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Influence of the Use of Diluents in the Technique of Homogenization of Beekeeping Seminal Material – Review

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Abstract: *Mixing bee semen is a necessary step in collecting the genetic structure from individuals with high production values, a method that serves with great success the intention of a very closed, intense and long-term selection program. Whether we talk about the technique of mechanical-gentle homogenization, more recently proposed, or the one by centrifugation, the use of diluents or extensions for the seminal material of the species Apis mellifera can significantly influence the results of instrumental insemination. The study looked at the influence of the use of diluents in relation to the adhesion, viability, mobility and density of sperm stored in the sperm deposit. The results indicate that the choice of diluent has a relative effect on optimizing the sperm mixing procedure and instrumental insemination.*
Keywords: Apis mellifera, instrumental insemination, sperm diluents, sperm homogenization.

Introduction

In the act of natural polyandric reproduction, the queen receives in the spermatheca a quantity of sperm coming randomly from a different number of drones (5-24), which she will later disseminate in the future genetic structure of the family. By using the method of sperm homogenization and instrumental insemination, male genetic information can be introduced into the queen's spermatheca, directly proportional to the number of drones participating in the act of artificial fertilization. By using the method of sperm homogenization and instrumental insemination, male genetic information can be introduced into the queen's spermatheca, directly proportional to the number of drones participating in the act of artificial fertilization , which obviously leads to an explosion of genetic information. This closed variability can be directed towards a precise long-term selection goal thus avoiding the inbreeding pressure on the biological material. But all these techniques and procedures are effectively supported by the identification of the best performing specific methodologies applied in relation to different diluent formulas.

Homogenization techniques in relation to Apis mellifera sperm diluents

The scientific research undertaken so far has highlighted the use of diluent formulas, namely:
- trisodium citrate-2-hydrate (2.43%), glucose monohydrate (0.3%), NaHCO3 (0.21%), sodium sulfacetamide (0.3%) which replaces sulfanylamides (0.3%), and in distilled water is added KCI which is brought to a pH of 7.8-8.4. Sodium penicillin G (0.1 %) is added to this composition [2];
- NaCl (1.1%), glucose (0.1%), L-Arginine HCI-(0.1%), L-Lysine -HCL (0.01%), Tris hydroxymethyl aminomethane hydrochloride, then the whole solution is brought to a pH of 8.7-8.9 and subsequently medicated with streptomycin sulfate (0.02%) and penicillin sulfate G-K (0.01%) [1];
- hydrated sodium citrate 2.43 g, sodium bicarbonate 0.21 g, potassium chloride 0.04 g, sulphanilamide 0.3 g, D-glucose 0.3 g, distilled water 100 ml and boiling the entire solution for sterilization at the temperature of 90 ° C

Although many authors, considered that the influence of the sperm factor in the success of the handling of bee semen plays an overwhelming role, later, research has shown that other factors are much more decisive .

Regarding the technique of homogenization of the sperm of the species Apis mellifera, two forms of mixing are practiced: by centrifugation and technique of gentle homogenization by paddle.

The technique of breeding and maintaining drones play a very important role in the success of the instrumental insemination technique.

The sperm, which is diluted in order to facilitate a better mixing, so that the semen is then reconcentrated by recentrifugation, is very affected in terms of viability and mobility of spermatozoons.

Thus, the gentle mixing process of the seminal material responds much more optimally to the requirements of the purpose of the instrumental insemination: the mobility and vitality of the honey bee sperm at the highest possible levels.

The homogenization edition was considered to be completed after the expiration of the exposure time of the semen depending on the choice of mixing method: centrifugation method - 2500 rpm for 10 minutes and mixing method - slow paddle for 5min / direction / up-down .

The queens were inseminated on day 7-10 after hatching, in order to allow the best physiological maturity of the reproductive system.

The queens were inseminated with 8 microliters of semen for each queen. The onset of laying was checked on average 5-7 days after insemination . A partial evaluation was made at 45 days, and the final evaluation regarding the vitality, viability and prolificacy of the brood was determined after the end of a complete beekeeping season [. Sperm mobility was assessed visually with a microscope, and the viability with SYBR-14.

As the semen was subjected to different dilution ratios, the results obtained were different. Thus the ratio of 1:1; 3:1; 6:1; 9:1; 12:1 was tried and the observation was that the use of diluents or extenders of bee semen does not dramatically change the parameters of viability, but only those related to the quality of mobility and density. The viability parameter does not appear to be affected either by the different diluent formulas or by the amount used in relation to the semen.

Fig. 1 Gentle sperm mixing session

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Other scientific studies have shown that the degree of dilution of sperm in different percentages, from 2-120%, changes the degree of sperm adhesion [2, 3, 15, 18]. Thus at a percentage lower than 2% of diluent, the degree of sperm adhesion is still very high, losing a significant amount of semen during the homogenization procedures. Also at this low mixture percentage, the degree of mixing is very inefficient. At a mixture of 10% diluent, the degree of sperm adhesion is very low, not affecting its mobility and density . Use of a diluent ratio (Hyes solution) and sperm of 1:1 had the best results.

The determined data were correlated in relation to the amount of sperm stored in the spermatheca, the beginning of laying and the quality of the mixture in correlation with the complete dispersion of the genetic diversity of the collected semen.

In the case of sperm homogenization, the use of a mixed solution containing energy medium recorded a better mobility of sperm both in the use of ad-hoc semen for harvesting, homogenization and insemination and in the case of the attempts of cryopreservation .

Another aspect is related to the ratio of the use of diluents during the procedures and the onset of laying. It was found that the onset of laying is not influenced by the use of diluents during the process of homogenization and insemination, but exerts changes only on the sperm density in the spermatheca. This impediment has been observed to be corrected by two successive inseminations of two classic doses of semen, respectively 6-8 microliters at intervals of one or two days .

The onset of laying in all experimental groups occurred on average between 5-11 days after insemination.

Conclusions

For a long time, the technique of homogenization and dilution of bee semen was adapted to the customs of the livestock sector. However, recently specific studies and researches have been intensified due to the need to start breeding and selection programs in closed populations, which is why the techniques of mixing and implicitly diluting the honey bee semen require constant improvements in the entire process of bee instrumental insemination. Studies have shown that homogenization of honey bee sperm by centrifugation has been shown to affect sperm motility regardless of the use of any diluent. In this sense, the trend is to identify means of mixing sperm, which should limit as little as possible the handling and exposure of bee semen to various diluents.

Thus, the gentle mixing process of the semen would respond much more optimally to the requirements of the purpose of instrumental insemination: this is not a decisive factor in the final result. Obviously, researches will continue to capture new knowledge and solutions for optimizing the process and e mobility and vitality of the honey bee sperm at the highest possible levels. This aspect needs to be more intensively explored and scientifically substantiated.

The study shows that in the success of the technique of homogenization of bee semen, the choice of diluent used can improve the results of the final procedure, but the phenomenon studied.