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Effect of α-Tocopherol Supplementation in Rooster Semen on Sperm Quality Parameters during *in-Vitro* Storage at 4°C

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ABSTRACT

The purpose of this study was to evaluate the probable effects of the α-tocopherol (Vitamin E) addition in different levels to the Tris based egg yolk plasma (T-EYP) extender in semen of Rhode Island Red roosters on spermatozoa quality during storage of semen at 4°C for 0, 24,48 and 72h. The collected semen from roosters was mixed together and extended with T-EYP. The extended 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) was added respectively. Treatments were evaluated for sperm motility, sperm viability, morphological defect, membrane integrity and acrosome integrity after 0, 24, 48 and 72 h of incubation at 4°C. The evaluations of spermatozoa immediately after semen collection, revealed no significant differences among values the 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 spermatozoa in the groups supplemented with different levels of α-tocopherol were significantly ($P<0.05$) lower than the control group. It was found that 0.5% level of α-tocopherol in extended rooster semen improved the sperm motility, viability plus to reduce the morphological defect rates of the spermatozoa up to 72 h storage time at 4°C.

Key words: Chilled Semen, α-Tocopherol, RIR breed, Sperm Attributes

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INTRODUCTION

Artificial insemination in the poultry industry is the key to intensive rearing of breeding roosters. The use of high fertility males is an integral part for artificial insemination. Semen preservation during cold shock causes damages to the membrane of the sperm cell. The damage to the membrane increases the risk of being attacked by reactive oxygen species (ROS) which can engender oxidative stress. Several strategies have been used to increase the lifespan of spermatozoa which reduces the metabolism of the sperm and motility via chilling the sperm at

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lower temperatures (Allai *et al*., 2018). Moreover, during cryopreservation sperm membrane is more prone to lipid peroxidation by free radicals such as O_2 and H_2O_2 which leads to structural damage of sperm membrane (Mavi *et al.,* 2020a; Kaur et al., 2020; Bisla *et al*., 2021a; Kumar *et al*., 2021; 2022). The major detrimental changes due to oxidative stress mainly occur at the plasma membrane and nucleus of the spermatozoa (Bisla *et al*., 2020; 2021b; Ngou *et al*., 2020; Rautela *et al*., 2022). Dietary supplementation of fatty acids had previously been reported to improve semen quality in different species (Gholami *et al*., 2010). Vitamin E has been regarded as a potent chain-breaking antioxidant as well as immuno-stimulator for both cell-mediated and humoral immunity (Abd El-Ghany, 2022). α-Tocopherol present in the plasma membrane provide energy, modulate the structure and composition of lipid rafts, regulate plasma membrane proteins, maintain and normalize plasma membrane function, sustain sperm viability and fertility during chilling and freezing (Shevchenoko and Simons, 2010). However, studies in poultry semen are sparse and limited. Hence, this study was undertaken to assess the effects of α-Tocopherol supplementation in extender of poultry semen and its effect on seminal parameters during *in vitro* storage at 4°C till 72h.

MATERIALS AND METHODS

Roosters (36–40 weeks old) were kept in individual cages and were given poultry feed and water ad libitum. Experiment was conducted on two generations of RIR breed (n=80) of first generation and evaluated for semen attributes and fertility. Based on semen and fertility analysis, roosters of second generation were selected (Mavi *et al*., 2019a; 2019b). Semen of ten roosters (32-36 weeks)was collected twice a week and checked for motility and only with > 70 % motility was pooled in Tris-citric acid-fructose buffer with EYP (Egg Yolk Plasma, Cheema *et al*., 2021). The extended semen of each ejaculate in six fractions of α-Tocopherol was equilibrated at 4°C in a cold cabinet and evaluated for different semen characteristics such as motility (subjective), viability (Eosin-nigrosin staining), membrane integrity and acrosome integrity (Giemsa staining) at different intervals of time.

Statistical analysis

Data of various sperm attributes was analyzed by one way ANOVA using SPSS21 program (Student version for windows, SPSS Inc.233 South Wacker Drive, 11th floor Chicago, IL 60606-6412) to find out difference among the treatments. Normality of the data was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using the Levene test. Data of various sperm attributes was analyzed by one way analysis of variance (ANOVA) test.

RESULTS AND DISCUSSION

The supplementation of the α-Tocopherol in different levels to the Tris based egg yolk plasma (T-EYP) extender in poultry semen on spermatozoa quality was evaluated during storage of semen at 4°C for 0, 24, 48 and 72h. T1 was a control group without any α-Tocopherol addition. For T2 to T5 groups 0.5 %, 1 %, 2 % and 3 % vitamin E (w/v) was added respectively. Treatments were evaluated for sperm motility, sperm viability, morphological defect, membrane integrity and acrosome integrity after 0, 24, 48 and 72h of incubation at 4°C. The evaluations of spermatozoa immediately after semen collection, 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. Morphological defect rates of spermatozoa in the groups supplemented with different levels of α-tocopherol were significantly ($P<0.05$) lower than the control group. Semen obtained from rooster supplemented with vitamin E 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 72 h. 0.5% level of α-tocopherol in extended rooster semen improved the sperm motility, viability plus to reduce the morphological defect rates of the spermatozoa up to 72 h storage time at 4°C. Mavi *et al*. (2017, 2020b) reported that only roosters with higher sperm attributes selected for artificial insemination, resulted in significant improvement in the fertility of artificially inseminated hens and thereby reduce maintenance cost of large number of cocks.

CONCLUSIONS

Addition of antioxidants such as Vitamin E to the preservation media could improve longevity and quality of sperm in poultry semen.The appropriate level of vitamin E advised for supplementation to the extended semen of poultry in order to improve the sperm motility and viability as well as reduce the morphological defect rates of the spermatozoa up to 72 h during storage at 4°C was 0.5% (w/v). Selection of roosters on the basis of combination

Table 1: Effect of vitamin E in EYP Extender of rooster Semen on Motility and Viability

	MOTILITY					VIABILITY				
-	T 1 (Control group)	T2 (0.5%)	T 3 (1%)	T4 (2%)	T5 (3%)	T 1 (Control group)	T2 (0.5%)	T 3 (1%)	T4 (2%)	T5 (3%)
0 hr	81.33[a] ± 3.33	83.44[a] ± 4.02	82.48[a] ± 3.32	80.82[a] ± 3.14	81.33[a] ± 3.33	80.20[a] ± 5.89	80.61[a] ±1.56	80.39[a] ± 2.56	79.17[a] ± 3.86	80.33[a] ± 3.33
24 h	68.33[b] ± 3.33	80.14[a] ± 3.29	74.50[b] ± 4.08	73.03[b] ± 2.24	70.33[b] ± 3.33	65.67[b] ± 2.85	81.48[a] ±0.83	78.25[a] ± 4.36	68.59[b] ± 4.81	68.33[b]± 3.33
48 h	59.00[c] ± 5.00	75.32[a] ± 3.52	71.67[a] ± 4.65	65.90[b] ± 5.85	63.00[b] ± 5.00	51.67[c] ± 0.67	77.03[a] ±1.40	75.86[a] ± 3.15	66.68[b] ± 3.93	62.00[b]± 5.00
72 h	45.33[c] ± 6.01	68.09[a] ± 4.13	60.90[a] ± 5.85	55.23[b] ± 4.50	53.33[b] ± 6.01	42.00[d] ± 1.00	72.21[a] ±1.39	63.20[b] ± 4.56	53.83[c] ±2.67	50.33[c] ± 6.01

Superscripts a, b, c, d different significantly (P<0.05) within row

Table 2: Effect of vitamin E to EYP Extender of rooster Semen on HOST And Acrosome Integrity

Semen attributes	HOST					Acrosome Integrity				
	T 1 (Control group)	T2 (0.5%)	T 3 (1%)	T4 (2%)	T5 (3%)	T 1 (Control group)	T2 (0.5%)	T 3 (1%)	T4 (2%)	T5 (3%)
0 hr	77.33[a] ± 3.33	80.44[a] ± 4.02	78.00[a] ± 2.89	76.33[a] ± 1.67	77.33[a] ± 1.67	79.33[a] ± 3.33	82.23[a] ± 2.08	81.04[a] ± 2.83	79.37[a] ± 2.43	80.67[a] ± 3.33
24 h	69.33[b] ± 3.33	78.14[a] ± 3.29	76.33[a] ± 4.41	71.67[b] ± 1.67	70.00[b] ± 2.88	68.33[b] ± 3.33	78.04[a] ± 2.83	74.60[a] ± 4.55	74.73[b] ± 0.84	75.00[b]± 2.88
48 h	59.29[d] ± 5.00	77.32[a] ± 3.52	70.67[b] ± 4.41	63.33[c] ± 1.67	61.67[c] ± 1.67	52.21[d] ± 5.00	74.60[a] ± 4.55	68.10[b] ± 4.01	61.03[c] ± 1.50	60.33[c] ± 1.67
72 h	48.33[d] ± 6.01	70.09[a] ± 4.13	61.67[b] ± 3.33	56.58[c] ± 1.67	54.33[c] ± 1.67	47.33[d] ± 6.01	69.19[a] ± 4.01	60.08[b] ± 3.47	54.27[c] ± 2.85	51.33[c] ± 1.67

Superscripts a, b, c, d different significantly (P<0.05) within row.

Table 3: Effect of vitamin E to EYP Extender of ROOSTER Semen on sperm morphological defects (%)

Experimental period	T1 (control)	T2 (0.5%)	T3 (1%)	T4 (2%)	T5 (3%)
0 hr	**14.86[a] ± 2.85**	**10.22[b] ±1.39**	**10.50[b] ±1.28**	**14.12[a] ±2.41**	**13.60[a] ±3.05**
24 h	**21.63[a] ±2.53**	**11.39[c] ±2.20**	**11.26[c] ±1.96**	**16.10[b] ±2.31**	**18.29[b] ±3.17**
48 h	**34.55[d] ±3.17**	**12.13[c] ±1.81**	**16.93[b] ±2.15**	**17.73[b] ±2.86**	**23.19[b] ±2.54**
72 h	**57.60[a] ± 4.31**	**19.20[c] ± 2.47**	**20.47[c] ± 4.55**	**30.86[b] ±3.66**	**32.26[b] ±3.93**

Superscripts a, b and c different significantly (P<0.05) within row.

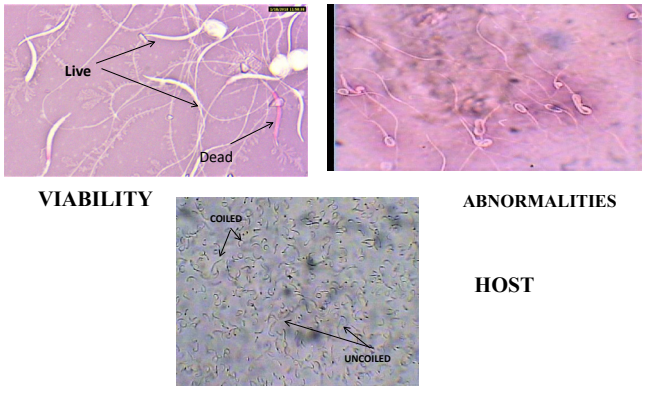

Fig. 1: Showing live/dead (a) and coiled / un-coiled spermatozoa (b) indicating viability and membrane integrity of RIR Bre

of sperm function tests for artificial insemination may improve the overall fertility rate and would be beneficial for breeding programmes.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest with this manuscript.

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