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Review Article

Effects of antioxidant use on semen storage in honey bees

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Abstract

Although there are many studies for the storage of semen in honey bees, the desired success has not been achieved, and more study is needed in this area. It has been reported that the percentage of bee egg-laying in queen bees fertilized with stored semen, especially in long-term storage conditions is below the expected rate despite the viability rate. The reason for this situation is that honey bee spermatozoa are negatively affected by freezing processes due to their very fragile and sensitive structure. However, due to natural mating, honey bee semen can remain healthy for years in the queen bee's spermatheca. It is known that there are many enzymatic antioxidants and special proteins in the spermatheca, as well as a suitable environment for spermatozoa. Manipulating made during the stored of honey bee semen has adverse effects on spermatozoon function and fertility. In particular, current antioxidant substances against cold shock, which are among the damages that occur during freezing of semen, are being investigated, and their protective effects on semen are determined. For this purpose, it is aimed to improve the storage conditions of honey bee semen by using substances with antioxidant properties. The purpose of this review is to give information about the use of antioxidant substances in the storage of honey bee semen.

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1. Introduction

Free radicals include reactive oxygen species and reactive nitrogen compounds. They are essential for important physiological activities in the body. To achieve this, the harmony between free radicals and antioxidants must not be disturbed. In case this situation deteriorates in favor of free radicals, negative effects due to oxidative stress may develop (Lobo et al., 2010).

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Free radicals arise as a result of metabolic byproducts and activities such as phagocytosis. They contain unpaired electrons and are unstable. In aerobic respiration cells, they occur as hydroxyl radicals, superoxide radicals, hydrogen peroxide, and transition metals. It is known that when reactive oxygen species (ROS) originating from free radicals are within the required limits, they involve in a series of metabolic events required for spermatozoa to gain fertilization ability such as activation, capacitation, and oocyte fusion, which occurs when encountering the ovum. However, harmful effects are seen when ROS is produced more than necessary. Besides causing some differences in the membrane structure of the spermatozoa, ROS also cause DNA damage in the spermatozoon. Moreover, low motility may cause impaired oocyte penetration ability and decrease the fertilization ability of spermatozoa. (Duru et al., 2000; Tremellen, 2008). The decrease in the antioxidant capacity of the cell, the disruption of the enzyme activities necessary for the cells to receive oxygen and the ion transition in the cell wall, the increase in metabolic activities in the cell, the occurrence of inflammatory conditions in the cell, and the presence of negativities such as radiation cause cell damage by ROS compounds. The cold shock that is seen during the cryopreservation of semen also increases the formation of ROS. Hence, it has been stated that antioxidant substances added to semen during semen storage have protective effects against ROS (Petruska et al., 2014).

2. Storage of Honey Bee Semen and Oxidative Stress

Queen honey bees can retain spermatozoa in their spermatheca for years after mating with drones. In this process, it was stated that antioxidants in the body of the queen honey bee have protective effects against the harmful effects caused by the interaction of ROS (Collins et al,, 2004). In particular, it was emphasized that antioxidant functions developed in the spermatheca of the mating queen bee in the order Hymenoptera. Furthermore, after becoming an adult, fertilized queen bees have the ability to store spermatozoa in their spermatheca throughout their lives (Gotoh et al., 2017). Honeybee semen contains antioxidative enzymes and proteins that regulate the functions of enzymes and balance various metabolic activities (Zareie et al., 2013). ROS production occurs due to the negative effects (such as pollen, nectar and oxidation of various chemical agents) that honey bees are exposed to outside the colony. The antioxidant level in the cell also changes according to the level and content of the harmful effect that causes the formation of ROS (Korayem et al., 2012). Besides, it was stated that there are antioxidative defense systems against free radicals in semen (Storey, 1997). However, all this internal balance

can be disrupted by various external negative and pathological effects. For this reason, the importance of using synthetic antioxidants from outside was emphasized (Lobo et al., 2010).

As in mammals, various freezing methods are used for the long-term storage of semen in honey bees. For this purpose, the positive effects of the use of antioxidants added to different diluents were reported (Taylor et al., 2009). When the honey bee spermatozoa are examined morphologically, it is seen that the proportional length between the head and the flagellum is quite high compared to the spermatozoa structure of mammals (Gontarz et al., 2016). In addition, it was reported that honey bee semen is negatively affected by environmental factors due to its high density and tendency to aggregate (Cobey et al., 2013). It has been reported that applications such as cold shock, which occur during the cryopreservation of semen, different diluent contents, cryoprotectant substances, and centrifugation adversely affect honey bee spermatozoa (Taylor et al., 2009; Paillard et al., 2017). It was reported that the levels of catalase, glutathione peroxidase, superoxide dismutase and malondialdehyde (MDA), which is the end product of lipid peroxidation increase in spermatozoa when exposed to chemical agents that cause oxidative stress in honey bees (Abdelkader et al., 2019). Furthermore, it was observed that there was a decrease in semen density and protein amount in drones due to stress-related reasons. On the other hand, it was stated that there was an increase in the level of superoxide dismutase (Abdelkader et al., 2014).

Queen honey bees are exposed to the effects of ROS due to the fact that the spermatozoa in the spermatheca need the oxygen necessary for respiration. The negative effects of this situation can be minimized by various antioxidative enzymes. It was observed that the levels of catalase and glutathione transferase in mated queen bees were 10 times higher than in unmated queen bees (Collins et al., 2004). It was determined that the addition of some antioxidant and protective substances against the harmful effects of oxidative stress in honey bees is effective in the protection of honeybee semen (Taylor et al., 2009). Balieira et al. (2018) reported that caffeine in honey bees caused a partial decrease in MDA level due to its antioxidant property against oxidative stress caused by the effect of imidacloprid. In a study conducted to determine the effects of soy lecithin and egg yolk added to honeybee semen diluent on semen storage, Dadkhah et al. (2016) reported that there was a positive effect on semen motility and viability in the study groups where different doses of egg yolk and soy lecithin were added compared to the control group. Besides, Almeda and Espencer (2002) stated that coconut water added to the semen diluent under short-term storage conditions is effective on the storage of semen. In the

study investigating the efficacy of trehalose added to the Kiev solution, which is a semen diluent, it was reported that there was an increase in the short-term retention time and semen motility values in honeybee semen (Yániz, et al., 2019). It was also reported that trehalose had a protective effect against damage to the spermatozoon plasma membrane (Ahmad and Aksoy, 2012). In a study investigating the effects of TL HEPES-based diluent with BSA added to the semen diluent at different rates in honey bees, it was reported that the diluent had a positive effect on semen motility, spermatozoa plasma membrane integrity and acrosome integrity. In addition, it was demonstrated that with the increase in the ratio of the substance used in the study, there was a positive increase in spermatological parameters (Alçay et al., 2019a).

3. Antioxidant Substances Used in Studies on Honey Bees

Over the past 40 years, various extenders and antioxidants have been used to improve honey bee spermatologic parameters in the cooled or cryopreserved. Because of temperature changes, cold shock, and ice formation, cryopreservation techniques damage spermatozoa. The sperm quality characteristics of spermatozoa are reduced as a result of these consequences. Furthermore, during the cryopreservation process, ROS are produced as a result of lipid peroxidation in the cytoplasmic membrane. The sperm membrane is stressed in various ways by the free radicals that arise. Antioxidants have been added to honey bee semen extenders and nutrients in recent years to prevent lipid peroxidation and induce spermatologic quality parameters (Wegener and Bienefeld, 2012). Antioxidant substances used in studies on honey bees are shown in Table 1.

Table 1. Antioxidant substances used in studies on honey bees

Spermatological Parameters	Antioxidant Substances	Effects	References
Semen Paremeters (motility, viability)	Catalase	Insignificant	(Taylor et al., 2009)
Sperm Concentration	Pollen	Insignificant	(Rousseau and Giovenezzo, 2016)
Sperm Motility	Soybean Lecithin	Beneficial effects	(Dadkhah et al., 2016)
Semen Parameters (motility, plasma membrane and, acrosomal integrity)	Bovive Serum Albümine (BSA)	Beneficial effects	(Alcay et al., 2019a)
Semen Parameters (motility, plasma membrane and, acrosomal integrity)	Royal Jelly	Beneficial effects	(Alcay et al., 2019b)
Semen Parameters (motility, plasma membrane, integrity, mitocondrial function)	L-Carnitine	Beneficial effects	Alcay et al., 2021)

5. Conclusion

As in mammals, the use of antioxidant substances in studies related to the storage of honey bee semen is an important factor that increases the success of semen freezing. When the structure of honey bee spermatozoa is evaluated morphologically, it is quite fragile and sensitive to environmental factors compared to mammalian spermatozoa. Many factors cause oxidative stress, especially the cold shock that occurs during the freezing and storage of semen, the centrifugation process applied to the semen, and the negative effects of different diluents on spermatozoa. Moreover, the stress environment that drones are exposed to outside the colony, contamination of nectar and pollen sources in the region with harmful external factors such as pesticides or carbon monoxide, spraying of colonies against parasites such as varroa destructor and similar conditions negatively affect semen quality. Although the enzymatic antioxidants and some proteins in honey bee semen help reduce the oxidative effects, they cannot eliminate all the damage that occurs depending on the content and duration of the damage. In this case, additional antioxidant substances are needed from the outside, especially for the storage of honey bee semen. For this purpose, various antioxidants or substances that strengthen the antioxidant effect are added to the nutrients or semen diluent in honey bees. As a result, it is

seen that antioxidant substances have important protective effects on the storage conditions of honeybee semen and spermatological parameters after solution. Studies on the storage of semen in honey bees should be increased. In studies to be conducted in this area, the activities of antioxidant substances are key in improving the storage conditions of honeybee semen.

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