



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

The Use of Certain Grape Constituents for Improve Semen Quality and Storage Ability of Diluted Roosters' Semen

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ABSTRACT

This study was conducted to determine the probable suppressive role of certain grape components on detrimental effects of lipid peroxidation which accompanied with liquid storage of roosters' semen. A total of 60 White Leghorn roosters, 32 weeks of age, randomly divided into 6 groups of 10 roosters each were used in this experiment. Semen samples for each treatment group were collected on a weekly basis during the whole experiment period which lasted 10 weeks. Treatment groups were as follows: T1 = fresh semen (control group); T2 = semen diluted 1: 1 with Lake diluent (LD) alone; T3 = semen diluted with LD and supplemented with 4 ml of grape flavonoids; T4 = semen diluted with LD and supplemented with 4 ml of grape phenols; T5 = semen diluted with LD and supplemented with 4 mg of grape seed proanthocyanidins extract (GSPE); and T6 = semen diluted with LD and supplemented with 4 ml of grape juice concentrate (GJC). Results revealed that after 1, 7 and 14 d *in vitro* storage, the supplementation of roosters' semen diluent with certain grape components (T3, T4, T5 and T6) resulted in significant ($p < 0.05$) improvement in mass and individual motility of spermatozoa in comparison with control group (T1) and significant improvement in regard to percentages of live spermatozoa and normal spermatozoa and acrosomes compared with T1 and T2 groups. Besides, T3 and T5 groups surpasses all other treatments involved in the present study as concerns all of semen traits involved in the present study. In conclusion, grape components especially flavonoids and GSPE gave a good suppression against the detriments of lipid peroxidation during *in vitro* storage of roosters' semen for up to 14d.

Key words: Grape constituents, semen quality, storage ability, roosters' semen

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Received 12 August 2012, Accepted 24 August 2012

Please cite this article in press as: Al-Daraji HJ, The use of certain grape constituents for improve semen quality and storage ability of diluted roosters' semen. American Journal of PharmTech Research 2012.

INTRODUCTION

Free radicals are being generated in organism body in there thousands every day. The organism body does not have a big reserve of antioxidant defense capacity and all the time it produces free radicals and other oxygen derived molecules (such as hydrogen peroxide)¹. Today, people and animals continuously subject to many things that increase the level of free radicals in their bodies and so the body can easily become overpopulated with oxygen – derived species that upset cell biochemistry. This imbalance of free radicals in the body is called oxidative stress². However, oxidative stress is caused by: Increase in free radicals meaning in the body's antioxidant defense system can't cope, antioxidant in the body decreasing with age, increased free radical exposure and an increase in the available transition metal ions, which causes more of the free radicals, produced in the body to be changed to more reactive peroxy species³. Major oxidative stress can cause grave disturbances in cell metabolism and contribute too many diseases. Tissue damage and injury can also lead to oxidative stress. Oxidative stress can cause DNA damage, lipid peroxidation, protein damage, and ischemic (lack of oxygen) injury⁴.

Systems that satisfy the metabolic requirement for oxygenation of avian semen during storage have improved the maintenance of fertilizing ability, but have led to consideration that the limiting factor of sperm maintenance may now be the deleterious effects of oxygen free radicals – and resultant lipid peroxidation. This has been identified as significant and problematic for both chicken and turkey spermatozoa, which, having a high proportion of polyunsaturated fatty acids (PUFAs), are therefore considered to be likely to be particularly susceptible to lipid peroxidation⁵. Indeed, even at low temperatures, spermatozoa of both chickens and turkeys form lipid peroxide on aerated storage⁶. However, a positive correlation between semen quality and fertilizing ability and lipid peroxides following storage has yet to be made. Blesbois⁷ pointed out that in chicken peroxides are already present at the time of ejaculation, in equal concentrations between spermatozoa and seminal plasma (2 to 4 X 10⁻² nM malonaldehyde / 10⁹ spermatozoa). However, sperm are subject to oxygen toxicity resulting from lipid peroxidation, which can result in membrane damage, reduced motility, and lower fertility⁸.

Grape extracts are excellent sources of essential fatty acids and tocopherol as well as being a powerful antioxidant. Grape seeds contain a mixture of compounds called bioflavonoids, also found in other fruits and vegetables in lesser concentrations. These exhibit powerful antioxidant properties that are superior to other known antioxidants like vitamins A, C and E or CoQ10. Grape seed extract contains oligomeric proanthocyanidins (OPC's) perhaps the most powerful

natural antioxidant free – radical scavenger known⁹. As bioflavonoids they help to increase the effectiveness of vitamins C and E in the body. Grape seed extracts remains in the body for up to 3 days and is up to 20 times and 50 times more powerful an antioxidant than vitamins C and E, respectively¹⁰. Grape extracts are one of the most necessary antioxidant supplements for those concerned about free radicals elimination. However, grape OPC's also reduce lipid peroxidation and provide a measure of protection against free radicals. Day et al.¹¹ reported that grape extracts enhance the effectiveness of other antioxidants such as vitamins A, C and E. Because they are many times more powerful as a free radical scavenger they free these vitamins up to perform their other functions. Bagchi et al.¹² stated that grape OPC's is a significantly more potent antioxidant than vitamins C, E and beta – carotene, and inhibits damage to cell lipids, proteins and DNA caused by free radicals. Consequently, it becomes very important for anyone concerned about lipid peroxidation in fowl semen to supplement their semen diluents with grape compounds which contain abundant levels of potent natural antioxidants. Therefore, the aim of the present study was to evaluate the efficacy of the grape components to preserve roosters spermatozoa during *in vitro* storage for up to 14 d. Grape components were chosen because they are the most powerful antioxidants known and because grape OPC's are able to take up and suppress most of the free radicals of oxygen within biological systems¹³.

MATERIALS AND METHODS

This experiment was carried out to determine the effect of diluent supplementation with certain components of grape on semen quality during liquid storage of roosters' semen. A total of 60 White Leghorn roosters, 32 weeks of age were used in this study and randomly distributed into 6 treatment groups of 10 roosters each. Roosters fed a commercial ration (16% protein and 2850 Kcal metabolic energy / kg of diet) ad libitum. The semen was collected from all roosters manually by dorsal – abdominal massage¹⁴, one time a week regularly, for consecutive weeks (32 – 42 weeks of age). Semen samples for each treatment pen were divided into 3 test tubes of 1 ml each to provide 3 replicates pooled samples per each treatment group. Therefore, there were 30 replicates for each treatment group during the total period of experiment. In order to maximize the quality and quantity of the collected semen, collection was always performed under the same conditions (environment, time, person, massage method). Only clean ejaculates were used for evaluating. The experimental groups were as follows: T1 = fresh semen (control group); T2 = semen diluted 1:1 with Lake diluent (LD;¹⁵) alone; T3 = semen diluted with LD and supplemented with 4 ml of grape flavonoids; T4 = semen diluted with LD and supplemented

with 4 ml of grape phenols; T5 = semen diluted with LD and supplemented with 4 mg of grape seed proanthocyanidins extract (GSPE); and T6 = semen diluted with LD and supplemented with 4 ml of grape juice concentrate (GJC). The pH of diluents was adjusted to be 6.6 – 6.8 by using phosphate buffer solution. However, the levels of these components of grape involved in the LD were chosen on the basis of the preliminary experiments that were conducted before the initiation of the present study (unpublished data). Semen samples were then stored at refrigerator temperature (4 – 6 °C) for certain storage periods (1, 7 and 14 days). Besides, grape flavonoids were extracted according to the procedure mentioned by Harborne¹⁶, grape phenols were extracted by using the method of Bourzeix et al.¹⁷, GSPE were extracted on the basis of the procedure described by Pekic et al.¹⁸, while GJC was prepared according to the method mentioned by Osman et al.¹⁹.

An aliquot of semen from each treatment group was evaluated directly after semen collection or at 1, 7 and 14 days of *in vitro* storage for mass activity, individual motility, and percentages of dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities. Spermatozoa motility (movement in a forward) was estimated on a percentage basis by using the microscopic method of Sexton²⁰. The measurement of dead spermatozoa was achieved by using a fast green stain – Eosin B stain – glutamate extender²¹. Percentage of abnormal spermatozoa was determined by using a Gentian violet – Eosin stain²². As an alternative to evaluate acrosomal abnormalities in birds, staining procedure for fixed samples have been developed to distinguish which spermatozoa have retained or lost acrosome²³. Data of experiment were evaluated by analysis of variance. Differences between experimental groups' means were analyzed by Duncan's multiple range test, using the ANOVA procedure in Statistical Analysis System²⁴.

RESULTS AND DISCUSSION

When semen samples were evaluated directly after collection, it was noticed that T2, T3, T4 and T5 surpassed other treatments (T1 and T6) with relation to mass activity and individual motility. Whereas there were no significant differences between T1 and T6 groups, and T3 and T5 groups were superior to other treatments as regards these two traits (Figures 1 and 2). After *in vitro* storage of 1, 7 and 14 days, it was found that supplementation of grape components into LD (T3, T4, T5 and T6) resulted in significant ($p < 0.05$) increase in mass activity and individual motility compared with control group (Figures 1 and 2). However, when a comparison was made between grape treatments (T3, T4, T5 and T6), it was seen that T3 recorded the best results with regard to these two traits followed by the results of T5, while the worst results were found with

T6 group. In addition, there were no significant differences between T2 and T4 groups (Figures 1 and 2).

The data obtained from this study in relation to livability and normality of spermatozoa and acrosomes denoted that T3, T4 and T5 groups were superior to ($p < 0.05$) control group (T1) when semen samples were tested just after semen collection. However, no significant differences were observed between T2 and T4 regarding these 3 characteristics, whereas there were no significant differences between T1 and T6 as concerns percentages of live and normal spermatozoa (Figures 3, 4 and 5). On the other hand, when semen samples were evaluated after 1, 7 and 14 day of *in vitro* storage, results revealed that inclusion of grape components (T3, T4, T5 and T6) into semen diluent resulted in significant ($p < 0.05$) amelioration in percentages of live spermatozoa and normal spermatozoa and acrosomes as compared with T1 and T2 groups (Figures 3, 4 and 5). In addition, T3 and T5 surpass other two grape treatments (T4 and T6) in regard to the three traits mentioned hereinbefore.

Results of this experiment clearly indicated that after 1, 7 and 14 day *in vitro* storage, the addition of grape components to semen diluent resulted in significant improvement in spermatozoa motility in comparison with control group (T1) and significant improvement with relation to percentages of live spermatozoa and normal spermatozoa and acrosomes compared with control group (T1) and T2. Besides, T3 and T5 groups recorded the best results concerning semen characters included in this study when compared with the results of all other treatments (T1, T2, T4 and T6).

The wholesome effects in semen quality that accompanied with the enrichment of semen diluent with grape components may be accounts for its very rich content of antioxidants material²⁵. These results are in agreement with the results of other authors^{26,27} who found that the supplementation of some antioxidants (Vitamins A, C or E) to the semen diluents resulted in significant improvement in motility, survivability, morphology and fertilizing ability of roosters' semen after *in vitro* storage for different periods. Bagchi²⁸ pointed out that grape OPC's are totally assimilated into the body within seconds and at work very quickly. It enhances the effectiveness of other antioxidants such as vitamins A, C and E. because it is many times more powerful as a free radical scavenger, it free them up to perform their other functions. Borse et al.²⁹ reported that flavonoids exert its antioxidant capacity by neutralizing all types of oxidizing radicals – including the superoxide – and hydroxyl radicals – and by chelatin. A chelator binds to metal ions to prevent them being available for oxidation. Flavonoids can also act as powerful chain – breaking antioxidants due to the hydrogen – donating capacity of their phenolic groups.

The antioxidant properties of flavonoids reside in their scavenging of free radicals and their ability to chelate metals; these two properties are the basis of biological effects such as inhibition of lipid peroxidation, protection of low density lipoprotein (LDL) against oxidation, inhibition of enzyme activities, and suppression of tissue free radical injury³⁰. Flavonoids extracted from grape are highly effective scavengers of harmful free radicals. These compounds seem particularly helpful in curbing free – radical damage (from peroxidation) which can alter fats and lipids (LDL) embedded in cell membranes.

Meyer et al.³¹ stated that grape seed extract (OPC's) are bioavailable and provides significantly greater protection against free radicals, free radical – induced lipid peroxidation, free radical – mediated tissue injury, oxidative stress and DNA damage than vitamins C, E and beta – carotene. Consumption of grape which rich in flavonoids is associated with a reduced risk of various chronic diseases. The protective benefits of dietary flavonoids may be due to in part to their antioxidant properties and ability to reduce oxidative stress³². In some experiments the antioxidant capacity of flavonoids was shown to exceed that of Trolox or α – tocopherol³³. Because of the diverse chemical structures of flavonoids and their metabolites, they can have hydrophilic or relatively lipophilic properties and may interact with plasma proteins as well as the polar surface region of phospholipids bilayers in lipoproteins and cell membranes³⁴. Because of the nature of these interactions, flavonoids may have the ability to protect against free radical attack in both aqueous and lipid environments, thus providing an effective antioxidant defense in biological systems.

Grape juice is a rich source of the antioxidant flavonoids catechin, epicatechin, quercetin, and anthocyanins. *In vitro* studies showed that grape juice has significant antioxidant activity and can inhibit oxidation of LDL¹³. O'Byrne et al.³⁵ found that 10 ml Concord grape juice. Kg⁻¹.d⁻¹ increased serum antioxidant capacity and protected LDL against oxidation to an extent similar to that obtained with 400 IU α – tocopherol / d.

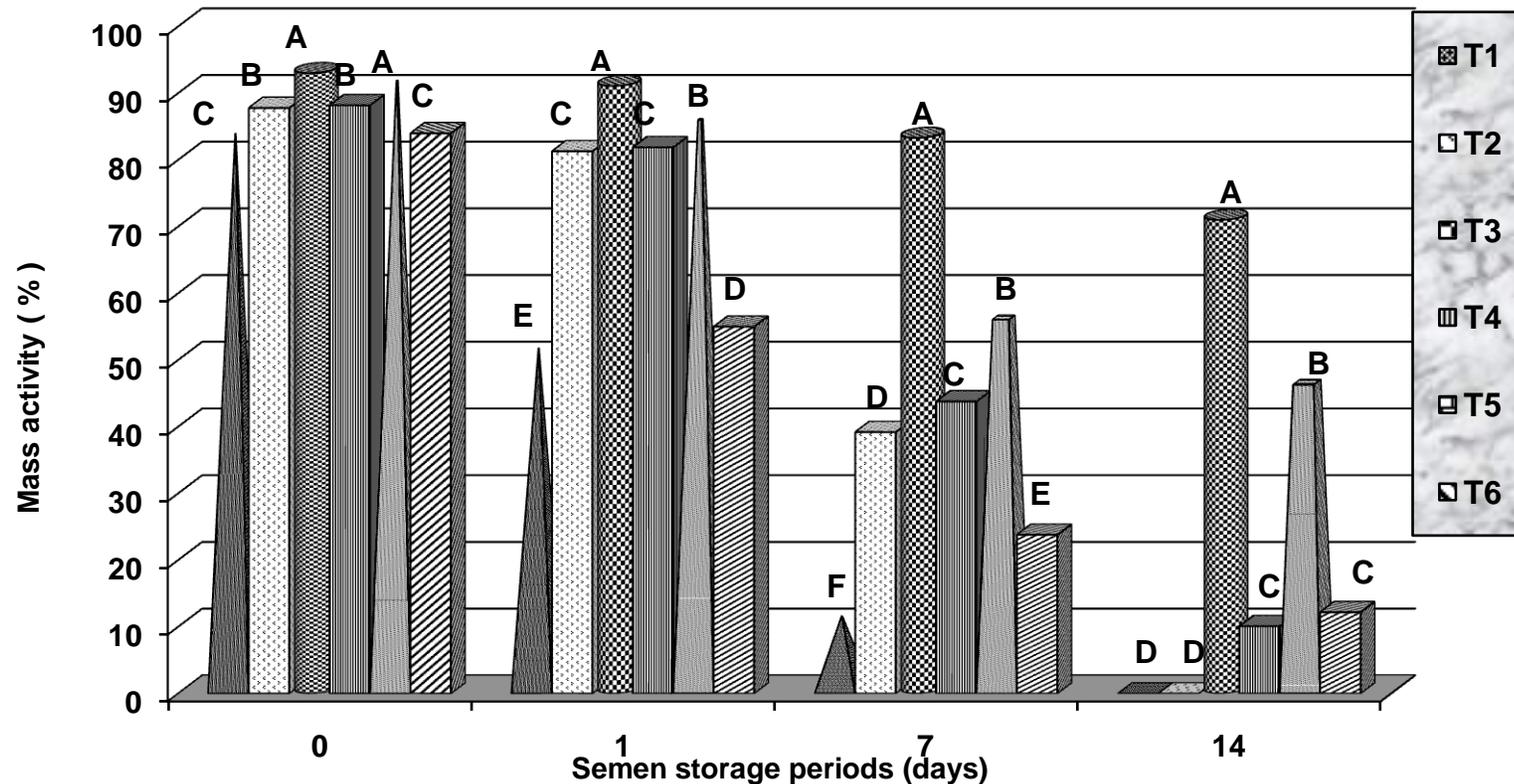
Oxidative stress is the general phenomenon of oxidant exposure and antioxidant depletion, or oxidant – antioxidant imbalance. The products of oxidation reactions can cause much damage including loss of function. This can occur from direct oxidation, such as the oxidation of membrane lipids, and leads to altered membrane permeability or loss of enzyme regulation or activity from the oxidation of proteins. Products of oxidation can also result in inappropriate cell responses. Therefore, the ingestion of certain antioxidant nutrients may be of use to prevent the damage caused by these processes³⁶. Rapport et al.³⁷ carried out *in vivo* study on mice to compare the antioxidant properties of GSPE, vitamin C, vitamin E succinate and β – carotene,

and found that GSPE provided the best protection in all categories at the doses used and suggest that GSPE is not only an efficient antioxidant *in vitro*, but also *in vivo* where it absorbed and distributed in target organs and be very useful in preventing cell membrane damage by lipid peroxidation. Young et al.³⁸ reported that short term, high intake of black currant juice had a prooxidant effect on plasma proteins and increased glutathione peroxidase activity, whereas lipid oxidation in plasma seemed to decrease. In rats, dietary supplementation with phenolic antioxidant has been found to significantly increase the plasma and LDL α – tocopherol concentration³⁹. Frémont et al.⁴⁰ suggested that the intake of dietary flavonoids is beneficial not only when diets are rich in PUFA but also when they are rich in MUFA. It seems likely that these substances contribute to the oxidant defense and reduce the consumption of α – tocopherol in both lipoproteins and membranes. Manach et al.⁴¹ concluded that the chief reason for the interest in using grape polyphenols is the recognition of its antioxidant properties, their great abundance, and their probable role in the prevention of various disorders associated with oxidative stress. The antioxidant properties of the phenolic compounds in grapes and grape juice reduce the propensity of LDL to undergo peroxidation and substantially prolong the lag time required for initiation of LDL oxidation¹⁹. By acting as free radical scavengers grape proanthocyanidins inhibit lipid peroxidation, a free – radical chain reaction that can produce cytotoxicity, disrupt lipid – containing membranes, and initiate LDL oxidation⁴². However, by reducing oxidative stress, OPC's from grape seed exert a cardioprotective effect against ischemia reperfusion injury and also protect gastric mucosal and glial cells from oxidative – stress induced injury.

CONCLUSION

The present results lead us to conclude that grape components especially flavonoids and GSPE produce good repression against lipid peroxidation during *in vitro* storage of roosters semen. However, the addition of grape compounds to semen diluent was a suitable tool for preserving semen quality when semen stored in the refrigerator for up to 14 d.

Figure 1. The effect of addition Flavonoids, Phenol, GSPE, and GJC to LD on mass activity of roosters semen stored for different periods.

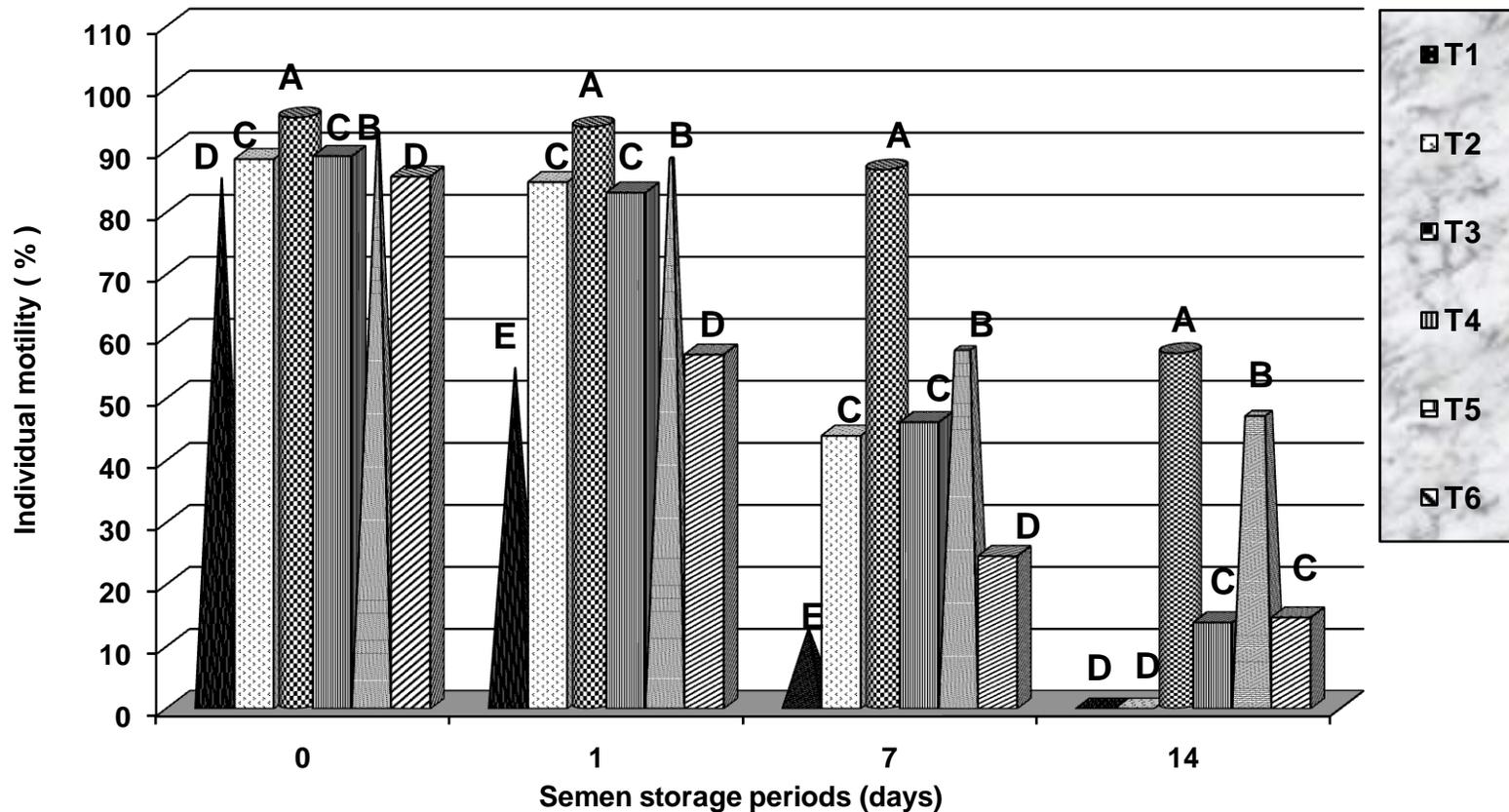


T1=Fresh semen, T2 – T6 = Semen diluted with LD alone, LD + Flavonoids, LD + Phenol, LD + GSPE, and LD + GJC, respectively.

Bars with different superscripts differ significantly ($p < 0.05$).

GSPE = Grape seeds proanthocyanidins, GJC = Grape juice concentrate and LD = Lake diluents

Figure 2 .The effect of addition Flavonoids, Phenol , GSPE , and GJC to LD on individual motility of roosters semen stored for different periods .

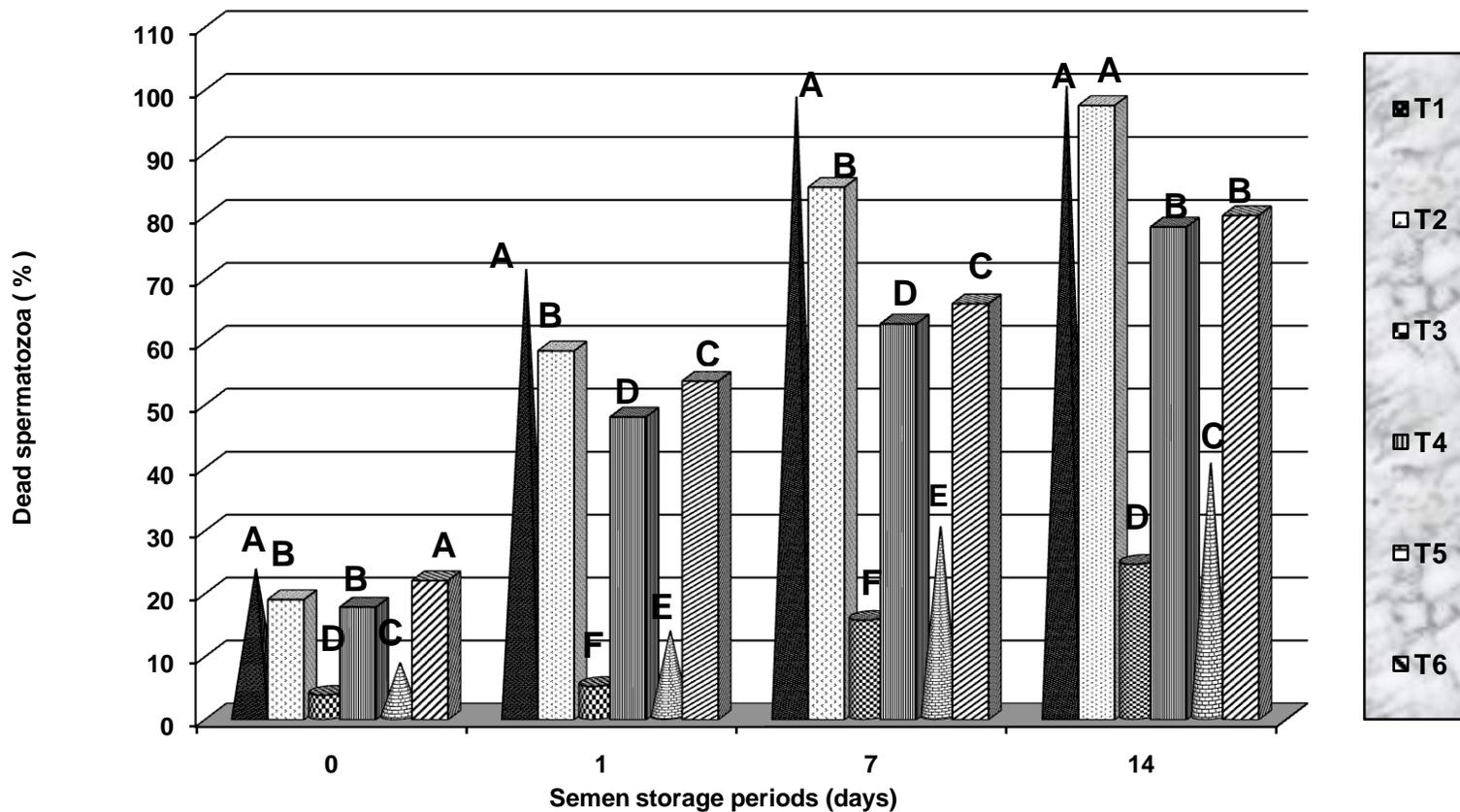


T1 = Fresh semen , T2 – T6 = Semen diluted with LD alone , LD + Flavonoids , LD + Phenol , LD + GSPE , and LD +GJC , respectively .

Bars with different superscripts differ significantly ($p < 0.05$)

GSPE = Grape seeds proanthocyanidins, GJC = Grape juice concentrate and LD = Lake diluents

Figure 3. The effect of addition Flavonoids , Phenol , GSPE , and GJC to LD on dead spermatozoa of roosters semen stored for different periods .

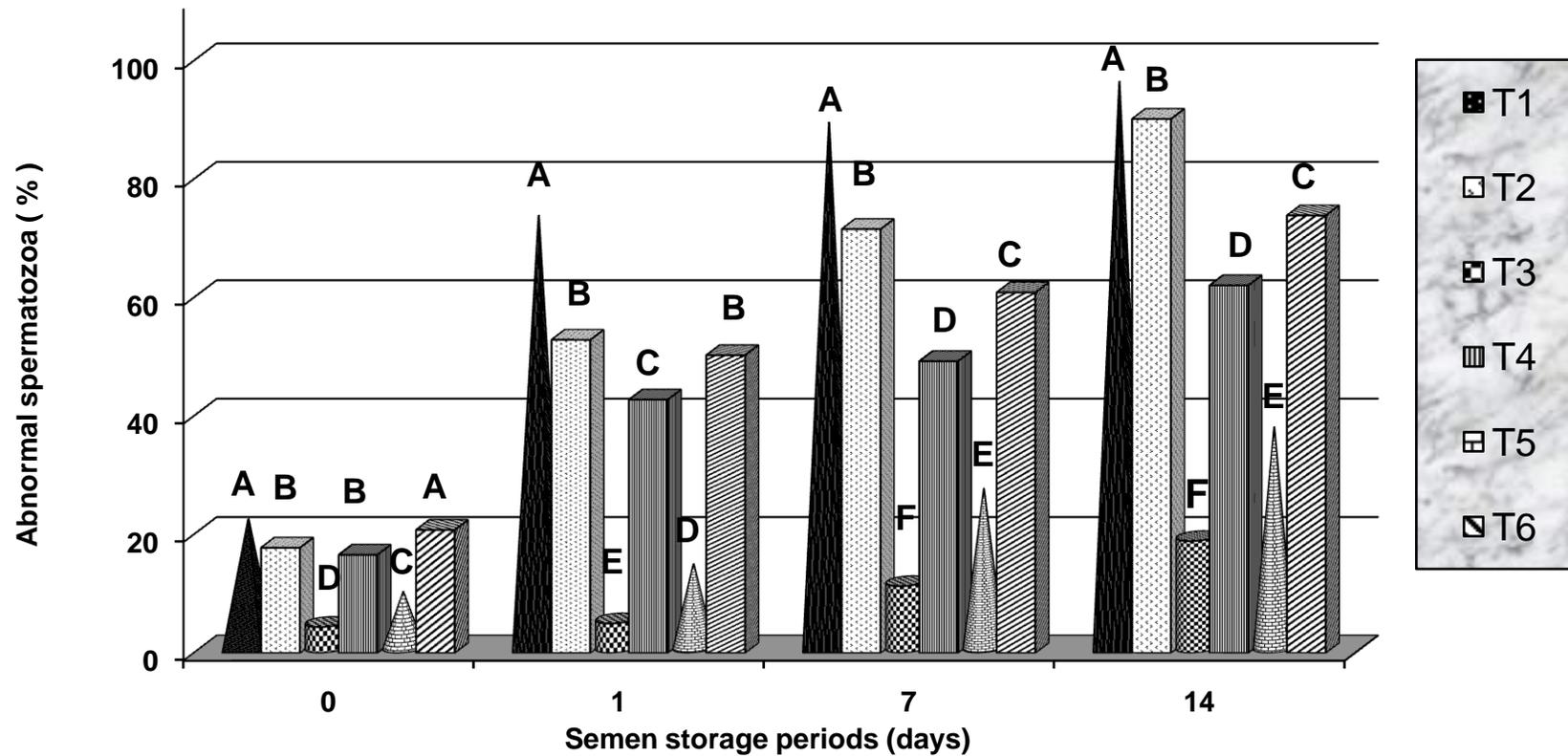


T1 = Fresh semen, T2 – T6 = Semen diluted with LD alone, LD + Flavonoids , LD + Phenol , LD + GSPE , LD + GJC , respectively .

Bars with different superscripts differ significantly ($p < 0.05$).

GSPE = Grape seeds proanthocyanidins, GJC = Grape juice concentrate and LD = Lake diluents

Figure 4. The effect of addition Flavonoids , Phenol , GSPE , and GJC to LD on abnormal spermatozoa of roosters semen stored for different periods .

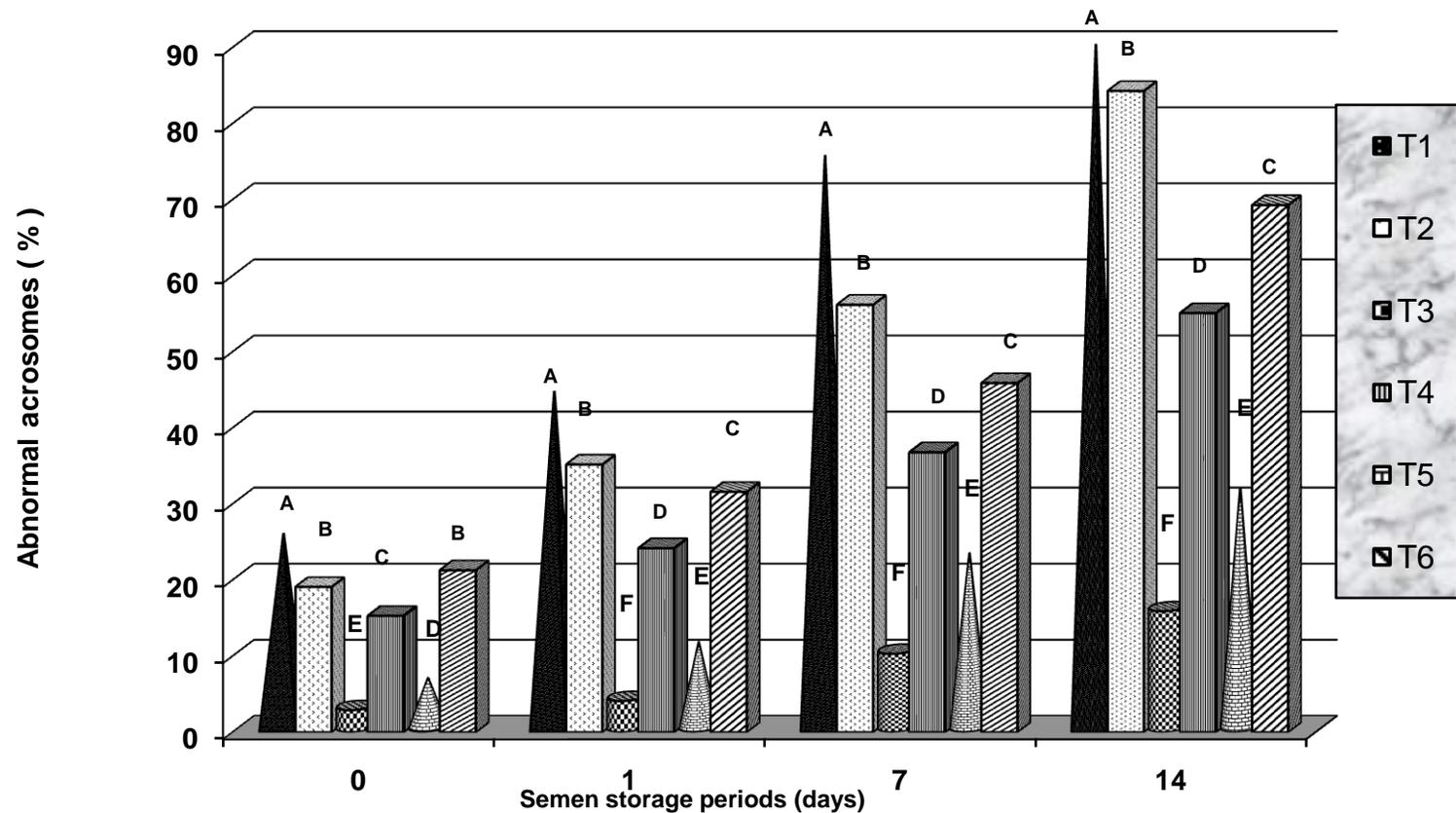


T1 = Fresh semen, T2 – T6 = Semen diluted with LD alone, LD + Flavonoids , LD + Phenol , LD + GSPE , LD + GJC , respectively

Bars with different superscripts differ significantly ($p < 0.05$).

GSPE = Grape seeds proanthocyanidins, GJC = Grape juice concentrate and LD = Lake diluent

Figure 5. The effect of addition Flavonoids , Phenol , GSPE , and GJC to LD on abnormal acrosomes of roosters semen stored for different periods .



T1 = Fresh semen, T2 – T6 = Semen diluted with LD alone, LD + Flavonoids , LD + Phenol , LD + GSPE , LD + GJC , respectively differ significantly ($p < 0.05$).

Bars with different superscripts d

GSPE = Grape seeds proanthocyanidins, GJC = Grape juice concentrate and LD = Lake diluent

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