



Current Approaches to Short and Long-Term Storage of Honey Bee Semen

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Abstract: There are many studies on the storage of honey bee semen. Due to its unique structure, bee semen varies according to the storage period but is suitable for short-term storage conditions. However, in terms of long-term storage conditions, developments in mammals have not been caught. It is known that more studies are needed on this subject. The great advantages of semen storage are its effective role in eliminating the problems of inbreeding after a while in pure breed enterprises, increasing the yield characteristics and preventing gene pollution. To solve these problems, beekeepers have to buy breeding queens from abroad with very high costs, and the expected results cannot always be obtained from the bees brought. Especially considering the genetic risks faced by regional breeds in Turkey, it is necessary to preserve semen, to protect colonies in pure breed enterprises, as well as to develop genetically yield characteristics and to prevent inbreeding. It can also prevent the spread of diseases compared to natural mating by contributing to artificial insemination. By improving the storage conditions of honey bee semen, long-term use of semen from well-known colonies will be possible. Producers will be able to benefit from the advantages of artificial insemination at a cheaper cost, with the semen being sent to distant regions as in mammals under appropriate storage conditions. The purpose of this review is to give information about the storage conditions of honeybee semen.

Keywords: Honey bee, semen, storage.

Bal Arısı Spermasının Kısa ve Uzun Süreli Saklanmasıyla İlgili Güncel Yaklaşımlar

Öz: Bal arısı spermasının saklanabilmesiyle ilgili pek çok çalışma bulunmaktadır. Arı sperması kendine özgü yapısı nedeniyle saklama süresine göre değişimle birlikte kısa süreli saklama koşulları için elverişlidir. Ancak uzun süreli saklama koşulları açısından memeli hayvanlardaki gelişmeler yakalanamamıştır. Bu konuda daha fazla çalışmaya ihtiyaç olduğu bilinmektedir. Spermanın saklanabilmesinin büyük avantajları saf ırk bulunan işletmelerde bir süre sonra akrabalı yetiştiriciliğin verdiği problemlerin giderilebilmesi, verim özelliklerinin artırılabilmesi ve gen kirliliğinin önlenbilmesindeki etkin rolüdür. Arı yetiştiricileri bu problemleri giderebilmek için yurt dışından çok yüksek maliyetlerle damızlık kraliçe satın almak zorunda kalmakta ve getirilen arılardan her zaman beklenen sonuçlar alınamamaktadır. Özellikle ülkemizde bölgesel ırkların karşılaştığı genetik riskleri düşünürsek spermanın saklanabilmesi, saf ırk işletmelerdeki kolonilerin korunabilmesinin yanında genetik olarak verim özelliklerinin geliştirilmesi, akrabalı yetiştiriciliğin önlenbilmesi için de gereklidir. Ayrıca suni tohumlamaya katkıda bulunarak doğal çiftleşmeyle karşılaştırıldığında hastalıkların yayılmasını önleyebilmektedir. Bal arısı spermasının saklanma koşullarının geliştirilmesiyle özellikleri bilinen kaliteli kolonilerden alınan spermanın uzun süreli kullanımı mümkün olacaktır. Üreticiler alınan spermanın uygun saklama koşullarında memeli hayvanlardaki gibi uzak bölgelere gönderilebilmesiyle birlikte daha ucuza suni tohumlamanın sunduğu avantajlardan yararlanılabileceklerdir. Bu derlemenin amacı bal arısı spermasının saklanma koşullarıyla ilgili bilgi vermektir.

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Anahtar kelimeler: Bal arısı, sperma, depolama.

INTRODUCTION

Studies on the storage of honey bee semen are important for the development and dissemination of artificial insemination in honey bees. Improving the storage conditions is key, especially to increase the yield characteristics of pure breed bees and to contribute to the development of beekeeping with the transport of semen.

It has been reported that honey bee semen can be stored at room temperature for up to 1 week (Collins, 2000). According to Cobey, (2007), honeybee semen can be stored at room temperature for a maximum of 2 weeks. The acrosome length of the honeybee semen is 5 µm and the total length including the flagellum is around 250-270 µm (Lensky et al., 1979; Lino-Neto et al., 2000). Honeybee semen is sensitive to adverse events that may occur during freezing (Wegener et al., 2014a). The low percentage of worker bees in the eggs of the fertilized queen bees as a result of keeping the semen in storage conditions for a long time was found to be remarkable (Harbo, 1979; Cobey, 2007). It has been stated that antioxidants have a positive effect on honey bee semen as in mammals. It has been reported that glutathione peroxidase, catalase and superoxide dismutase have a positive effect on semen quality, especially against the damages of oxidative stress (Abdelkader et al., 2019).

Semen Diluents: Diluents added to the semen are very important for the storage of semen for artificial insemination in honey bees. For this purpose, many studies have been conducted and the effects of different diluents on semen have been evaluated.

In a study of queen bees inseminated with semen diluted with Tris, Hayem, and Kiev diluents, it was found that diluted and centrifuged semen extended the ovulation time at different rates. Moreover, it has been reported that diluents change the rate of drones in inseminated bees (Moritz, 1984). Amino acids and antibiotics added to the diluent have a beneficial effect on storage conditions. Besides, the success of using the diluted semen by centrifugation varies depending on the diluents (Moritz, 1984). In the cryopreservation application applied for the storage of honey bee semen, the semen can be diluted with suitable diluents and the dilution rates may vary according to the solution used. Dilution ratios such as 1:1, 5:1, 3:2 can be preferred (Paillard et al., 2017). It is also used in different ratios from cryoprotectant agents such as glycerol, ethylene glycol, DMSO under long-term storage conditions (Taylor et al., 2009). Harbo's successful diluent contains 60% semen, 10% DMSO and 30% saline solution (Harbo, 1979). To increase the effectiveness of diluents, it has also been tried to use substances such as egg yolk and coconut water for semen diluents. Hopkins et al., (2012) emphasized the importance of the fact that egg yolk added

to the diluent can cause infection because it is an animal product, as well as it can negatively affect the life of the queen bee by causing obstruction in the reproductive canal. It has been observed that coconut water had a supportive effect on the semen vitality rate. In this way, no significant loss of vitality was observed for up to 14 days (Almeda & Soarez, 2002). It has been stated that antioxidants added to semen diluent increase the semen quality (Cobey, 2007; Taylor et al., 2009). Moreover, it has been reported that the buffer content of the diluent contains amino acids and sugar, as well as a pH value of 8.5 for honey bees.

Protocol Regarding Filling Honeybee Semen into Straws: To ensure healthy storage conditions, the semen diluted with the appropriate dilution rate and method are filled into straws in the form of diluent-air-space-semen-air-space-diluent, and the open end of the straw is pressed and closed (Hopkins et al., 2012).



Figure 1. Filling of honey bee semen into the straw (Gül et al., 2017).

Approaches to the Short-Term Storage of Honeybee Semen: It has been reported that no serious semen loss was observed when honey bee semen was stored under room temperature conditions for as little as a week. It has been stated that it can be used by keeping it waiting for artificial insemination in this way. After the diluted semen was loaded into the capillary tube, it could be stored for up to 6 weeks with around 80% spermatozoa viability preserved. It was emphasized that the success rate increased due to the improvement of short-term storage conditions at 12-16°C (Collins, 2000). It has been observed that viability and motility decrease as the storage period increases under short-term storage conditions (Looke & Peng, 1993). For artificial insemination, honeybee semen can be stored for a short time at 15-36°C for up to 36 hours. It has been reported that semen can be stored for up to 48 hours at 21°C (Harbo & Williams, 1987). The semen, which is diluted and transferred to capillary tubes, can be stored as diluent-air-semen-air-diluent. The prepared capillary tubes can be stored in special incubators at 16 ± 1 °C, protected from light (Paillard et al., 2017). Since the queen bee can be adversely affected by the composition of the diluent, it can be centrifuged at 1000 rpm for 10 minutes in a 1.5 ml eppendorf tube, and the semen can be purified from the diluent (Wegener et al., 2014b). Centrifugation of diluted semen can damage spermatozoa. It has been reported that it is better to mix gently with an instrument rather than a centrifuge (Lodessani et al., 2004).

It has been stated that temperatures between 16°C and -196°C are suitable for storing honeybee semen. The important thing is to obtain healthy results when the stored semen is used in artificial insemination (Paillard et al., 2017). The semen taken from drones can be temporarily stored at room temperature for a few days and can be practically used in artificial insemination. For this purpose, the two ends of the capillary glass tubes used to collect semen are temporarily closed with petroleum jelly or similar material. Since light can damage semen, it can be stored in dark conditions (Cobey, 2013).

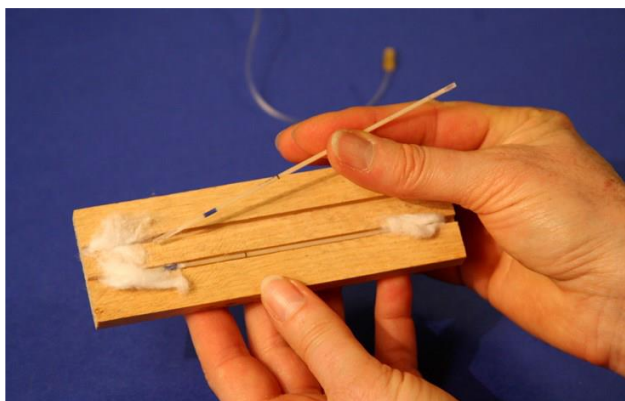


Figure 2. Short-term storage of semen for artificial insemination (Cobey, 2013).

Approaches to the Long-Term Storage of Honeybee Semen: With the long-term storage of semen, artificial insemination is possible not only during the season but also when drones are not available (Cobey, 2013). Honeybee semen has a very long flagellum. Furthermore, the head is long and the acrosome and nucleus lengths are the same. The mitochondrial helix functions by stretching along the flagellum (Lino-Neto et al., 2000). The secretions of the attachment glands that participate in the structure of the semen in drone and the special proteins it contains play an important role in the survival of the spermatozoa (den Boer et al., 2009). In honeybees, long-term semen can be stored using freezing methods (Harbo, 1979). Semen can be stored in liquid nitrogen using various cryoprotectants (Dimethyl sulfoxide, glycerol etc). However, it has been determined that a significant part of the eggs of the queen bees fertilized in the sperm stored for a long time in this way are male eggs (Wegenner & Bienefeld, 2012). It has been reported that the reason for this situation is the genetic damage suffered during the freezing of semen (Harbo, 1981). The main factors affecting the success of cryopreservation methods are thought to be the toxicity of cryoprotectants, temperature sensitivity, freezing speed ratio and cold shock (Hopkins & Herr, 2010). Therefore, it is necessary to develop appropriate storage techniques for the long-term storage of honey bee semen (Cobey, 2007). Many studies have been done on this subject and different

semen freezing methods have been tried. Hopkins and Herr (2010) reduced the temperature gradually from room temperature to -40°C with a temperature loss of 3°C per minute and then transferred it to -196°C liquid nitrogen. In another semen freezing study, semen, which was gradually cooled to 5°C, was stored directly in -196°C liquid nitrogen (Dadkhah et al., 2016). In their study, Alçay et al., (2015) reduced the temperature of semen to 5°C and kept them in the cooler for 2 hours at 5°C for the equilibration process. They were then cooled to -110 °C in liquid nitrogen vapor for 10 minutes and stored in liquid nitrogen at -196 °C. Thawing of frozen bee semen was done by keeping it at 37°C for 30 seconds (Paillard et al., 2017).

It was observed that the percentage of drones in artificially inseminated queen bees decreased when honey bee semen was frozen and stored for a long time. As the storage period increases, the percentage of drones also decreases. The percentage of drones was determined as 22% in semen stored frozen for 4 days and 8% in semen stored for 2 years (Harbo, 1983). DMSO has been found to be safer for honey bees among other cryoprotectant agents (Harbo, 1979; Taylor et al., 2009). In a study on storing bee semen at -196°C for 1 year by freezing with a diluent containing DMSO, it was reported that storage conditions below freezing temperature were more successful. Besides, the importance of frozen and thawed semen in terms of protecting the health of the queen bee was mentioned (Paillard et al., 2017).

CONCLUSION

It is seen that short-term storage of bee semen has very successful results compared to long-term storage. The most important determinant of success is the percentage of drones in the eggs of the queen bee inseminated with the stored sperm. It is a great advantage that bee semen can be stored at room temperature for up to 1 week after collection. On the other hand, when stored for a long time, semen must be frozen and cryoprotectant must be added to the semen diluent. Due to its unique structure, honey bee semen suffers serious damage during freezing and thawing. In the storage of semen, the substances added to the semen diluent and antioxidants contribute to the improvement of the storage conditions. With the storage of semen in honey bees, the cost of forming colonies with the desired genetic characteristics can be reduced. Disease and parasitic diseases can be prevented. Pure breeds can be maintained in controlled beekeeping. Artificial insemination can be expanded. There are many studies on the storage of honey bee semen, and new studies shed light on this field every day. However, it is thought that more studies are needed on the long-term storage of bee semen.

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