

EFFECT OF VITAMIN E (ALPHA-TOCOPHEROL) ADDITION TO RABBIT SEMEN on SURVIVAL AND FERTILITY

Kishk W.¹, S. Yousif² and J. Rafay³

¹ Suez Canal University, Faculty of Agriculture, Animal Production Dept., Ismailia, Egypt

² Veterinary Research Institute, Ministry of Agriculture, Ismailia, Egypt

³ Research Institute of Animal Production, 949 92 Nitra, Hlohovska 2, Slovakia

ABSTRACT

Five Nitra male rabbits were subjected to artificially semen collection. Samples with percent motility above 70 were pooled for subsequent dilution and processing. Pooled semen samples were diluted (1:5) with Tris extender free of egg-yolk. Three different levels of vitamin E were added to extended semen samples. These levels were 2, 4, 8 mg vitamin E for each 5 ml extended semen in addition to two control groups (control 1 without vitamin E and control 2 with ethanol and without vitamin E), respectively. Diluted samples with different levels of vitamin E were kept at 15 °C for one day. Sperm motility, live and dead sperm, broken head and broken tail sperm percentages, and acrosome integrity criteria were measured for diluted samples of different groups after dilution by 0, 2 and after 24 hours of incubation at the same temperature. It was found that vitamin E addition significantly improved sperm motility % after two hours of incubation at all added levels. In which sperm motility were 70% for different levels of vitamin E and 65% for control 1 & 2 groups, respectively after 2 hrs incubation periods. Also acrosome integrity % was differed among different groups. Where, it was higher in correlation with higher doses of vitamin E. Average values of acrosome integrity % (A.I) were 91.67, 92.00 and 95.33 for three different levels of vit E respectively. Whereas, they were (A.I) 81.33 and 79.33 for control 1 and control 2, respectively. As for live and dead sperm percentage, broken head and broken tail sperm, did not differ significantly according to different groups. It could be concluded that, vitamin E can be included in rabbit semen diluters as a sperm metabolism protectant especially for fine organelles and structures such as galea caputis (acrosome). In addition, fertility rate for different levels of vitamin E addition to extended semen was 75, 75, 70 and 85 % for control, first, second and third level, respectively.

Keywords: Semen, rabbit sperm, vitamin E, acrosome reaction, motility, fertility.

INTRODUCTION

There are many deleterious and lethal cellular changes can occur to sperm cells during incubation and storage. These changes include peroxidation of membrane lipids, inactivation of enzymes and DNA damage (Elhassan and Wright, 1995). The by-products of aerobic metabolism can cause direct damage to sperm cell compartments which render it to infertile state. Vitamin E (alpha-tocopherol) is the primary lipid-soluble antioxidant found naturally in animal cells. In addition, vitamin E acts at tissue level in which, it can produce the reduction of fat oxidation and in a tendency for reducing drip loss (Schwarz *et al.*, 1998). Many authors have pointed out the effect of vitamin E supplementation in culture media for IVM/IVF oocytes

(Dalvit et al., 1998; Elhassan and Wright, 1995) and bovine embryos (Olson and Seidel, 1995). Some experiments showed that supplementation with vitamin E resulted in significantly more early, expanded and hatched blastocysts. While vitamin E and vitamin C can act synergistically as antioxidants, under some conditions vitamin C is a prooxidant, and may have offset the beneficial effect of vitamin E (Olson and Seidel, 1995).

There are some changes in sperm cell acrosome membranes during *in vitro* incubation or conservation. These changes could be related to surface-absorbed materials, redistribution of intramembranous protein particles and changes in the membrane lipids (Bedford, 1970; Gwatkin, 1977; Bedford and Cooper, 1978). Most of the previous cellular changes can lead to sperm acrosome reaction which is considered as a necessary biochemical process for multiple fusions between the outer acrosomal membrane and the overlaying plasma membrane. This enables sperm to release and exposure of accrosomal enzymes possibly necessary for sperm passage through the egg investments (Fraser and Ahuja, 1988).

The aim of the present work is to study the effect of vitamin E addition to semen on sperm survival after different periods of preservation at 15 °C. As well, to determine fertility and prolificacy rate of artificially inseminated does with diluted semen supplemented with vitamin E.

MATERIALS AND METHODS

Rabbit bucks management:

Male Nitra rabbit bucks (5 bucks) were kept and reared at a closed rabbitry system, Research Institute of Animal production, Nitra, Slovak Republic. Light / dark ratio was adjusted to be (16 L / 8 D per day) throughout the year to ensure high reproductive performance and semen production. Bucks were fed a pelleted diet *ad libitum* and had *ad free* access to water through provided nipples in metal cages. The average age of Nitra bucks was 2 years with an average body weight of 4.5 Kg. Males were subjected to routinely veterinary examination to maintain them in healthy condition throughout the experimental period.

Semen collection and examination:

Semen samples were collected by gelatinous artificial vagina (G.A.V) after adjusting its temperature to 40 °C. Bucks were subjected to regularly semen collection (three times per week). After semen collection, samples were kept at 37 °C in a water bath. Microscopic examination was carried out to assess sperm motility percent and sperm concentration / ml using haemocytometer. Samples with sperm motility above 70 % and sperm count not less than 150 million sperm / ml were pooled for next dilution and conservation.

Semen extender preparation:

Tris extender free of egg-yolk was used to dilute pooled semen samples in a dilution rate 1:5. The composition of 100 ml Tris extender was

prepared as the following: T, hydroxymethylaminomethane, 3.028 gram, glucose 1.25 g; citric acid 1.7 g, , 30,000 IU procaine penicillin-G; 10,000 IU sodium penicillin-G and 50,000 micrograms streptomycin based (as sulphate). Vitamin E (alpha-tocopherol, Rodisma- Med Pharma, GmbH- Koln, Germany) was added at three different levels in which 400 mg of vitamin E were dissolved in 0.5 ml ethyl alcohol (90 %). Three levels of prepared vitamin E solution were added, the first was 2.5 µl of vitamin E solution (100 µM vitamin E / 5 ml extended semen), 2nd level contained 5 µl of vitamin E solution (200 µM vitamin E / 5 ml extended semen) and 3rd level contained 10 µl of vitamin E solution (400 µM vitamin E / 5 ml extended semen). In addition, there were two different control groups in this experiment. The 1st group was free of vitamin E and the 2nd group contained 10 µl ethyl alcohol (90 %) / 5 ml extended semen.

Sperm viability measurements:

Sperm motility percent was measured microscopically (magnification 400 X) after dilution by 0, 2 and 24 hours for all treatments. Smears of diluted semen were carried out for next staining with alkaline methyl violet stain to determine acrosome integrity %. Smears were also prepared for staining with eosin - nigrosin stain to evaluate live and dead sperm percent. In addition, broken head and broken tail sperm were counted for the same slides (eosin - nigrosin stain) for all treatments.

Fecundity experiment and farrowing rate :

A total number of 80 multiparous Nitra does were allocated to four groups. Does were synchronized hormonally for ovulation induction by using PMSG / HCG system for each doe. After hormonal preparation, does were artificially inseminated (1 ml of diluted semen with 30 million sperm for different groups according to the following: a) Semen diluted (1: 5) with Tris-extender (control group). b) Semen diluted (1: 5) with Tris-extender supplemented with 100 µM vitamin E (second group). c) Semen diluted (1: 5) with Tris-extender supplemented with 200 µM vitamin E (third group). d) Semen diluted (1: 5) with Tris-extender supplemented with 400 µM vitamin E (fourth group). The inseminated does were subjected to evaluation for the following traits:

- 1- Fertility percent
- 2- Total litter size born
- 3- Number of born alive
- 4- Number of born dead
- 5- Litter size after 21 days of lactation
- 6- Litter weight at parturition.

Statistical analysis

Data of sperm viability measurements (sperm motility, acrosome integrity, dead sperm, broken head and broken tail sperm %) were analyzed for the effect of vitamin E supplementation by using SPSS program for analysis of variance. Means of previous sperm characters during different preservation periods were compared according to multiple range tests (Duncan test with

significance levels 0.05). As regards fecundity experiment, data of different groups were subjected to ANOVA test for the effect of vitamin E supplementation on litter size born, litter size born alive, litter size born dead, litter size after 21 days of age and litter weight at parturition. Chi-Square was carried out to test significant differences as regard to fertility percent between control and different treatments.

RESULTS AND DISCUSSION

Sperm motility criteria

Data presented in Table 1 showed that, there were no significant differences among different means of various treatments of vitamin E addition in respect of sperm motility % compared to control groups. Whereas, there were significant differences ($P < 0.05$) in sperm motility % due to the period of sperm preservation. In which sperm motility percentages were 73.00, 68.00 and 48.00 for extended semen after dilution by 0, 2 and 24 hours, respectively. The change percent was 6.85 and 29.41 % for 2 and 24 hours after preservation at 15 °C, respectively. Sperm motility can be reduced through period of sperm preservation and this may be mainly attributed to metabolic activities and accumulation of by-products of aerobic and anaerobic metabolism which may change media of extended semen (Fraser and Ahuja, 1988). Vitamin E addition in this experiment is based on its character as a primary lipid-soluble antioxidant. Its addition slightly increased sperm motility % in the treated groups in comparison to the control groups (Table 1). The highest sperm motility % was recorded for first level of vitamin E (65.00 %). The second and third levels of vitamin E recorded the same value of sperm motility being 63.33 %. The lowest value of sperm motility was that of control 1 and control 2 which were equals (61.67%). The harmful effects of ethanol could not be noticed in this experiment, this may be due to low concentration of ethanol addition (10 μ l / 5ml semen extender).

Sperm metabolism via glycolytic and / or oxidative metabolic pathways can produce energy to support sperm motility. The marked preference of sperm for glycolyzable substrates, even under aerobic conditions, makes them an unusual cell type; the introduction of glucose to an oxidatively metabolizing suspension leads to a reduction in oxygen consumption as the cells begin to metabolize glucose preferentially, a phenomenon known as the Crabtree effect (Mann, 1964). A glycolyzable substrate is sufficient to support fertilization under both aerobic and anaerobic conditions and neither pyruvate nor lactate will substitute (Hoppe, 1976; Fraser and Quinn, 1981). In addition Rogers *et al.*, (1979) have suggested that, there are some mammalian sperm species use pyruvate plus lactate instead of glucose and it is due to the Crabtree effect, with preference given to glycolytic rather than oxidative metabolism. From the previous discussion, it could be pointed out that vitamin E addition to rabbit semen extender could reduce aerobic metabolism rate and reduce consequently producing of pyruvate and lactate. This reduction in oxidation and oxygen consumption

Table 1 effect of vitamin addition to semen and storage period after dilution on some sperm characters (x±s.e)

Storage period (hrs) Treatment	Sperm Motility%		Mean±SE*	Acrosome Integrity%	Mean±SE		Dead Sperm%		Mean±SE	Broken Head S.		Mean±SE		Broken Tail S.		Mean ±SE
	1	2			0	2	1	2		1	2	1	2	1	2	
Control 1	70***	65	61.67 ^A ±6.12	86	83.00 ^A ±2.56	15	20	23.33 ^A ±0.90	5	6	8	6.33 ^A ±0.90	3	10	5.33 ^A ±2.38	
Control2 with Ethanol	70	65	61.67 ^A ±6.22	84	79.33 ^A ±2.65	18	25	27.67 ^A ±1.48	6	9	11	8.67 ^A ±1.48	4	8	7.67 ^A ±2.06	
1 st level of Vit E	75	70	65.00 ^A ±7.77	96	91.67 ^B ±2.65	11	17	17.00 ^A ±3.53	7	10	11	9.33 ^A ±1.22	4	6	6.67 ^A ±1.80	
2 nd level of Vit E	75	70	63.33 ^A ±9.45	95	92.00 ^B ±2.12	10	15	22.33 ^A ±10.12	4	7	7	6.00 ^A ±1.02	4	4	5.00 ^A ±1.02	
3 rd level of Vit E	75	70	63.33 ^A ±9.55	98	95.33 ^B ±1.8	12	20	24.33 ^A ±8.81	5	7	9	7.00 ^A ±1.18	3	6	6.00 ^A ±1.76	
Mean**	73.00 ^A	68.00 ^B	63.00 ±1.22	91.80 ^A	88.27 ±1.05	13.20 ^A	19.40 ^A	22.93 ±3.25	5.40 ^A	7.80 ^B	9.20 ^B	7.47 ±0.52	3.60 ^A	5.40 ^A	6.13 ±0.68	
±SE	±1.22	±1.42	±3.48	±2.84	±3.08	±1.46	±1.69	±3.51	±0.51	±0.73	±0.80	±0.87	±0.24	±0.87	±0.82	

* Means within each column with different letters superscript differ significantly (P<0.05)

** Means within each row with different letters superscript differ significantly (P<0.05)

*** Each figure based on the average of three observations.

rate by sperm cells through vitamin E addition can maintain and preserve energy sources for a long period (mainly fructose and glucose from both seminal plasma and exogenous components of semen extender). In addition, it can explain the superiority of sperm motility and fertility percent (Table 1 & 3) for groups which contained vitamin E comparing to control groups.

Acrosome integrity criteria & litter size traits

As regard to acrosome integrity (A.I) percentages, there were significant differences ($P < 0.05$) due to vitamin E addition to semen as shown in Table 1. The highest A.I was recorded for the 3rd level of vitamin E addition. The A. I percentages were 95.33, 92.00 and 91.67 for third, second and first level of vitamin E addition, respectively. While, it averaged 83.00 and 79.33 % for control 1 and control 2, respectively. Although the two controls were not significantly different, the ethanol addition in control 2 decreased A.I than control 1 by about 3.8 %. Sperm cells can undergo some chemical and biochemical reactions during preservation period which can lead to capacitation and acrosome reaction (O'Rand, 1982). The high rate of acrosome integrity especially for vitamin E addition groups can be attributed to its role as an antioxidant substrate. Mujica and Valdes-Ruiz (1983) found that some mammalian sperm when preincubated in the presence of glucose, washed, and transferred to medium containing pyruvate and lactate underwent rapid acrosome reactions. While others reported that glucose is the required substrate for sperm capacitation and acrosome reaction (Dravland and Meizel, 1981) basing on species – specific differences. In this experiment it could be noticed that vitamin E addition can maintain sperm cell membranes for a long period against cellular changes which can lead to sperm capacitation and acrosome reaction. In contrast to sperm motility criteria, there were no significant differences ($P < 0.05$) between different incubation periods as regard to acrosome integrity % for entire preservation term in Tris-extender at 15 °C as shown in Table 1.

There is general concept describing sperm capacitation, which involves, at least in part, the loss or removal of inhibitory molecules from the sperm surface (Oliphant *et al.*, 1985). These changes can take place either in vivo (female tract) or in vitro during preservation period. The beneficial effect of vitamin E is reducing the occurrence of acrosome reaction via reducing aerobic metabolic rate and oxygen consumption consequently. There are some indicators for the alterations to the medium composition, which support fertilizing ability, promote an immediate decline in oxygen consumption (Borland *et al.*, 1977).

Live and dead sperm criteria

Data of dead sperm percent during semen conservation showed that there were no significant differences ($P < 0.05$) between treated groups and control in dead sperm percent (Table 1). On the contrary, there were significant differences ($P < 0.05$) in dead sperm % due to the effect of semen storage period at 15 °C, where it averaged 13.20, 19.40 and 36.20% for the three different storage periods, respectively. The increase in dead sperm percent through storage period may be due to rabbit sperm sensitivity to

hypertonic solutions (Castelini *et al.*, 1992). Therefore, most of the rabbit semen extenders are designed to store semen for not more than 48 hours by cooling sperm cells at 5 to 25 °C.

Broken head and broken tail sperm percent criteria

As regard to these two characters, there were no significant differences ($P < 0.05$) between different groups. However, the storage period had significant effects on these traits (Table 1). These two characters (broken head and broken tail sperm percent) could be affected mainly by genetic makeup rather than semen processing and preservation (Hafez, 1993).

Doe litter traits

The present study showed that, there were no significant differences between different groups in this experiment as regard to conception rate by using Chi-Square where it averaged 75, 75, 70 and 85% for control, 1st, 2nd, and 3rd levels of vitamin E addition, respectively. But, there were significant differences ($P < 0.05$) between control and groups of vitamin E supplementation in respect of litter size, number of born alive, litter size after 21 days of lactation (Table 2). The only trait which contained no significant differences was number of born dead (Table 2). Diluted semen of first and second levels of vitamin E and used to inseminate does immediately after dilution gave higher values of litter size, number of born alive, litter size after 21 days of lactation and litter weight at parturition.

Table 2. Fertility rate and litter size traits of multiparous does after Insemination with diluted semen free of vitamin E or contained three different levels of vitamin E.

Treatment	Control	First Level of vit E	Second Level of vit E	Third Level of vit E
Conception rate%	75 ^a	75 ^a	70 ^a	85 ^a
Litter size	8.27 ^a	9.07 ^b	9.21 ^b	8.76 ^a
Number of born alive	7.80 ^a	8.40 ^a	8.57 ^b	8.29 ^a
Number of born dead	0.53 ^a	0.67 ^a	0.64 ^a	0.47 ^a
Litter size after 21 days	7.40 ^a	8.07 ^b	8.29 ^b	8.06 ^b
Litter weight at parturition	515.80 ^a	522.80 ^b	501.93 ^a	510.65 ^a

Means within each row with different letters superscript differ significantly ($P < 0.05$)

Correlations of semen characters

Most of the studied correlations which are presented in Table 3 showed high correlation coefficient ($P < 0.05$) especially between motility and other characters. While, there were negative correlations between sperm motility and broken head & broken tail sperm. There was medium correlation between sperm motility and acrosome integrity as shown in (Table 3). This is declare that, sperm motility assessment can be used alone to predict the most other semen characters to evaluate semen samples.

It could be concluded that vitamin E supplementation (100 or 200 µM / 5 ml semen extender) can be used in rabbit semen extenders as a tool to improve sperm cells characteristics, litter size traits as well.

Table 3. Estimates of correlations between semen traits.

Semen Characters	SM%	AI%	DS%	BHS%	BTS%
Semen Motility% (SM%)	-	0.52*	-0.94**	-0.61**	-0.82**
Acrosome Integrity%(AI%)	0.52 *	-	-0.56*	-0.44	-0.57*
Dead Sperm %(DS%)	-0.94**	-0.56*	-	0.55*	0.79**
Broken Head Sperm %(BHS%)	-0.61**	-0.44	0.55*	-	0.83**
Broken Tail Sperm %(BTS%)	-0.82**	-0.57*	0.79**	0.83**	-

* P < 0.05

** P < 0.01

REFERENCES

- Bedford, J. M. (1970). Sperm capacitation and fertilization in mammals. *Biol Reprod suppl* 2: 128-158.
- Bedford, J. M. and C. W. Cooper (1978). Membrane fusion events in the fertilization of vertebrate eggs. In Poste G, Nicolson GL (eds): *Cell Surface Reviews*. Amsterdam: North-Holland, Vol 5, pp. 65-125.
- Borland, R. M.; S.Hazara; J. D. Biggers; and C. P. Lechene (1977). The elemental composition of the environments of the gametes and preimplantation embryo during the initiation of the pregnancy. *Biol Reprod* 16: 147-157.
- Castellini, C.; M. Battagiani and P. Lattaioli (1992). Effects of cryoprotectants and freezing on rabbit sperm motility. *J. Appl. Rabbit Res.*, 15, 431-438.
- Dalvit, G.C.; P. D. Cetica and M. T. Beconi (1998). Effect of <<alpha>>-tocopherol and ascorbic acid on bovine in vitro fertilization. *Theriogenology* Vol. 49 (3) pp. 619-627.
- Dravland, E. and S. Meizel (1981). Stimulation of hamster sperm capacitation and acrosome reaction in vitro by glucose and lactate and inhibition by the glycolytic inhibitor α - chlorohydrin. *Gamete Res*, 4:515-523.
- Elhassan, Y. M. and Jr. Wright; R. W. (1995). The effect of selenium and vitamin E addition on cleavage rate of IVM/IVF bovine oocytes. *Theriogenology*, 43 (1): p. 206
- Fraser, L. R. and K. K. Ahuja (1988). Metabolic and Surface Events in Fertilization. *Gamete Research*, 20: 491-519.
- Fraser, L. R. and P. J. Quinn (1981). A glycolytic product is obligatory for initiation of the sperm acrosome reaction and whiplash motility required for fertilization in the mouse. *J. Reprod Fertil*, 61: 25-35.
- Gwatkin, R. B. L. (1977). *Fertilization Mechanisms in Man and Mammals*. New York : Raven Press, ch 6, 7, p. 67.
- Hafez, E. S. (1993). *Reproduction in farm animals*. 6th Edition, Lea & Febiger, Philadelphia.
- Hoppe, P. C. (1976). Glucose requirement for mouse sperm capacitation in vitro. *Biol Reprod*, 15: 39-45.
- Mann, T. (1964). *The Biochemistry of Semen and the Male Reproductive Tract*. London. Methuen.

- Mujica, A. and M. A. Valdes-Ruiz (1983). On the role of glucose in capacitation and acrosomal reaction of guinea pig sperm. *Gamete Res* 8: 335-344.
- O'Rand, M. G. (1982). Modification of the sperm membrane during capacitation. *Ann NY Acad Sci* 383: 392-404.
- Oliphant, G.; A. B. Reynolds and T. S. Thomas (1985). Sperm surface components involved in the control of the acrosome reaction. *Am J Anat* 174: 269-283.
- Olson, S. E. and Jr. Seidel; G. E. (1995). Vitamin E improves development of bovine produced in vitro. *Theriogenology*, Vol. 43 (1) pp. 289-289.
- Rogers, B. J.; L. Chang and R. Yanagimachi (1979). Glucose effect on respiration. Possible mechanisms for capacitation in guinea pig spermatozoa. *J Exp Zool*, 207:107-112.
- Schwarz, F. J.; C. Augustini; M. Timm; M. Kirchgkner and H. Steinhart (1998). Effect of vitamin E on α - tocopherol concentration in different tissues and oxidative stability of bull beef. *Livestock Production Science* Vol. 56 (2) p. 171.

تأثير إضافة فيتامين هـ للسائل المنوي على حيوية الحيوانات المنوية والخصوبة
في الأرناب

وليد كشك^١ ، سهير يوسف^٢ ، Rafay, J^٣
كلية الزراعة - جامعة قناة السويس^١ - مركز البحوث البيطرية بمحافظة الإسماعيلية^٢ - معهد بحوث
الإنتاج الحيواني بمدينة نترأ - سلوفاكيا^٣

خمس من ذكور أرناب النترأ تم استخدامها لجمع السائل المنوي. عينات السائل المنوي التي كان لها نسبة حركة مقدارها ٧٠% تم خلطها معاً لعمليات التخفيف التالية. عينات السائل المنوي التي تم خلطها تم تخفيفها بعد ذلك بنسبة ١:٥ باستخدام مخفف الترس الخالي من صفار البيض. وكان هناك ثلاثة مستويات من فيتامين هـ تم إضافتهم إلى عينات السائل المنوي المخففة. هذه المستويات كانت ٢ و ٤ و ٨ ملليجرام فيتامين هـ لكل ٥ ملي سائل منوي مخفف بالإضافة إلى وجود مجموعتين للمقارنة المجموعة الأولى كانت خالية من فيتامين هـ أما المجموعة الثانية فقد احتوت على الإيثانول وبدون فيتامين هـ على التوالي. عينات السائل المنوي المخففة والتي احتوت على المستويات المختلفة من فيتامين هـ تم حفظها على درجة حرارة ١٥ درجة مئوية لمدة يوم. وتم فحص النسبة المنوية لحركة الحيوانات المنوية ونسبة الحيوانات المنوية الحية ونسبة الحيوانات المنوية المكسورة الرأس ونسبة الحيوانات المنوية المكسورة الذيل. وكذلك تم تقدير النسبة المنوية للحيوانات المنوية ذات منطقة الأكروسوم السليمة وذلك بعد التخفيف مباشرة وبعد ساعتين وبعد ٢٤ ساعة من التخفيف. فقد وجد أن إضافة فيتامين هـ أدى إلى تحسين معنوي في النسبة المنوية لحركة الحيوانات المنوية وذلك بعد ساعتين من إضافته عند جميع المستويات المستخدمة. حيث كانت النسبة المنوية لحركة الحيوانات المنوية ٧٠% لمستويات فيتامين هـ المضافة بينما كانت ٦٥% فقط لمجموعات المقارنة وذلك بعد ساعتين من الحفظ. أيضاً فقد اختلفت نسبة الحيوانات المنوية سليمة منطقة الأكروسوم بين المجموعات المختلفة. فقد تحسنت معنوياً بإضافة المستويات المرتفعة من فيتامين هـ. حيث كانت متوسطات نسبة الحيوانات المنوية سليمة منطقة الأكروسوم كالآتي: ٩١,٦٧ و ٩٢,٠٠ و ٩٥,٣٣ للثلاث مستويات من الفيتامين على التوالي. بينما كانت النسبة ٨١,٣٣ و ٧٩,٣٣ لعينة المقارنة الأولى والثانية على التوالي. أما نسبة الحيوانات المنوية الميتة ونسبة الحيوانات المنوية مكسورة الرأس ونسبة الحيوانات المنوية مكسورة الزيل فلم تختلف معنوياً بين المجموعات المختلفة. بصفة عامة يمكن استنتاج أن إضافة فيتامين هـ في مخففات السائل المنوي للأرناب يؤدي إلى المحافظة على درجة تمثيل الحيوانات المنوية ويؤدي إلى المحافظة على منطقة الأكروسوم للحيوانات المنوية. كذلك فقد كانت نسب الخصوبة المتحصل عليها للمستويات المختلفة من إضافة فيتامين هـ إلى السائل المنوي المخفف هي ٧٥ ، ٧٥ ، ٧٠ ، ٨٥ لكل من مجموعة المقارنة والمستوى الأول والثاني والثالث من مستويات إضافة فيتامين هـ على التوالي.