COMPARATIVE STUDY OF SEMEN QUALITY AND FREE AMINO ACIDS CONTENT IN SEMINAL PLASMA BETWEEN HIGH AND LOW MOTILE SPERM RABBIT BUCKS

By

A.E. Elkomy*; M.E. El-Sbeiy and K.I. Kamel****

Arid Lands Cultivation And Development Research Institute, Mubarak City for Scientific Research and Technology Applications, Egypt**.

Animal Prod. Res. Inst., Agric. Res. Center, Ministry of Agric., Egypt**

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Summary: *The present study was aimed to investigate semen quality, seminal plasma protein and free amino acid in V-Line rabbit bucks of low (LSM) and high (HSM) sperm motility. Twenty mature males at 7-month old with average initial weight of 3.254 kg were used during winter season.*

The low sperm motility (LSM) rabbits showed a significant decline in ejaculate volume, while, sperm concentration slightly increased compared to the HSM rabbits. Total sperm out-put was significantly higher in HSM group. Seminal plasma pH significantly decreased in the HSM rabbits compared to the other group. Percentage of live and normal sperm was significantly increased in the HSM compared with the LSM group.

Seminal plasma total protein, albumin, acid phosphatase (AcP) and Alkaline phosphatase (AlP), were significantly higher in the HSM group while, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly lower than the other group. Seminal fluid of HSM rabbits had higher levels of total free amino acids to those of the LSM rabbits and the major constituent is cystein then arginine. Seminal fluid from HSM male rabbits appeared to be higher in lysine and methionine content compared to LSM rabbits, while, LSM rabbits seminal fluid contained higher amount from Glutamic acid and Histidine. Seminal plasma proteins electrophoresis showed that protein band with molecular weight 100.7 kd have high intensity in HSM rabbits than in LSM rabbits which associated with increased total motile sperm in the first group. Also, protein bands at molecular weight (78.8, 47.5, 40.7 and 36 kd) were expressed in the HSM rabbit.

INTRODUCTION

Biochemical estimates of seminal plasma are used for semen evaluation, to obtain a satisfactory semen appraisal (Mann and Lutwak-Mann, 1981). Early studies on the biochemistry of semen have been done by Mann (1964) who noted that there was evidence that amino acid and proteases present in the seminal plasma play an important role in the survival of spermatozoa. The function of seminal plasma free amino acids is not totally established although they were shown to act as fuels for the spermatozoa, to create favorable conditions for cell survival and to be probably involved in detoxifying functions (Al-Hakim et al., 1970; Ibrahim and Boldizsár, 1981). Comparison of the amino acids in seminal plasma from normal and from vasectomized rams (Setchell *et al.*, 1967) indicated that most of the amino acids originated in the testes or epididymides.

Hopwood and Gassner (1962) noted a positive correlation between seminal plasma free amino acid concentration and the fertilizing capacity of bull semen. Addition of amino acids has been reported to overcome to some extent the deleterious effects of excessive dilution on semen (Mann, 1964). In addition, Newmark and Schindler, (1967) reported that the amino acids could serve as a readily oxidizable substrate for energy yielding reactions in semen.

Oltjen et al. (1971) showed that high amounts of total free amino acids are important for the semen quality and the fertility of the animal. Ibrahim and Boldizsár (1981) classified a population of bulls in two groups according to their total amino acid concentrations and found that the average fertility score of the group containing elevated free amino acid concentration was slightly higher than the group of low amino acid levels.

Many investigators found significant relationship between levels of GOT in bull seminal plasma and percentage of motile spermatozoa in fresh collected semen (Zahariev, et al., 1973), sperm concentration (Singal et al., 1976; Strzezek et al., 1982) and fertility rate (Strzezek and Swidowicz 1986). On the other hand, Yousef et al., (2001) found that, in V-line strain, grouped to high, medium and low motile sperm groups, the ejaculate volume increased and reaction time decreased in high motile sperm group than the low motile group. Also, sperm concentration per ml, total sperm output and total motile sperm per ejaculate were all higher in high motile sperm group than in medium or low fertile bucks and there was a positive correlation coefficient between conception rate and pervious semen characteristics. Yousef et al., (2001) reported that in rabbits it is possible to select buck

s for breeding programs on basis of number of motile sperm per ejaculate, but not on percentage of progressive motility per buck.

The aim of this work is to identify the seminal plasma free amino acid and their association with rabbit seminal quality in V-line male rabbits (Spanish strain).

MATERIAL AND METHODS

This study was carried out at El-Sabhia Poultry Research station, belong to Animal production Research Institute, Agriculture Research Center and Arid Lands Cultivation and Development Research Institute, Mubarak City For Scientific Research and Applied Technology, Egypt.

Eighteen mature males of V-line rabbits (7-month old with average initial weight 3.325 kg) were used in this experiment during winter season for 8 weeks. Rabbits were individually housed in cages. Feed and water were provided *ad libtum*. The composition of the ingredients of pellet concentrate feed (% on a dry matter basis) is shown in Table (1).

Semen from each rabbit was collected weekly using an artificial vagina and a teaser doe (Tesh and Tesh, 1971). Motility was determined and semen with a progressive motility ≤ 50% was used as a low motile sperm group (LMS), while semen with a progressive motility ≥ 75% was used as a high motile sperm group (HMS).

The volume of each ejaculate was recorded nearest 0.1 ml (using a graduated collection tube) after removal of the gel mass. A weak eosin solution (Smith and Mayer, 1955) was used for evaluation the sperm concentration by the improved Neubauer haemocytometer slide (Germany). Total sperm output ejaculate was calculated. The percentage of motile sperm was estimated by visual examination under low-power magnification (10×) using a phase-contrast microscope with heated stage. Total number of motile sperm (TMS) was calculated by multiplying percentage of motile sperm and total sperm outputs. Total functional sperm fraction (TFSF) parameter was also calculated as the product of total sperm output by motility by normal morphology sperm (Correa and Zavos, 1996). Assessment of live, dead, and abnormal spermatozoa were performed using an eosin-nigrosine blue staining mixture (Blom, 1950). Initial hydrogen ion concentration (pH) of semen samples was determined just after collection using a pH cooperative paper ranging from 0 to 14 with 1 grades (pH 0-14 Merck, Germany). Reaction time was determined as the moment of

subjecting a doe to the buck until the completion of erection using a stopwatch; it was measured in seconds.

Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4 °C, and was stored at -20 °C until later analysis. Seminal plasma samples were analyzed for total protein (TP) according to Henry et al. (1974). Albumin (A) concentration was determined by the method of Doumas et al. (1977). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). For assaying acid phosphatase (AcP) activity, the method of Moss, (1984) was used. Alkaline phosphatase (AlP) activity was measured using the methods of Principato et al., (1985).

Heparnized tubes were used to collect the blood samples which obtained from the ear vein of each buck every week. Blood samples were centrifuged at 3500 rpm for 20 min to obtain plasma, and stored at −20 °C. Testosterone concentration in plasma was measured using immunoassay commercial Elisa kit (Biosource Europe S.A. Co.).

Seminal plasma protein electrophoresis methods was carried out by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) as a technique used to resolve proteins in a mixture based on their molecular size according to the methods of Sambrook *et al.* (1989). Free amino acids in seminal plasma were extracted according to the method described by Hamilton (1962) and the individual free amino acids were measured using a method which described by Spackman et al. (1958) using amino acid analyzer system (model: SYKAM S 7130).

Data were analyzed as a completely randomized design (Steel and Torrie, 1980) using the general linear model procedures of SAS (1986). Significance of the effects was tested at level $p<0.05$(*) and $p<0.01$ (**) with the appropriate F statistic. Duncan's multiple range test was used to detect any significant differences among the experimental means (Duncan, 1955).

RESULTS AND DISCUSSIONS

Semen Characteristics:

Results in Table (2) are data of this study on sperm motility (SM), ejaculate volume (EV), pH and reaction time (RT) of buck semen classified as low (LMS) and high motile sperm rabbits (HMS) of V-line rabbit bucks. A significant increase in SM of the HMS group was observed compared to LMS. The data showed that the LMS significantly declined in semen ejaculate volume compared to the HMS group.

pH semen value significantly less in the HMS compared with the LMS group and this differences might be due to a increase in sperm metabolite activities, which affected on hydrogen ion concentration in the seminal plasma. From Table (2) it can be observed that the reaction time was significantly shorter with the increase of rabbit sperm motility it means that rabbits with HMS are faster to arrive to libido than the low motile sperm rabbits (LMS).

Sperm concentration appeared to be slightly higher in the LMS than the HMS group, but the differences did not significant (Table 3). The total sperm output was significantly higher in HMS group and this may due to the increase of semen ejaculate volume in this group (Table 2). Percentage of dead sperm significantly decreased in the HMS compared to the LMS group. On the other hand, the percentage of normal sperm increased and the percentage of abnormal sperm decreased in the HMS compared to LMS group but the difference was not significant. Increased total output sperm and normal sperm in the HMS than LMS group increased total motile sperm and total functional sperm fraction (TFSF) and increase was statistically significant (Table 3).

Our results was in agreement with the results of Yousef et al., (2001) who found that, in V-line strain which divided based on motile sperm to high, medium and low motile sperm, the ejaculate volume increased and reaction time decreased in high motile sperm group than the low group. Also, they mentioned that sperm concentration per ml, total sperm output and total motile sperm per ejaculate were higher in high motile sperm group than in medium or low fertile bucks and there was a positive correlation coefficient between conception rate and pervious semen characteristics.

Mohamed et al., (1986) reported that the percentage of progressive motile spermatozoa appeared to be the best prediction of human male fertility potential. Furthermore, sperm motility was found to be one of the most important criteria of semen quality and a determinant in the success of fertilization (Ijaz et al., 1994). Saacke et al., (2000) in cattle and Castellini and Lattaioli (1999) in rabbits found that sperm number and number of motile sperms are positively associated with both fertilization rate and embryonic quality. In addition, Yousef et al., (2001) reported that in rabbits it is possible to select bucks as sires for breeding programs depending on number of motile sperm per ejaculate, but not percentage of progressive motility per sires.

Seminal plasma characteristics:

Seminal plasma total protein and albumin levels (Table 4) for HMS were significantly higher compared to the LMS group, this increase in seminal plasma total protein and albumin was associated with increasing percentage of motile and normal sperm. Many studies showed that low content of seminal plasma proteins was associated with poor semen quality (Verma et al., 1985; Dhami and Kodagali, 1989). Taha et al., (2000) reported that there is a positive relationship between semen quality parameters and level of seminal plasma total proteins. Kulkarni et al., (1996) showed that, seminal plasma total proteins are mainly composed of albumin and globulin, in addition to small quantities of nonprotein nitrogen, amino acids and peptides. These compounds make up the amphoteric property of seminal plasma proteins, thus, low protein content in seminal plasma reduce its buffering capacity and in turn semen quality (Dhami et al., 1994). A similar results were found by Osama and El-Sahn (2006) who found a positive relationship between increasing seminal plasma total proteins and albumin and increasing total number of sperm output.

Data presented in Table (4) indicated that seminal plasma aspartate aminotransferase (AST) and alanine transaminase (ALT) activities significantly decreased in the HMS when compared with the LMS group. Pursel et al. (1968) reported that one of the consequences of acrosomal damage is the leakage of enzymes from the sperm. Yousef et al. (2003) reported that there was a negative correlation between increased ALT and AST activities and ejaculate volume, sperm concentration, total sperm output, sperm motility index, total motility index, total motile sperm, Therefore, the decrease in the activities of these enzymes coincided with the increase of semen quality. Positive correlation between enzyme release and acrosomal damage was reported with goat's sperm (Chauhan et al., 1993). Yousef and Zeitoun (1998) found that there were negative correlation coefficients between sperm motility and AST and ALT release. They reported that the activities of these enzymes could be used as an indicator of sperm integrity. Flipse, (1960) mentioned that, The fact that the amino acid found in greatest concentration in semen, glutamic acid, is accompanied by a high level of Glutamic oxaloacetic transaminase activity which suggests a rapid metabolism of this amino acid.

Data presented in Table (4) revealed that the HMS had significantly higher activities of seminal plasma AcP and AlP enzymes when compared with the LMS. These enzymes play a pivotal role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism, in fertilization process and in the maintenance of constant

osmotic pressure during preservation (Dhami and Kodagali, 1987). According to Dhami et al., (1994) Phosphatases enzymes in semen play an important role in phosphorylation processes in sperm metabolism and thus explain the differences observed in the semen quality. Also, Kamel (2005) found that, the rabbits which have higher seminal quality index the AcP activity increased in their seminal plasma.

Data in Table (4) showed significant differences between low motile sperm and high motile sperm in testosterone concentration. Testosterone concentration level appeared was significantly higher in HMS than LMS. Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testes, whereas it is responsible for the development of secondary male sex characteristics. Testosterone is needed to initiate spermatogenesis at puberty and for the maintenance of this process in the adult. It also required for the completion of meiosis and for the differentiation of the spermatids (Procida, 1994). Spermatogenesis depends on the action of testosterone (Sharpe et al, 1988). High local level of testosterone was found surrounding the somniferous tubules due to the close proximity of the later to the leydig cell (Sharpe et al, 1988). Seminal vesicle weights increased significantly with the increase of testosterone dosage (Ewing et al 1976). Low levels of testosterone can be found with the hypopituitarism, testicular feminization and some autoimmune diseases (Granoff and Abraham, 1979).

The significant increase in testosterone level which found in the high motile sperm compared to the low one may be due to spermatogenesis activation in the somniferous tubes. The improvement in semen quality of HMS group may be due to a great level of testosterone hormone. The previous results revealed that high fertile rabbits had a higher level of testosterone than the low fertile rabbits, since the increase in testosterone hormone level increases the sexual desire.

Seminal plasma free amino acids components:

Data in Table (5) showed that seminal fluid in HMS rabbits had higher levels of total free amino acids compared to the LMS group, and seminal fluid in both HMS and LMS rabbits contained approximately 16 free amino acids. The major constituent is cystein then arginine which constitute approximately 55% from all free amino acids. Seminal fluid from HMS rabbits appeared to be higher in lysine and methionine content compared with LMS rabbits, which contained higher amount from Glutamic acid and Histidine than the HMS.

Increasing total free amino acids content in seminal plasma of the HMS group was associated with increase in motility of sperm and normal sperm, and this increase in total free amino acids may be required for vital processes by spermatozoa. Mann (1964) noted that there is some evidence that the amino acid present in the seminal plasma play an important role in the survival of spermatozoa. The function of seminal plasma free amino acids is shown to act as fuels for the spermatozoa, to create favorable conditions for cell survival and to be probably involved in detoxifying functions (Al-Hakim et al., 1970; Ibrahim and Boldizsár, 1981). Oltjen et al. (1971) and Roussel and Stallcup (1967) showed that high amounts of total free amino acids are important for the semen quality and the fertility of the animal. Ibrahim and Boldizsár (1981) found that the average fertility score of the group containing elevated free amino acid concentration was slightly higher than the group of low amino acid levels. On the other hand, there was a relationship between increasing Glutamic acid in seminal plasma of LMS rabbits and increase AST enzyme activity. This relationship was also found by Flipse, (1960) who mentioned that, the fact that the amino acid found in greatest concentration in semen, glutamic acid, is accompanied by a high level of Glutamic oxaloacetic transaminase activity which suggests a rapid metabolism of this amino acid.

SDS-PAGE of seminal plasma proteins:

Figures (1) represent SDS-PAGE of seminal plasma proteins of HMS and LMS rabbits. It is obvious from protein bands pattern that the protein band with molecular weight (100.7 kd) showed a high intensity band in the HMS rabbit samples than in the LMS samples. As well as, the bands with molecular weight 78.8 kd was found in the HMS rabbit samples but it did not found in the LMS rabbits. It can be seen that, the protein with molecular weight (47.5, 40.7 and 36 kd) which expressed in HMS group was completely absent from the LMS rabbit. The protein at molecular weight 33 kd showed high expression protein with both LMS and HMS samples. As well as, the protein at 16.5 kd was expressed in both LMS and HMS rabbit samples but that was highly expressed in the HMS rabbit than the LMS samples. However, the protein at 9 kd was observed in the both groups. On the other hand, the LMS rabbits appeared a protein band at molecular weight 45 kd that disappeared from the HMS group. Mohan et al., (1995) reported that chicken seminal plasma contain a high molecular weight protein (> 100 kd) which neutralizes the motility inhibiting property of spermatozoa motility inhibiting factor. This result is agreement with that in the present study, whereas, the protein band with molecular weight 100.7 kd have a high intensity in HMS than the LMS rabbit which associated with

increase total motile sperm in the first group. This protein seemed to be similar to the peptide band having 105 kd which detected in chicken seminal plasma by Osama and El-Sahn (2006).

On the other hand, it can be observed that, the two protein bands at molecular weight (78.8, 47.5, 40.7 and 36 kd) that expressed in the HMS rabbit only may have an enzyme role which involved to maintenance motility of sperm.

Table (1): Proximate analysis of pellet concentrates feed (% on a dry matter basis)

Pellet concentrate		Chemical analysis*	
Ingredients (%)		Crude protein (%)	17.5
Berseem hay	30.0	Crude fiber (%)	14.0
Yellow corn	25.0	Crude fat (%)	2.7
Wheat bran	26.2	Nitrogen free extract	56.4
Soybean meal	14.0		
Molasses	3.0		
$CaCl_2$	1.0		
NaCl	0.4		
Vit.&Min. mix.	0.3		
Methionine	0.1		

*The chemical analysis of the pellets (AOAC, 1990)

Table (2): Overall means (±SE) of sperm motility (SM), ejaculate volume (EV), pH and reaction time (RT) for semen classified as low (LMS) and high motile sperm (HMS) of V-Line rabbit bucks.

	HSM	LSM
SM (%)	78.1±1.07^a	42.5±0.78^b
EV (ml)	0.72±0.02^a	0.65±0.03^b
pH	7.39±0.06^b	7.54 ±0.05^a
RT (Sec)	5.3±0.21^b	7.1±0.42^a

^{ab} means the same row have the different superscript are significantly different at ($p \leq 0.05$).

Table (3): Overall means (±SE) of sperm concentration, dead sperm, live sperm, normal sperm, abnormal sperm, total motile sperm (TMS) and total functional sperm fraction (TFSF) for semen low (LMS) and high motile sperm (HMS) of V-Line rabbit bucks.

	HSM	LSM
SC ($\times 10^6$/ml)	270.63 ± 4.12	276.3±2.52
TSO ($\times 10^6$/ml)	199.0 ± 5.39^a	175.9±7.62^b
Dead Sperm (%)	20.5 ± 0.74^b	24.1±0.76^a
Normal Sperm (%)	87.1±0.29	87.0±0.24
Abnormal Sperm (%)	12.9±0.29	13.0±.024
TMS ($\times 10^6$/ml)	152.1±4.37^a	75.9±3.14^b
TFSF ($\times 10^6$/ml)	132.5±3.88^a	66.0±2.74^b

^{ab} means the same row have the different superscript are significantly different at ($p \leq 0.05$).

Table (4): Overall means (±SE) of seminal plasma of TP, Alb, ALT, AST, AcP and AlP and blood serum testosterone for low (LMS) and high motile sperm (HMS) of V-line rabbit bucks.

Items	HSM	LSM
Total protein (TP) g/dl	6.58±0.08[a]	5.91±0.07[b]
Albumin (Alb) g/dl	3.24±0.01[a]	2.85±0.02[b]
Alanine aminotransferase (ALT) U/l	12.59±0.22[b]	16.96±0.40[a]
Aspartate aminotransferase (AST) U/l	9.28±0.25[b]	13.89±0.53[a]
Acid phosphatase (AcP) U/l	34.34±0.28[a]	31.10±0.07[b]
Alkaline phosphatase (AlP) U/l	57.52±0.29[a]	46.48±0.52[b]
Testosterone (ng/ml)	3.40±0.12[a]	1.05±0.02[b]

[a,b] means the same row have the different superscript are significantly different at ($p \leq 0.05$).

Table (5): Free amino acids composition in seminal plasma of low (LMS) and high motile sperm (HMS) of V-line rabbit bucks.

Free amino acid composition	HSM	LSM
Aspartic acid	0.81	1.12
Threonine	1.84	1.05
Serine	-	-
Glutamic Acid	2.86	6.09
Proline	1.88	0.87
Glycine	9.89	10.46
Alanine	1.36	0.84
Cystein	46.82	48.44
Valine	2.41	3.5
Methionine	2.35	0.76
Isoleucine	3.24	3.12
Leucine	0.43	0.36
Tyrosine	3.01	3.29
Phenylalanine	12.5	11.85
Histidine	3.07	7.42
Lysine	15.6	4.65
Arginine	22.78	21.41
Total free amino acids	**130.85**	**125.23**

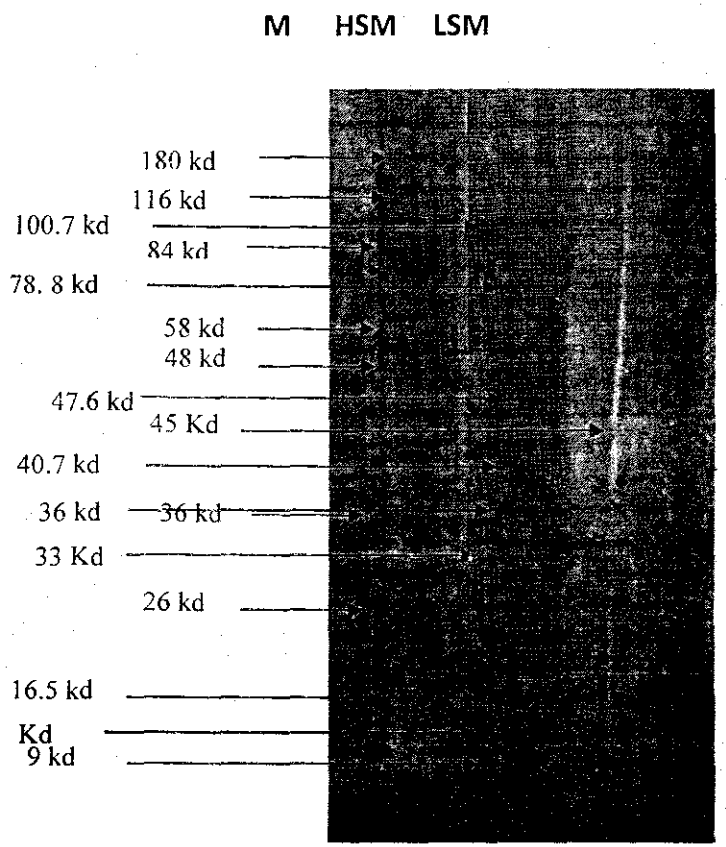

Figure (1): SDS polyacrylamide gel electrophoresis (PAGE) of seminal plasma proteins in High and Low motile sperm rabbits:
M= protein marker
HMS= High motile sperm rabbits
LMS= Low motile sperm rabbits

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الملخص العربي

دراسة مقارنة لمحتوي الاحماض الامينية في البلازما المنوية لذكور الارانب ذات حيوانات منوية منخفضة وعالية الحركة

*علاء الكومي-**محمد السيد السبيعى- **كامل إبراهيم كامل

*مدينة مبارك للابحاث العلمية والتكنولوجي

**معهد بحوث الإنتاج الحيواني- مركز البحوث الزراعية- وزاره الزراعة - مصر

تهدف هذه الدراسة في البحث في خصائص السائل المنوي ومحتواه من الاحماض الامينية والهجرة الكهربائية لبروتينات السائل المنوي في ذكور الارانب الفيلايين البالغة. عشرون من ذكور الارانب عمر 7 شهور استخدمت اثناء فصل الشتاء. قسمت الارانب علي حسب حيوية الحيوانات المنوية الي المجموعة الاولي منخفضة الحيوية (اقل من 50% حيوية) و المجموعة الثانية عالية الحيوية (اعلي من 75% حيوية).

اظهرت النتائج ان الارانب ذات الحيوانات المنوية منخفضة الحيوية كانت اقل معنويا في حجم القذفة بينما تركيز الحيوانات المنوية كان اعلي قليلا في الارانب الاخري.عدد الحيوانات المنوية الكلية كان اعلي معنويا في الاسبرمات الاعلي حيوية والتي كانت اقل معنويا في قيمة الاس الهيدروجيني . نسبة الاسبرمات الحية والطبيعية اعلي في الارانب ذات الاسبرمات الاعلي حيوية مقارنا بالاقل حيوية.

محتوي البلازما المنوية من البروتين والالبيومين وانزيم الفوسفايت الحامضي والقاعدي كان اعلي في المجموعة الاولي بينما الانزيمات اسبرتيت ترنزامينيز والانيين ترنزامينيز كان الاقل في المجموعة الثانية.

الهجرة الكهربائية لبروتينات السائل المنوي مع الوزن الجزئي kd100.7 كان اعلي كثافة في الارانب ذات الاسبرمات عالية الحيوية مقارنا بالارانب الاخري.

محتوي الاحماض الامينية في الارانب ذات الاسبرمات عالية الحيوية كان الاعلي. تميزت الاسبرمات عالية الحيوية بارتفاع محتواها من الاحماض الامينية الليسين والمثيونيين بينما كان الحمض الاميني الجلوتمك والهستيدين الاعلي في الاسبرمات المنخفضة الحيوية.