THE PUBLISHING HOUSE
OF THE ROMANIANACADEMY

Supplement 1/2015, 4th ISAA

ARTIFICIAL INSEMINATION ON *APIS MELLIFERA* – ASPECTS OF ARTIFICIAL INSEMINATED QUEEN PERFORMANCES AND FACTORS THAT MAY AFFECT THEIR PERFORMANCE

Elena BUESCU (LIPAN), Maria Rodica GURĂU and Alin Ion BÎRȚOIU

University of Agronomical Science and Veterinary Medicine Bucharest, Faculty of Veterinary Medicine, Bucharest, Romania.
Corresponding author: Elena BUESCU (LIPAN), E-mail: helenabue2006@yahoo.com.

Accepted November 12, 2015

In international literature, there are many data about artificial insemination on queenbee. This informations clarify in all aspects, the problems relating with rearing queen and insemination technique. Instrumental insemination is an important tool that provides complete control of honey bee mating for research and breeding purposes. With improvements in instrumentation equipment, the instrumental insemination technique is repeatable and successful. Honey bee queens are polyandrous and mate in flight, so, the ability to control mating has been a challenging aspect of queen honey bee breeding. Artificial inseminated queen performance can be affected by some factors like: rearing conditions, mating age, treatment of queens before and after insemination, semen dosage and handling, pheromone development, effects of CO_2 treatments and environmental conditions.

Keywords: artificial insemination, queenbee, rearing queen

INTRODUCTION

Romanian literature data on artificial insemination of queenbees are quite low in comparison with international literature, therefore a retrospective study in this regard is requiring. Instrumental insemination technique is the method with whom the mating honeybee queens can be controled.
Within honeybee selection and improvement programmes, artificial insemination has an important role. Naturally, drones that mate with the queen are genetical unknown. But with instrumental insemination, geneticaly improvement can be realise faster during selection programme. Other advantages of this tehnique are: selection of desired character and decreasing of unwanted characters[1], it help to preserve bee native race[2].
Queen bees are characterized by polyandry and mate in flight with an average of 10 to 20 drones[3] or 12 drones[4] so the natural mating of honey bees represents a challenge in controling it. Drones going in congregation areas consisting over 20000 drones.They come from diverse genetic sources. The mating is the principal role of an adult drone honey bee (*Apis mellifera*). During the mating, semen is transferred from a drone into the genital orifice of a virgin queen. After mating, queen has a „mating sign" represented by parts of the last drone reproductive organs[5]. More drones will mate with a single virgin queen. Copulations follow one another very quickly (every two seconds). During mating, each drone ejaculates about 6 – 12 million sperm. Sperm accumulates in the queen's lateral oviducts but just a small part migrate into the spermatheca[5,6].
Artificial insemination gives control over mating[3] and also can reduce the risk of spreading pathogen agents and pests by passing the semen instead of live honeybees[7]. In 1920's started the development of artificial insemination technique. The method was improved over time and now instrumental insemination is a success and repeatable technique. For research purpose this technique is used for a very long time[7,8,9].
There are many studies that compare natural mated queen performance with instrumental inseminate queen performance. Queen performances can be influence by many factors. For example, natural mated queen (NMQ) performance can be influence by abiotic factors (weather and geographic area), biotic factors (drone availability, sperm quality of males), management practices, chemical treatments and the conditions inside the hive[4]. Instrumental inseminated queen (IIQ) performance can be influenced by factors like: treatment of queens, semen dosage and handling, pheromone development, effects of CO_2 treatments, rearing conditions, mating age[3]. The aim of the study was to bring into attention the importance of honey bee queen artificial insemination, in order to control and improve genetic breeds, for the preservation and improvement of local breeds and to create disease-resistant lines and lines with high productivity.

MATERIAL AND METHODS

For artificial insemination of *Apis mellifera* queen the equipment include: insemination instrument - Schley instrument[3] or Laidlaw instrument[8] , binocular stereozoom microscope, lamp with cool light, carbon dioxide source with flow regulator and tubing, sterile

vials, saline solution 0,9%, syringe – Harbo or Harbo-like[3] or Mackensen[9], bidistiled water, ethanol 95%, sterilizer, queen cages, drone holding cages and drone flight box, small nuclei and hives. The semen is collected from mature drones from the endhophallus into syringe, and it is used to inseminate the virgine queen[3,8,9].

The endhophallus is everted with hand (partial eversion and full eversion) by torax and abdominal massage with great care because they can defecate during this action[3]. The creamy, marbled colour semen of mature drone it is drawn into the syringe, carefully, not to take the underlying mucus[3,8]. A little drop of saline solution it is drawn into the syringe before start collecting semen.

Between loads of drones semen the tip is kept in saline solution to avoid drying[3,9]. When the syringe is full, a small amount of liquid with antibiotic can be drawn in the tip. This liquid is used like lubrificant for queen insemination, but also to prevent the infection[8].

The virgine queen is placed in a holding tube and CO_2 is used like anesthetic[3]. CO_2 can influence the queen egg-laying[9]. Usually, it takes two treatment with CO_2, in the same day or with one or two days before insemination. The first treatment is short (few minutes) and the second it is used during insemination procedure.

During insemination the tip is insert in the vaginal orifice and passes by valvefold – tissue that cover median oviduct. Semen dosage per queen is about 8 - 12µl. After insemination it returned in the cage and than in the colony[3,8,9] .

RESULTS AND DISCUSSIONS

Studies between IIQ and NMQ are made for a long time and continues today. When we talk about queen quality and performances we keep in mind aspects like: the productivity of the colony, queen longevity, onset of oviposition, the quality of oviposition[7].

All these aspects can be influence by many factors.Some of these factors are:

1.Rearing conditions of queen and drones and environmental conditions.

The quality of virgin queen and drones is very important for the production of IIQ. The quality of queen is correlated with the larva age selected to obtain a virgin queen. Also depends on the food that the nurses bee give to the larva. If the queen are inseminate to late after emerged this can influence negative the quality of inseminated queen. The rearing conditions of the queen influence its quality (size of the queen, the number of ovariole and the capacity of spermatheca)[10].

Rearing and the condition of drones strorage until they become mature affect the quality of drones used for queen insemination[7]. It is very important to reduce the storage period because the drones wears out quickly. Also is important to eliminate the stress factors, especially impact of pathogens, parasites, chemical substance and cold[3]. For example, in some study to avoid the stress of the lack of nutrition the drones were feed throughout the entire period of study with pollen and syrup sugar the rearing family[5].

2. Queens mating age.

Virgin queens have a optimal receptive period for mating (naturaly or artificial). Some authors reveal like optimal period the age of 4 - 13 days post-emergence[7]. Others authors consider that this optimal period is about 5 – 10 days after emergence[11],4 – 10 days[9], 6 days[12] or 7 days[13]. It is very important to respect this period, because if it is to early (under 5 days) the queen is not mature and if it is to late (14 days) the queen quality is low so the risk of supersedure is high.

3. Semen dosage and number of sperm stored in spermatheca.

It is very important that the queen keep enough sperm in her spermatheca. This allows her to maintain a strong colony for her entire lifetime (3 – 5 years). Spermatheca is a globular organ, with a diameter of 1,1mm. It is surrounded by a dense tracheal net. Spermathecal fluid contains proteins, sugars and antioxidants, and the surrounding tracheal net provides oxygen and it help sperm cells to remain viable in the queen's spermatheca for several years[12].

The efficiency of sperms migration from lateral oviducts into spermatheca it depends on semen doses. There are different opinion about optimal doses of semen: 10µl inseminate in four steps (each step with 2,5µl)[9], 8µl[12] or between 8 - 12µl[3].

Higher doses up to 8µl of semen migrate more slowly (over 40 hours). Smaller doses, migrate faster (in about 8 hours)[6,7].

A major factor that can affect queen performance and quality is the treatment of queen after insemination. Most of the sperm get into the spermatheca in the first ours after insemination. The activity, temperature and hive conditions, the size of colony can help or can inhibit the sperm migration[7]. If after insemination the queen is put in a hive with small population it tend to retain semen in the oviduct. The movements of the queen (abdominal contractions), sperm activity, the care of worker bees, after insemination, help the queen to clear her oviducts[6,14].

4. Sperm quality

Apis mellifera drones develop from unfertilized eggs after 24 - 25 days (depending of temperature)[15]. This period is divided in the following steps: the egg 3 days, larva 6 days, prepupa and pupa 15 days[16].

Spermatogenesis begin in the larval stage (third larval period) and is finished before the drones emergence[17]. Spermiogenesis ends at the pupal stage. In the first seven days of adult life, sperm migrate from testes (after that tests are flattened) to seminal vesicles. Here are stored temporaly, until copulation[16,17].

After emergence, drones are not capable to copulate because they are not sexual mature. Sexual maturation it occurs inside the nest in about 8 days after emergence[9], 10 – 12 days[15] or in 9 – 12 in opinion of others authors[5]. After this age it is possible the transfer of spermatozoa from the seminal vesicles to the everted endophallus.

The optimal period for semen collection is about 10 – 21 days. Earlier, drones are not mature and after 21 days semen become dense. This can create problems to inseminated queen in eliminate the excess sperm from oviducts[16,17,18].

The nutrition of drones during their larval stages can influence number and viability of their spermatozoa. The lack of polen during larval development decrease the reproductive value[15].

Studies have showed that the amount of semen released, depends mostly on drones genetics and the season in which drones were reared have the greatest effect on sperm numbers[5].

The way drones are kept until maturity, until the collection of material for insemination can influence the quality of IIQ. Heating the drone (imature drone) or the seminal material can decrease the sperm cells viability. Studies reflect that mature drones sperm is more toughness in heat stroke than sperm of 10 days drones[19].

5. Semen handling and storage.

The chemical properties of diluents used in insemination technique, pH, osmolarity and nutrients can affect semen quality. Also the handling methods can increase or reduce sperm cell survival[20].

After collecting honeybee drone semen it can be hold for more days (about two weeks) at room temperature in sealed capillary tubes. The optimal temperature for short holding semen is 21◦C, but may vary between 13 - 25◦C, temperature being an important factor. Sunlight can also affect semen quality that is way it must be kept in the dark[3, 7, 21].

For long term storage of semen it can be use cryopreservation techniques. Studies have not been completed and continue to improve this techinque. For cryopreservation it can be used cyroprotectants to prevent intracelular ice (damaging agent for sperm) such as: DMSO (dimethyl sulfoxide) – better results and ethylene glycol[3, 21].

Research reveal that, slowly cooling to prevent heat stroke and -3°C freezing, cause 93% viability of sperm cells.If the cooling was made rapidly to zero degrees, the viability was only about 13% and the motility absent[21].

Other studies used crioconserved sperm in liquid nitrogen, but the majority of the inseminated queen layed more drones egg and next generations had evident genetic disorders[7].

6. Pheromone development and queen acceptance.

The queen sufer many changes physiological and behavioral. The composition of queen pheromone changes quantitative and qualitative between virgin and mated queens[7,22]. Queen pheromone is a mix of chemical substances like 9 – oxo - (E) - 2-decenoic acid, (R) - and (S) – 9 –hydroxy - (E) - 2-decenoic acid, methyl p -hydroxybenzoate, 4 – hydroxy – 3 - methyoxyphenylethanol, methyl oleate, coniferyl alcohol, palmityl alcohol, and linolenic acid[23].

This pheromones has many functions including aggregation, alarm and mate attraction. Also help honeybee to distiguish between a virgine and a mated queen. Queen pheromone is produce by mandibular and Dufour's glands. Research show that factors like CO2, manipulation of genital tract during insemination procedure, insemination volume have effects for a long-time on mandibular gland chemical profiles. This can influence the acceptance of the queen[22,24]. An essential component of queen pheromone is 9-oxo-(E)-2-decenoic acid, that acts like long-distance sex pheromone[23].

In instrumental inseminated queen, the absence of a mating flight and the treatment, may influence queen pheromone development, but differences in pheromone levels between IIQ and NMQ are not significant when queens are laying and established in the colony[7,22,25].

Physiological changes of laying queens are not depending of natural mating and the way queen is inseminated - naturaly or instrumentaly – has now influence over pheromone production[22,25].

7. Effects of carbon dioxide treatments.

There are different opinions about effects of CO_2 used for queen anesthesia. Some authors hold that CO_2 has the positive effect of inducing egg laying and stimulate the neurosecretory production of juvenile hormone. This contributes to the initiating of oviposition[7,9,12]. Others state that in the first phase, CO_2 cause queen weight loss[7].

Studie reveal that in queen wich received two CO_2 doses, one in the day of insemination and one the day before, the sperm migration was more effective[26].

Different concentrations of CO_2 used for queen narcosis, influences the immobilization (can increase or decrease the time of isemination), the length of unconsciousness and the regaining of consciousness. CO_2 may also accelerates the introduction of the semen into the oviducts[27].

CONCLUSIONS

Research shows the advantages of instrumental insemination of *Apis mellifera* queen starting with genetic and mating control and continuing with the possibility to create controlled crosses that naturally may not occur, to enhance certain traits (productivity, overwintering resistance, disease resistance), etc. Instrumental inseminated queen can be used not only in research centers but even by beekeepers. Many studies demonstrated their equal or even higher performance compared to natural mated queen, taking into account all the factors that can affect their performance.

Acknowledgement *This work was cofinaced from the European Social Fund through Sectoral Operational Programme - Human Resources Development 2007-2013,project number POSDRU/1871.5/S/155631, entitled "Doctoral programs at the forefront of research excellence in prioritary domains: health, materials, products and innovative processes", Beneficiary – "Carol Davila"University of Medicine and Pharmacy Bucharest*

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