples were treated with acid alcohol (methyl alcohol–glacial acetic acid _ 3:1, vol/vol) for _2 hours. Immediate preparation is necessary to prevent natural oxidation of thiols in sperm nucleoprotein and the acid alcohol was made on the day of the experiment. Immediately after air drying, approximately 1 mL of working solution was mounted on each slide glass for 5 minutes at room temperature and the samples were then washed with distilled water. The samples were observed under an epifluorescent microscope (Olympus U-PMTVC5D 00637, Olympus, Tokyo, Japan) immediately after a coverglass was put in position. A total of 100 to 200 spermatozoa were observed and classified by type as green, red, or yellow, which is the intermediate type, based on differences in their fluorescent color.

Statistics: Data were analysed using SPSS statistical program version 14.0. (2005) Analysis of variance was used to detect significance effect of treatment on semen quality after cooling with a confidence limit 95%.

RESULTS AND DISCUSSION

Our results revealed significant improvement in the quality of tris - extended semen (sperm motility, alive and sperm abnormalities ($P<0.0001$) and DNA integrity ($P<0.01$) of male rabbits supplemented with L- carnitine as shown in Table 1.

The present results exhibited significant improvement in the quality of galap extended semen (sperm motility $P<0.03$, alive sperm $P<0.05$ and sperm abnormalities $P<0.0001$) of male rabbits supplemented with L-carnitine as shown in Table 2. However, the difference in DNA integrity was non significant and after 72 hours chilling, semen quality decreased, this may be due to exhaustion of sperms as L-carnitine is a bioenergetic. These results are in agreement with Xuan *et al*. [28] who documented that maturation, respiration, motility and fertility are dependent on the progressive increase in epididymal and spermatozoa carnitine, critical for mitochondrial fatty acid oxidation, as sperm pass from the caput of the epididymis. Newsletter [29] showed that L-carnitine increased the number of viable sperm cells.

Table 1: Effect of L-carnitine on semen characteristics of preserved rabbit semen using tris-extender

		Control	Treated	Overall
Sperm motility %	Cooled semen after 2 hours	73.75±1.250	82.50*±0.90	78.13^{a}±1.36
	Cooled semen after 24 hrs	50.63±1.480	48.13±0.920	49.38^{b}±0.90
	Cooled semen after 48 hrs	20.38±1.780	13.13*±0.90	16.75^{c}±1.35
	Overall	48.25^{A}±4.63	47.92^{A}±5.93	
Alive sperm %	Cooled semen after 2 hours	82.63±1.390	89.13*±1.14	85.88^{a}±1.21
	Cooled semen after 24 hrs	62.50±0.950	74.00*±0.38	68.25^{b}±1.56
	Cooled semen after 48 hrs	50.00±1.890	42.50*±2.84	46.25^{c}±1.91
	Overall	65.04^{B}±2.92	68.54^{A}±4.17	
Abnormal sperm %	Cooled semen after 2 hours	18.00±0.780	12.00*±0.76	15.00^{b}±0.93
	Cooled semen after 24 hrs	19.00±0.380	14.00*±0.76	16.50^{b}±0.76
	Cooled semen after 48 hrs	21.50±0.950	24.00*±1.51	22.75^{a}±0.92
	Overall	19.50^{A}±0.51	16.67^{B}±1.24	
DNA integrity %	Cooled semen after 2 hours	91.50±0.570	94.00±1.510	92.75^{a}±0.84
	Cooled semen after 24 hrs	67.50±0.950	72.50*±0.93	70.00^{b}±0.91
	Cooled semen after 48 hrs	57.50±1.890	55.00±0.900	56.25^{c}±1.07
	Overall	72.17^{A}±3.01	73.83^{A}±3.43	

*LSD significant (between control and treated) at P<0.05

Different superscripts (a, b…) are significantly different within column concerning each parameter at P<0.05

Different superscripts (A, B…) are significantly different within raw concerning each parameter at P<0.05

Table 2: Effect of L-carnitine on semen characteristics of preserved rabbit semen using galap-extender

		Control	Treated	Overall
Sperm motility %	Cooled semen after 2 hours	83.25±0.80	87.13*±1.29	85.19^{a}±0.89
	Cooled semen after 24 hrs	62.13±0.93	68.50*±1.35	65.31^{b}±1.14
	Cooled semen after 48 hrs	52.50±2.50	56.50*±2.62	54.50^{c}±1.82
	Cooled semen after 72 hrs	50.63±3.83	44.38*±2.40	47.50^{d}±2.33
	Overall	62.13^{A}±2.58	64.13^{A}±2.99	
Alive sperm %	Cooled semen after 2 hours	89.50±1.02	91.38±0.96	90.44^{a}±0.72
	Cooled semen after 24 hrs	70.88±1.61	75.13*±1.64	73.00^{b}±1.24
	Cooled semen after 48 hrs	61.50±2.14	61.25±1.25	61.38^{c}±1.20
	Cooled semen after 72 hrs	55.00±3.78	47.50*±2.99	51.25^{d}±2.52
	Overall	69.22^{A}±2.59	68.81^{A}±3.06	
Abnormal sperm %	Cooled semen after 2 hours	16.00±0.38	11.00*±0.40	13.50^{c}±0.70
	Cooled semen after 24 hrs	19.00±0.30	14.50*±0.57	16.75^{b}±0.67
	Cooled semen after 48 hrs	20.75±0.41	17.00*±0.38	18.88^{a}±0.55
	Cooled semen after 72 hrs	20.25±0.45	19.63±0.71	19.94^{a}±0.41
	Overall	19.00^{A}±0.38	15.53^{B}±0.62	
DNA integrity %	Cooled semen after 2 hours	91.63±0.94	91.75±0.70	91.69^{a}±0.57
	Cooled semen after 24 hrs	82.00±1.13	80.88±0.83	81.44^{b}±0.70
	Cooled semen after 48 hrs	72.50±0.95	74.00±0.46	73.25^{c}±0.54
	Cooled semen after 72 hrs	65.00±1.89	55.00*±1.50	60.00^{d}±1.83
	Overall	77.78^{A}±1.90	75.41^{B}±2.46	

*LSD significant (between control and treated) at P<0.05

Different superscripts (a, b…) are significantly different within column concerning each parameter at P<0.05

Different superscripts (A, B…) are significantly different within raw concerning each parameter at P<0.05

The improved semen quality in L-carnitine supplemented animals is related to the bioenergy coming from the beta-oxidation of activated long-chain fatty acids in the inner mitochondrial matrix. Mayes [30] detailed that L-carnitine plays a major role as a cofactor in the transportation of free fatty acids from cytosol to the mitochondria.

Vicari and Calogero [31] showed that carnitine reduces ROS and increases sperm forward motility and viability in infertile males. Inoue *et al*. [32] and Chang *et al.* [33] revealed that L-carnitine exhibits antioxidative protective effects on mitochondria and cells against oxidative injuries by decreasing the free forms of long-chain fatty acids that damage the membrane of the mitochondria.

Semen characteristics showed significant decline with the progress of the time of chilling in tris and galap extended semen of male rabbits although these results are satisfactory to be used in artificial insemination of rabbits.

In conclusion the administration of L-carnitine has ameliorated the semen characters of bucks, especially when diluted with galap than using tris-extender due to its long duration in preservation of good semen characters.

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