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Original Article

Effects of Vitamin E Addition to Chicken Semen on Sperm Quality During in Vitro Storage of Semen

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Abstract

The purpose of this study was to evaluate the probable effects of the vitamin E addition in different levels to the extender of chicken semen on spermatozoa quality during storage of semen at 4°C for 0, 3, 6, 10 and 24 hours. Eight young Ross broiler breeder strain 308 roosters were used in this experiment. The collected semen from all roosters was mixed together and diluted with modified a Ringer's solution. The diluted pooled semen was divided into 5 treatments (T). T1 was a control group without any vitamin E addition. For T2 to T5 groups 0.5 %, 1 %, 2 % and 3 % vitamin E (w/v), were added respectively. Treatments were evaluated for sperm motility, sperm viability and probable morphological defects after 0, 3, 6, 10 and 24 hours of incubation at 4°C. The evaluations of spermatozoa immediately after semen collection, were revealed no significant differences among values of treatment groups**,** whereas after incubating the treatments for different spans of time, the sperm progressive motility and viability rates for groups supplemented with vitamin E were significantly ($P < 0.05$) higher than that of the control group. In addition, morphological defect rates of chicken spermatozoa in the groups supplemented with different levels of vitamin E were significantly ($P < 0.05$) lower than that in control group. According to the results of this study we conclude that, the most excellent level of vitamin E for supplementation to the extended semen of chicken in order to improve the sperm motility and viability plus to reduce the morphological defect rates of the spermatozoa up to 24 hours storage time at 4°C is 2 % (w/v).

Key words: Chicken, Semen, Vitamin E, Sperm quality, Semen storage

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Introduction

Lipids are a basic component of semen. They are present in both the spermatozoa and seminal plasma, probably playing different explicit roles. Originally, the interest in sperm lipid began because these compounds were supposed to be an energy substrate for spermatozoon metabolism.[1] Currently, it is clear that, lipids are involved not only in sperm energy metabolism[2] but also in all the main functions and events that lead to fertilization. Lipid composition of semen is unique in its content of long chain polyunsaturated fatty acids (LCPUFAs) and these LC-PUFAs are essential components of all cell membranes and also give rise to many bioactive molecules, such as eicosanoids.[3] Because of their highly unsaturated lipids, spermatozoa are very susceptible to peroxidative damage resulting from the acts of free radicals and reactive oxygen species[1]. Oxidative stress is known to play a most important role in the etiology of defective sperm function via mechanisms involving the induction of peroxidative damage to the plasma membrane,[4,5] a subsequent reduction of sperm motility[6] and a decline in cell quality which results in insufficient records of viable spermatozoa and fertility.[4] The use of chilled-stored semen is limited by its relatively short time fertilizing capacity.[7] Oxidative damage of spermatozoa during storage is a potential cause of the decline in motility and fertility during hypothermia storage of liquid semen. The survival of ejaculated sperm in seminal plasma alone is limited to a few hours[8]. The antioxidants are playing important roles in avian reproduction. Vitamin E is a natural antioxidant capable of enhancing semen quality and fertilizing ability of chickens, when it is provided at a level some 500 times greater than the NRC requirements (15 IU/kg diet)[9]. In chicken vitamin E prevents lipid peroxidation of spermatozoa.[10] The association of vitamin E deficiency with impaired male reproduction has been established for three decades, and usually it is called the 'anti-sterility' vitamin. Vitamin E ameliorates oxidative stress in spermatozoa helping to maintain optimum fertilizing ability. Recently, vitamin E supplements have been widely used in poultry diets enhancing production and reproductive performance several folds[11]. The principal aim of this experiment was to study the effects of vitamin E addition to the extender of chicken semen on sperm motility, viability and morphological defect rates in terms of incubation time. The results of this study provided essential knowledge of using vitamin E as an antioxidant for improving sperm quality in chicken semen and could help artificial insemination industry in chicken.

Materials and Methods

The Experiments were performed in poultry research farm of Ramin Agriculture University in Khuzestan province, Iran. Eighteen Ross broiler breeder strain 308 roosters with the similar ages (30 weeks old) and nearly the same weight (3.2 kg) were used at this study. All roosters were maintained in enclosed houses and fed with standard breeder diet (2700 kcal kgG, 13 % protein, 1 % calcium, 0.45 % phosphorous). All males received 16 h light/day throughout the experiment. The roosters were trained to give semen 10 days before the collection began. Semen was collected by the abdominal massage method.[12] Modified Ringer's solution (sodium chloride: 68 g, potassium chloride: 17.33 g, calcium chloride: 6.42 g, magnesium sulphate: 2.5 g, sodium bicarbonate: 24.5 g, distilled water: 10,000 mL) were used as a semen diluent. Glass test tubes were used for semen collection. After exciting of the roosters with abdominal massage, the copulatory organ become protrudes out and downwards, then white semen seen in the central furrow of the organ. The semen was milked down by firm finger pressure

either side of the vent into the collecting tube. The semens discolored due to contamination were discarded. The collected semen from all roosters was mixed together and then diluted (1:100). The temperature of dilution was approximately 15°C and this temperature was maintained during the examination. The diluted pool semen was divided into 5 treatments (T) in separated tubes. For one of these groups no vitamin E was used (T1 or control group). For other samples were added 0.5 % (T1), 1 % (T2), 2 % (T3) and 3 % (T4) of vitamin E (w/v) (α-tocopherol, Sigma Chemical Co.), respectively. Treatment groups initially were evaluated for semen quality immediately after semen collection (before incubation) and then were stored in 4°C and evaluated for semen quality at 3, 6, 10 and 24 hours of incubation. The present study was performed in quadruplicate.

Semen quality assessment. The semen quality evaluations were comprised: progressive motility, viability and morphological defect rates in all of treatment samples at different incubation periods. For evaluation of motility, one drop of the diluted semen was placed on the slide and covered with a cover slide. The sperm motility was estimated by microscopic observation (400 × magnification). Motility was expressed as the percentage of motile spermatozoa with moderate to rapid progressive movement. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 sperm for each sample (according to protocol provided by others.[13, 14] Sperm viability was assessed by an eosin exclusion test. Five microliters of sperm dilution were mixed with 5 microliters of eosin solution. Immediate count of uncolored sperms was used to calculate the percentage sperm viability.[15] The number of 200 spermatozoa were evaluated to determine viability (according to protocol provided by Breininger et al. 2004[13]). A phase-contrast microscope with immersion was used for morphological examinations. Sperm morphology was examined in smears stained with eosin and nigrosin. At each preparation 300 cells were counted and the percentage of various defects calculated. The morphological defects of acrosome, head, mid-piece, tail and their proportions were evaluated.[14]

Statistical analyses. The spermatozoa quality parameters were including, percentages of sperm motility, viability and morphological defects among various treatment groups were evaluated by using one-way ANOVA and Duncan's Multiple Range Test to locate differences. Values were presented as Mean ± SEM and the level of significance was set at $P < 0.05$.[16]

Results

With different levels of vitamin E in extended chicken semen, the progressive sperm motility, sperm viability and morphological defect levels were determined at 0, 3, 6, 10 and 24 hours of the incubation periods at 4°C. These results are presented in tables 1, 2 and 3.

Spermatozoa motility. The evaluations of spermatozoa immediately after semen collection showed that the sperm motility rate differences were not significant among treatment groups ($P > 0.05$). After 3 hours of the incubation, the sperm motility of T2, T3, T4 and T5 groups were significantly higher than that of T1 (0%) ($P < 0.05$). At 6 hours, the sperm motility of T3 to T5 was significantly higher than that of T1 and T2. In this incubation period, sperm motility was significantly higher in T2 when compared with T1 ($P < 0.05$). At 10 hours, the sperm motility of T4 and T5 was higher ($P < 0.05$) than that of other groups, and, sperm motility of T2 group was significantly higher than that of T1 group ($P < 0.05$). After incubation for 24 hours, the sperm motilities of T4 and T5 were significantly ($P < 0.05$) higher than that of T1 to T3 groups. In this incubation period, sperm motilities of T2

and T3 were higher ($P < 0.05$) than T1 group (Table 1).

Table 1. Effects of vitamin E addition to extender of chicken semen on sperm motility (%) at different periods of incubation

Experimental period	T1 (control)	T2 (0.5%)	T3 (1%)	T4 (2%)	T5 (3%)
0 h	74.67 ± 1.45^a	72.85 ± 2.40^a	75.22 ± 1.20^a	76.10 ± 2.31^a	76.17 ± 1.73^a
3 h	62.45 ± 1.15^a	71.47 ± 3.16^b	73.67 ± 1.88^b	72.33 ± 1.70^b	74.09 ± 1.45^b
6 h	49.35 ± 3.86^a	61.61 ± 2.67^b	69.28 ± 2.53^c	71.11 ± 2.49^c	69.33 ± 2.12^c
10 h	36.86 ± 3.40^a	47.10 ± 4.03^b	60.08 ± 2.53^c	67.30 ± 2.90^d	68.67 ± 3.13^d
24 h	16.17 ± 5.15^a	35.35 ± 4.86^b	37.12 ± 3.40^b	65.33 ± 4.42^c	66.47 ± 5.10^c

Mean ± SE values with different superscripts in the same row differ significantly ($P < 0.05$)

Spermatozoa viability. The evaluations of spermatozoa immediately after semen collection reveal were revealed that the sperm viability percent differences were not significant among treatment groups ($P > 0.05$). After 3 hours, the sperm viability of control group (T1) was significantly lower than that of T2 to T5 groups. After the incubation for 6 hours, sperm viabilities of T4 and T5 groups were significantly higher than that of other groups ($P < 0.05$). At this period, sperm viability in T1 was lower ($P < 0.05$) than that of T2 group. At 10 hours, sperm motilities in T4 and T5 were higher ($P < 0.05$) than other groups. In T3 was higher ($P < 0.05$) than T1 and T2 groups and finally in T2 was higher ($P < 0.05$) than control (T1) group. At 24 hours of incubation, the sperm viabilities of T4 and T5 were higher than other groups ($P < 0.05$). In this incubation period, sperm viabilities of T2 and T3 were higher ($P < 0.05$) than that of T1 group (Table 2).

Table 2. Effects of vitamin E addition to extender of chicken semen on sperm viability (%) at different periods of incubation

Experimental period	T1 (control)	T2 (0.5%)	T3 (1%)	T4 (2%)	T5 (3%)
0 h	90.42 ± 3.72^a	92.93 ± 3.54^a	93.32 ± 3.86^a	91.68 ±4.15^a	94.07 ± 3.80^a
3 h	82.19 ± 2.26^a	83.29 ± 1.85^a	91.05 ± 2.30^b	90.27 ± 3.11^b	92.08 ± 2.90^b
6 h	71.22 ± 2.84^a	82.12 ± 2.86^b	83.33 ± 2.11^b	89.20 ± 2.12^c	90.13 ± 2.03^c
10 h	51.18 ± 2.89^a	62.37 ± 3.44^b	79.57 ± 3.60^c	88.46 ± 3.08^d	88.17 ± 2.88^d
24 h	33.04 ± 4.29^a	48.21 ± 3.24^b	49.02 ± 2.81^b	84.21 ± 3.07^c	85.44 ± 3.83^c

Mean ± SE values with different superscripts in the same row differ significantly ($P < 0.05$)

Morphological defects of spermatozoa. In the evaluations of spermatozoa immediately after semen collection, there were no significant differences ($P < 0.05$) among mean values of treatment groups**.** After 3 hours of incubation, the sperm morphological defects percent of T4 and T5 were lower than that of T1, T2 and T3 groups ($P < 0.05$), and the sperm morphological defects of T2 and T3 were lower than T1 (control) group. After 6 hours, the morphological defect values of spermatozoa in T4 and T5 were lower ($P < 0.05$) than other groups. At this incubation time, the level of this defect in T3 was lower than control group ($P < 0.05$). After 10 hours of the incubation, sperm defect of T5 was lower than other groups ($P < 0.05$) and the sperm defects of T3 and T4 were lower than that of T1 ($P < 0.05$). In this incubation period, morphological defect intensity of spermatozoa in T2 was lower ($P < 0.05$) than control group. After the incubation for 24 hours, sperm defect of T4 and T5 groups were significantly lower ($P < 0.05$) than the other groups. At this incubation period, sperm defect in T1 was higher ($P < 0.05$) than that of T2 group (Table 3).

Table 3. Effects of vitamin E addition to extender of chicken semen on sperm morphological defects (%) at different periods of incubation

Experimental period	T1 (control)	T2 (0.5%)	T3 (1%)	T4 (2%)	T5 (3%)
0 h	8.22 ± 1.45^{a}	8.43 ± 1.77^{a}	7.81 ± 1.30^{a}	8.66 ± 1.09^{a}	7.59 ± 1.80^{a}
3 h	14.86 ± 2.85^{b}	13.60 ± 3.05^{b}	14.12 ± 2.41^{b}	10.22 ± 1.39^{a}	10.50 ± 1.28^{a}
6 h	21.63 ± 2.53^{c}	18.29 ± 3.17^{bc}	16.10 ± 2.31^{b}	11.39 ± 2.20^{a}	11.26 ± 1.96^{a}
10 h	34.55 ± 3.17^{d}	23.19 ± 2.54^{c}	17.73 ± 2.86^{b}	16.93 ± 2.15^{b}	12.13 ± 1.81^{a}
24 h	57.60 ± 4.31^{c}	32.26 ± 3.93^{b}	30.86 ± 3.66^{b}	19.20 ± 2.47^{a}	20.47 ± 4.55^{a}

Mean ± SE values with different superscripts in the same row differ significantly ($P < 0.05$)

Discussion

The oxidative stress is one of the factors associated with decline in fertility during semen storage. The sperm plasma membrane contains a high amount of unsaturated fatty acids, therefore particularly is susceptible to peroxidative damages with subsequent loss of membrane integrity, imparted cell function and decreased motility of spermatozoa.[7, 8] Vitamin E is a lipid-soluble compound with an effective membrane-stabilizing antioxidant function within the cell membrane,[17] and is recognized as a natural component in male gametes. Studies on chicken, concerning the effect of vitamin E supplementation inside the extended semen on spermatozoa quality at different incubation periods of semen has not been published so far and, to our knowledge, the present report is the first one on this subject. The results of this study revealed that, supplementing of the vitamin E in semen extender of chicken improved spermatozoa quality parameters including motility, viability and morphological defect rates after incubating the semen at different periods. Rather similar results have also found in chickens,[18] boars,[19] humans,[20] rams [21,22] and rats,[23] where supplementation of food rich in Vitamin E improved semen quality. Marin-Guzman et al. (1997)[24] reported Vitamin E deficiency in boars adversely affected sperm motility. The results (Tables 1, 2 and 3) indicate that, the best concentration of vitamin E for supplementation to the extended semen of chicken in order to improve the sperm motility and viability as well as reduce the morphological defect rates of spermatozoa after all incubation periods is 2%. The beneficial effect of vitamin E on sperm motility was previously reported by Thuwanut et al. (2008) [25] in cat and Boonsorn et al. (2010) [4] and Pena et al. (2003) [26] in swine. They supplemented vitamin E analogue in an extender and found that this vitamin could improve the motility of cat frozen-thawed epididymal sperm and boar sperm, respectively. Michael et al. (2008) [27] studied the effect of antioxidant on the motility of canine sperm and found that catalase, NAC (N-acetyl-cysteine) and vitamin E could improve the motility and rapid steady forward movement of post-thaw canine sperm, in vivo. Askari et al. (1994) [28] reported minimal improvements in maintaining the human spermatozoa motility when the freezing media contained vitamin E. Marin-Guzman et al. (1997) [24] supplemented selenium in the diet of boar and found that the motility of the boar sperm increased. Moreover, Bucak et al. (2008) [29] supplemented trehalose, taurine and cysteamine in an extender of ram semen and found that, this could also increase the sperm motility. The results from this study are in conformity with the results of Cerolini et al. (2000) [30] who showed that, the addition of alpha-tocopherol (Vitamin E) in the semen extender could help improve the viability of boar sperm. Vitamin E tends to improve semen quality in chickens by increasing concentrations of spermatozoa and cell

viability.[31] Dietary supplementation of 250 or 500 mg/kg of L-carnitine (one of the antioxidants) increased the viability of the mature male Japanese quail breeder spermatozoa.[32] Complementary to our results, Biswas et al. (2007) [9] reported that, the semen characteristics include sperm motility and percentage of live spermatozoa did not differ statistically among the different dietary treatment with vitamin E. The relatively wide difference in response to vitamin E also indicate that, the quality of sperm samples from some persons might be improved with the addition of vitamin E to extender.[28] The beneficial effects of Vitamin E noted in the present study can be attributed to the antioxidant effects comparable to those reported by Sen et al. (2004).[33] The high levels of antioxidants in seminal plasma carried out an important role for the spermatozoa in vitro storage. Addition of antioxidants significantly decreased the amount of spermatozoa DNA damage induced by ROS in vitro.[34] Vitamin E as a lipid soluble antioxidant plays a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals (particularly strong scavenger of hydroxyl radicals) which are toxic byproducts of many metabolic processes in biological membranes.[20,35-38] A probable improvement in semen quality by addition of the vitamin E in semen extender is more likely related to an inhibition of lipid peroxidation of the sperm plasma membrane as was revealed in rats[23] and fish.[39] This possibility is supported by the type of sperm abnormalities that showed an improvement i.e., neck piece and tail piece defects, distal droplets. However, adding vitamin E to semen has resulted a variable success in improving the maintenance of motility. According to the study of Ball et al. (2001),[8] vitamin E did not significantly alter the maintenance of motility during cold storage of equine spermatozoa for either 72 or 96 hours. Parallel to our results, Aguero et al. (1995) [40] reported that, adding vitamin E to stallion semen improves the maintenance of motility and viability during 24 hours cold storage. The adding of vitamin E on ram semen improves the maintenance of motility in stored semen.[22] Vitamin E and Se act as antioxidants which in turn may provide direct protection of sperm cells from morphological damage by preventing free radical oxygen from damaging sperms. The vitamin E helps protecting the sperms cell membranes from damage.[19] Furthermore, the morphology and the motility of sperm cells would be preserved by the binding of Vitamin E to endoperoxides.[41] Our findings are in conformity with those of other authors reports, who stated that, Vitamin E supplementation leads to improved motility and percentage of normal sperm.[42] The positive effect of vitamin E on prevention of occurrence of abnormal spermatozoa which revealed by our study is in accordance with a previous report by Behne et al. (1996) [43], Köhrle et al. (2005) [44] and Olson (2005) [45].

Conclusions. 1) Semen obtained from Ross-308 broiler breeder roosters supplemented with vitamin E was characterized better resistance to storage at 4°C. This was reflected by better motility and viability as well as lower morphological defects of spermatozoa after semen storage for 24 hours. 2) The appropriate level of vitamin E advised for supplementation to the extended semen of chicken in order to improve the sperm motility and viability as well as reduce the morphological defect rates of the spermatozoa up to 24 hours during storage at 4°C was 2 % (w/v).

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