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The Applicability of Semen Collection in Drones of APIS Melifera Carpatica

KEYWORDS: Drone, semen, collection

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ABSTRACT *For beekeeping farms, the bee reproduction is one of the most important things. If it is well known and controlled, it leads to high productive performances. This study aimed to test the applicability of semen collection in drones, according to their age and collection technique. Of studied drones, in 55.5% the ejaculation occurred, and this way the semen collection was possible. In the rest of the drones, the endophallus was not revealed and the ejaculation did not occur. Of drones that served to collection, in 75.7% the ejaculation was total, while in 24.3% the ejaculation was partial. Based on age categories, in L1 group the collection succeeded in 52% of the drones (69.2% with total ejaculation and 30.7% with partial ejaculation) while in L2 group the results were better, semen being collected from 59% of the drones (81.4% with total ejaculation and 18.6% with partial ejaculation.*

By applying the biotechnologies in assisted reproduction in beekeeping it is aimed to raise the number of the individuals in the bee family so the family will have a higher capacity of picking when the environment offers high quantities of nectar and pollen. One of the main aspects of the bee growing and reproduction through which is realized maximum development of the families and consequently the increase of production is represented by assisted breeding of the getters originated from high biological value parents (5, 8).

As it's well known, the bee reproduction is sexual and partogenetic. By sexual way, worker bees and queen bee are born of fertilized eggs. The drones develop from non-fertilized eggs.

In order to achieve the mentioned goals (strong families and high yields), it is necessary to know very well the essential elements of the complex process of reproduction, namely: gametogenesis, mating or artificial insemination, fertilization and metamorphosis of the three classes of bees (queen bee, worker bees and drone).

Oogenesis occurs in ovaries, primary ovogonies develop and transform into oocytes, which by multiple divisions become mature egg capable of fertilization.

Spermatogenesis occurs in testicles. In drones, the spermatozoa are produced starting with the 6th day of the larval stage. Three days after hatching they are completely mature and suitable for fertilization, and they migrate in seminal vesicles.

The phenomena of polyandry met in mating process, have quite high importance in terms of the physiology of reproduction. This represents the best and the most complete hybridization, being known that drones are free to move from one family to another and from an apiary to another. They fly on a distance of 8-10 km and the mating flight occurs only outside the hive. Polyandry provides increased prolificacy and high vitality of the descendants (1, 4).

Heterosperm mating activates the fertilization of the eggs. 5-10 spermatozoa participate in fertilization of an egg, but only the most vigorous one fuses with female gamete.

These natural characteristics specific to the bees explain the preservation of the fertilization capacity of the sperm stored in spermateca over a long period (several years), and prevent degeneration of the species through inbreeding (2, 7).

The key to success in conservation of bee biological material is likely to be in spermateca. By studying the biochemical and physiological environment that ensures sperm survival for many years, the best way of long-term preservation of semen could be found.

Until now, most of the literature says that the drones eject two substances during revealing of endophallus: semen and mucus. (9, 10).

MATERIAL AND METHOD

For semen collection were used a number of 200 drones of Apis Melifera Carpatica. Drones originated from an apiary that comply with existing veterinary standards and is accredited as organic apiary after EU criteria. Sexual maturity occurs in 13 to 15 days after hatching, so there were formed two groups, by age (L1- 15 days old drones, and L2 - 30 days days old drones).

Semen collection was performed using the following technique: the collecting syringe was prepared by filling it's cusp with saline solution. As saline solution, it was used Hyes physiological solution. After extraction of one drop of saline solution, a small air bubble was aspirated and then the collection started. The drone used for collection was positioned at the cusp of the syringe, under microscope. Semen was aspirated separate from annexes glands secretion and the collection of the mucus was avoided. Between two crops an air gap was kept and some saline solution was aspirated to avoid drying.

The sperm is ejaculated along with homogeneous white mucus, of which is easy to differentiate by its yellowish color and different structure. Higher content of spermatozoa is reflected in more intense color and viscosity

RESULTS AND DISCUSSION

Of studied drones, in 55.5% (111/200) the ejaculation occurred, and this way the semen collection was possible. In the rest of the drones, (89/200), the endophallus was not revealed and the ejaculation did not occur. Of drones that served to collection, in 75.7% (84/111) the ejaculation was total, while in 24.3% (27/111) the ejaculation was partial. Based on age categories, in L1 group the collection succeeded in 52% of the drones (69.2% with total ejaculation and 30.7% with partial ejaculation) while in L2 group the results were better, semen being collected from 59% of the drones (81.4% with total ejaculation and 18.6% with partial ejaculation.

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There are currently few data regarding the proportion of drones from which semen can be collected manually by revealing the endophallus (2). Unlike our results (55.5% successful collecting) Collins and Pettis (2001) collected semen from 60% of 12-day-old drones, and Anderson (2004) reports a successful collection from 90% of 20 days-old drones.

Manual labor for collecting semen from drone involves contention and stimulation of abdominal contraction this way producing a partial erection (partial revealing of endophallus) in the first time and afterwards total. (Figure no 3, 4). Initially is revealed the bulb, which is empty and pushed out, and then filled with semen and content of mucous glands. Chitinous plates of the bulb appear to the left of the vestibular hole. Constant pressure in the abdomen determines the bulb to be completely revealed and leads to gradual and total endophallus revealing. As a result, a drop of creamy sperm appears at the end of it. This stage of endophallus eversion is essential in semen collection process. (figure no 1, 2).

Sperm collecting process is extremely important, because the success of semen preservation and artificial insemination may depend on it. Collection of the sperm must be under aseptic conditions, to avoid its infection with different pathogens. The equipment of collecting must be made of a material that does not affect the viability of spermatozoa. The used methods should be practical and easy to apply in the field or laboratory, and they should lead to obtaining a large, sperm-rich quantity of ejaculate.

During the operation of collecting sperm from drones, usually there are large differences between individuals in terms of ease of collection, quantity and quality of the ejaculate. Some of them ejaculate very easy, but in other individuals this operation turns to be extremely difficult or even impossible. Semen quantity is also very different, especially in drones originated from inbred lines. Some drones have no sperm at all, in others ejaculation occurs without full eversion when the process of artificial collecting was initiated; in some drones, the eversion with ejaculation is so violent that the penis "explodes" and the sperm is lost.

Triggering of the artificial sperm ejaculation can be obtained by massaging the chest and abdomen. The collection technique should be designed to stimulate abdominal contractions and not to squeeze the sperm out. A collecting process started correctly releases the sperm of the drone completely and not mixed with mucus, which appears entirely separated.

Ejaculation can occur in two modes: partial ejaculation and total ejaculation. Partial ejaculation is obtained by holding drone's head and thorax and pushing its abdomen. Stimulation is required and it is done by holding the head and thorax of the drone and turning the drone slightly. During partial ejaculation, the abdomen contracts and forms the antennas. Pushing the anterior part of the abdomen must not be powerful; otherwise the abdomen could be crushed.

Sometimes it is necessary to insist, compressing the abdomen to stimulate total ejaculation, which occurs with total revealing of the endophallus. This pressure forces endophallus glands which produce semen. The sperm is ejaculated along with homogeneous white mucus of which differs by yellowish color and by structure.

Emptying the genital tract occurs through contractions of the muscles from seminal vesicle wall and mucous glands, starting from the top, and the content is ejected with considerable pressure. In mature drones, this process is triggered after a slight pressure on the abdomen. A collecting process started correctly releases the sperm of the drone completely and not mixed with mucus, which appears entirely separated. It is important to collect only semen, not mucus.

Table 1
Success in semen collection process according to age of the drones

	Drones used to collection		Successful collection		Drones that did not ejaculate	
	No.	%	No.	%	nr	%
L1 (15 days)	100	50	52	52	48	48
L2 (30 days)	100	50	59	59	41	41
Total	200	100	111	55,5	89	44,5

Table 2
Types of ejaculation in drones according to their age

	Drones that ejaculated		Partial ejaculation		Total ejaculation	
	No.	%	No.	%	No.	%
L1 (15 days)	52	46,8	16	30,7	36	69,3
L2 (30 days)	59	53,2	11	18,6	48	81,4
Total	111	100%	27	24,3%	84	75,7%

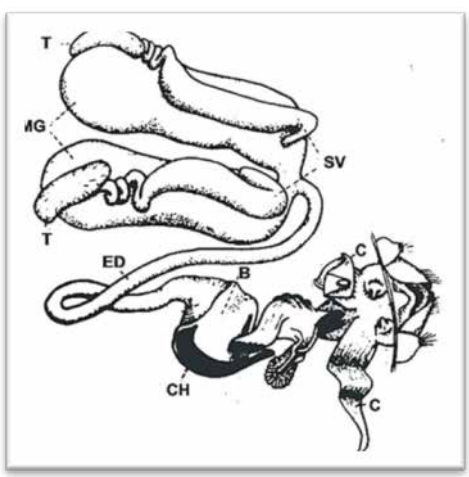

Fig no 1. Scheme of the genital apparatus in drone –after Woyke J. et al. 2001

T – Testicle, MG – Mucous glands, SV –Seminal vesicles, ED – Endophallus, B- Bulb, CH- Chitinous plates.

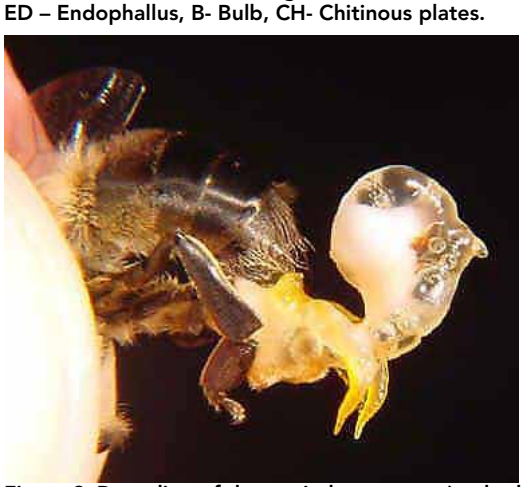

Fig no 2 Revealing of the genital apparatus (endophallus) with annexes glands

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Fig 3. Partial revealing of endophallus with partial ejaculation

Fig 3. Total revealing of endophallus with total ejaculation

CONCLUSIONS:

1. Manual labor of collecting semen in drones involves contention of their head and chest, pressing roundly and moderate, in order to stimulate contraction of the abdomen, thus producing a full erection (revealing the entire endophallus) which will assure the ejaculation. We do not recommend abdominal pressing, to avoid crushing.
2. In 55.5% (111/200) of subjects ejaculation occurred, and thus semen was collected. Of these, 75.7% (84/111) had total ejaculation.
3. By age, the best results were obtained in group L2, in which collection was successful in 59% (81.4% total ejaculation and 18.6% partial ejaculation). We recommend usage of drones older than 30 days for collecting semen.

REFERENCE 1. The Hindu Business Line, 21.11.2011 | 2. M.V. Prasad, Ananta Sarkar and J. Jameena (Directorate of Oil Palm Research, Pedavegi, A.P.), Performance of oil production Technologies, Indian Research Journal Ext. Edu 10(3). | 3. Prasad M.V.Rethinam, P.Kochu Babu.M and Dhander.D.G. (2008), Oil Technologies dissemination and its Adoption. Abstracts of National Conference on Oil Palm for farmer prosperity and edible oil security. pp 48-53. | 4. Chadha.K.L.(3006) Progress and potential of Oil Palm in India. Report of the Committee to reassess fresh potential areas of Oil Palm in India, Department of Agriculture and Co-operation, Government of India, New Delhi 22. | 5. Agricultural Statistics at a glance, Government of India, Ministry of Agriculture, Directorate of Economics and Statistics. | 6. Fertilizer Association of India (2012 a), "Fertilizer Profile 2012", Fertilizer Marketing News, Vol.43, No.5, May 2012, pp.27. | 7. Lal M (2003) Global Climate Change: India's monsoon and its variability, Journal of Environmental Studies and Policy, TERI 6:1-34.