Bee World

An Introduction to Instrumental Insemination of Honey Bees

--Manuscript Draft--

Full Title:	An Introduction to Instrumental Insemination of Honey Bees
Manuscript Number:	TBEE-2016-0033
Article Type:	Review article
Keywords:	honey bee; instrumental insemination; breeding; Apis mellifera
Manuscript Classifications:	Bee management; Genetics and breeding
Abstract:	Selective breeding and stock improvement of honey bees are an integral part of the long term solution to challenges facing the beekeeping industry. Honey bees present a unique challenge because the queen mates in flight with numerous drones. Thus controlled mating with instrumental insemination is essential for a successful and sustainable honey bee breeding program. The technique of instrumental insemination is practical and highly successful, yet remains under-utilized by the beekeeping industry. This chapter provides an overview of the technique and basic requirements are reviewed.
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An Introduction to Instrumental Insemination

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Selective breeding and stock improvement are recognized as an integral part of the long term solution to challenges facing the beekeeping industry. The continuing high loss of colonies and the worldwide movement of honey bee pests and pathogens adds to this urgency. Queen producers realize the need for more rigorous programs to select, improve and maintain their breeding stocks. Interest in programs to select locally adapted and survival stocks is also increasing.

Controlled mating is essential to achieve the goals of any breeding program. Honey bees present a unique challenge, because the queen mates in flight with an average of 15 to 20 drones in congregation areas consisting of 10,000 to 30,000 drones from diverse genetic sources. The queen mates during the first week of her adult life and stores the sperm collected in her spermatheca for use over her lifetime. She stores a small percentage of sperm from each drone with whom she mated and this is mixed in her spermatheca. All the drones the queen mated with are represented in the many subfamilies of her colony, although the ratio of these may change over time. Instrumental insemination (I.I.) provides a valuable tool to control this random mating process.

I.I. is a powerful tool for breeding as well as for research purposes. It also provides a means to create specific and novel crosses, beyond what occurs naturally. For example, a single drone can be mated to one or several queens, isolating and amplifying a specific trait. On the other extreme, semen from hundreds of drones can be mixed and inseminated to a batch of queens. Varying degrees of inbreeding can be created to produce different relationships. This ability provides a means to study and tease out the complexities of honey bees. Another advantage I.I. provides is the ability to hold fresh semen for several days, enabling the safe and easy transport of desired stock without the risk of introducing parasites. Also, we now have the capability to cryopreserve honey bee semen in liquid nitrogen for long term storage (Hopkins at al. 2012).

The technique of instrumental insemination is practical and highly successful. It is an essential skill for beekeepers to develop and maintain viable breeding programs. Yet, today the technique remains under-utilized, largely limited to the scientific community. The reluctance of the beekeeping industry to adopt the widespread use of I.I is due to several factors. The technique is specialized and very delicate, and requires advanced beekeeping skills, the rearing and care of virgin queens and drones. The unfounded reputation of poor performance of instrumentally inseminated queens, (IIQs), persists. Poor results are generally due to poor insemination procedures and inadequate care of queens. Contributing factors include:

- infection and injury
- poor banking practices
- inseminating queens beyond their appropriate age
- insufficient semen dosage

These problems can be corrected with proper training, care and attention to detail.

Using proper techniques, the performance of IIQs, is similar to that of naturally mated queens. Colony performance evaluated in terms of honey and brood production, and longevity of queens in field colonies are indistinguishable between the two groups. A comparison of studies from 1947 to 2007 and factors affecting performance of IIQs are reviewed, and the treatment of queens is clearly shown to influence results (Cobey, 2007).

In addition to controlled mating, stock improvement requires a program of systematic and scientific breeding methodology. To gain the benefits of I.I. also requires good beekeeping practices, the resources to run a selection and breeding program, and a long-term commitment. This is demanding, labor intensive and expensive. Most successful long term programs are a cooperative effort between universities or institutes and the beekeeping industry.

Of critical importance to consider and research is the supporting information and material on bee breeding methods. To ensure colony fitness, selection for a variety of traits such as productivity, temperament, overwintering ability, must be considered. The selection criteria should be weighted and balanced in terms of colony productivity and the "colony cost" of selection for resistance to pests and diseases, as *Varroa* mite tolerance/resistance is a major goal.

Honey bees are unique, given that selective breeding is based upon behavioral traits of a super organism with a complex social structure, in a constant state of change. *Varroa* mite and its associated pathogens are also undergoing constant change. For these reasons, honey bee breeding is challenging. Yet, with the current focus on bee breeding and advances of research and new scientific technologies, applying this knowledge in the field will hopefully offer real solutions. To gain the benefits of I.I., beekeepers must first build a solid foundation by establishing a breeding program or research project.

Learning the I.I. technique requires proper instruction, precision equipment, practice, fine motor skills, maintaining sanitary conditions, patience and a commitment. The process can initially be awkward and frustrating to learn, as meticulousness precision is essential. The beekeeping knowledge and skills to rear a plentiful supply of mature drones and virgin queens is also required. To gain proficiency; practice, manual dexterity, and attention to detail are necessary. The pre and post care of queens also plays a critical role in the success of I.I.

To gain confidence in the technique I suggest, inseminating 30 to 50 queens in the initial practice and dissect their spermathecas to evaluate success. Keep good notes on each insemination. Bank the queens in a nursery colony for 2 days to allow for sperm migration. Lack of vigor and mortality will indicate problems with injury or infection. Dissection of the spermathecas will determine the rate of success. The color and density of the spermatheca will indicate the quality of insemination, as described in the reference listed: Dissection of the Spermatheca (Cobey). Inseminate a second batch of queens and introduce these into nucleus colonies to establish them as laying queens.

There are several insemination instruments designs, these vary widely in quality and cost. There is no standardization in equipment and some parts are not interchangeable between instruments. Therefore, research the options. The technique requires precision and accuracy in fine adjustments and these features will determine the ease and repeatability of the procedure

The basic instrument consists if a stand, a set of hooks or forceps, queen holder assembly, syringe and syringe tips. A microscope with a magnification of 10X to 20X, cold light and compatible stand with sufficient depth of field and instrument clearance are required. A cold light prevents drying of tissues, a gooseneck L.E.D. light works well. A source of carbon dioxide with a flow regulator and flexible tubing to the instrument are used to anesthetize the queen during the procedure.

Modern instruments offer micro-manipulators that provide fine precision in movements. Large capacity syringes provide efficiency in semen collection, storage and shipment of semen. Various designs of sting manipulation tools offer personal choice in techniques. The Schley and the Loc Instruments are currently the most widely used instruments, valued for their fine precision and wide range of flexibility in adjustments. The Harbo large-capacity syringe, designed for ease of semen handling and storage, is also popular and compatible with these and most instruments.

Good sanitation and proper techniques are critical to success. Injury and infection are the most common problems for beginners. Drones tend to defecate during the eversion process of semen collection, and can be very messy. Maintaining highly sanitary conditions is essential.

The insemination procedure requires accuracy, precision and attention to detail. The manipulations: 1) positioning the queen, 2) opening the vaginal cavity, 3) bypassing the valvefold and 4) insertion of semen, must be accurate and brief. Queens vary physically, especially between subspecies, and these nuances of differences must be learned and recognized.

Care of queens, their pre and post insemination treatment and introduction methods require careful attention. The queen's complex pheromone blend signal changes in her reproductive status to the workers, from virgin to mated and laying. These changes vary more among IIQs and therefore queen introductions require more care. These factors are discussed in the reference; Comparison of instrumental inseminated and naturally mated honey bee queens and factors affecting their performance, (Cobey 2007).

Rearing a plentiful supply of select drones to maturity can be a major limiting factor. Most beekeepers are not accustomed to giving detailed attention to drone production. Drones are seasonally produced and vulnerable to stressors; such as parasites and pathogens, poor nutrition, miticide and pesticide residues, poor weather conditions, etc. Colonies will regulate their drone population based on many factors.

Strong healthy, well fed colonies are required for drone production. A colony will rear about 2000 drones during peak season, of which about half will be immature. Drones are mature at 2 weeks post emergence and peak at 3 weeks (Rousseau et al , 2015). Stressed colonies will eliminate mature drones and often continue to rear young drones. Colonies headed by older queens tend to rear and care for more drones.

The actual insemination procedure is very quick, timing is dependent upon the skill of the inseminator and the quality of the equipment used. Once the semen has been collected, the insertion of semen into the queen's oviduct takes a matter of seconds. The semen collection process is more tedious and determined by the quality and maturity of drones. Many semen loads will be discarded due to contamination during the eversion of the endophallus. Some drones may not be mature and some simply will not yield semen. The standard semen dosage per queen is 8 to 10 microliters. Often it takes twice the number of drones to attain this.

Success is dependent upon two major factors; the skill of the inseminator and the beekeeping skills to provide proper care of queens and drones. With proper techniques and practices, IIQs have the capability to preform as well or better than naturally mated queens. Better performance of IIQs is based upon the ability to do selection and control the semen dosage given. The pre and post-insemination care given to IIQs affects sperm migration, performance and longevity.

A major advantage of I.I is that honey bee semen can be held at room temperature, 13ºC for best results, with good viability for about 2 weeks. Short term storage of semen enables the transport of semen and insemination scheduling. The type of diluent used and the temperature of storage (do not refrigerate and avoid sunlight exposure) are important factors affecting viability, see (Cobey, S., Tarpy, D., Woyke, J. , 2013).

Advances in cryopreservation techniques for the long term storage of honey bee semen enables the conservation and reconstitution of select stocks (Hopkins et al. 2012). This ability also provides unique breeding and research tools, as it enables breeders to select across time and space. Cryopreserved semen can be held indefinitely. Research is continuing in this area, with worldwide interest in establishing honey bee germplasm repositories to conserve honey bee genetic diversity.

It is well recognized that genetic diversity increases colony fitness. The ability to pool and mix semen from a diversity of drone sources has advantages for breeding purposes and stock maintenance programs. Techniques to homogenize semen are being explored, although this remains a challenge. The natural mixing and migration of sperm from the queen's oviducts into the spermatheca is a complex process, requiring about 40 hours. This involves contraction of queen's muscles, mediated by the Bresslau's pump (the muscular system of the spermatheca duct) and specialized composition of fluids in the semen and the oviducts, as well as active movement of the queen.

Perfecting a technique to homogenizing bee semen is difficult because the sperm tails are very long and fragile, and the semen is highly viscous, very dense and tightly coiled. Mixing techniques require dilution, mechanical movement and reconstitution of the semen, in which some critical components are removed. Brauße J., & J.P. V. Praagh (2010) report success in mixing large volumes of semen, but the rate of semen recovery is low. This can also be very risky in terms of contamination if careful attention is not taken. For routine inseminations, it is recommended to mix active drones from different sources in a flight box for semen collection.

Techniques

To collect semen, the drone's endophallus is everted and semen exposed. This is a two-step process, the partial eversion and the full eversion. To stimulate the partial eversion, hold the drone dorsally - ventrally and apply pressure to the head and thorax. During this stage, the abdominal muscles will contract making the abdomen hard to the touch. The cornua, a pair of horn-like structures, appear. An orange-yellow color of the cornua indicates the drone is mature. If the drone is the immature, the cornua will have no color and the abdomen will remain soft.

The second step, the full eversion, exposes the semen. Once the partial eversion is obtained, apply pressure along the sides of the abdomen with the thumb and forefinger, starting at the anterior base of the abdomen. This pressure forces the endophallus inside out to expose semen. Hold the drone downward to prevent the endophallus from flipping back onto your fingers causing contamination. The semen is a creamy marbled color on a layer of white mucus.

To collect semen, a small drop of saline is expelled from the syringe tip to make contact with the semen. Skim the semen off the mucus layer of the endophallus. The mucus is more viscous and the semen will separate readily from this. Evert the next drone and repeat the process until the desired amount of semen is obtained. Each drone will yield about one microliter of semen. The standard semen dosage is 8 to 10 µl per queen.

The queen is anesthetized with carbon dioxide and placed in the holding tube of the instrument under magnification of 15X to 20X. The vaginal cavity is exposed by using a pair of hooks or forceps. The dorsal hook, or sting hook is designed to lift the sting structure to expose the vaginal opening of the queen. The choice if a perforated sting hook or forceps can be used.

The sting structure is lifted to expose the vaginal opening. When the hooks are properly positioned a "V" of tissue defines the location of the valve fold. The valve fold, an invaginated flap of tissue which blocks passage of semen into the oviduct, is not readily visible. The valve fold must be bypassed and semen delivered directly into the median oviduct.

The syringe tip is positioned above and slightly to the right of the "V". Using a slight zig zag motion, the tip is inserted and moved slightly to the left, increasing the incline of the tip to bypass the valvefold. A measured amount of semen, as discussed above, is inserted into the median oviduct.

To gain proficiency in the art of instrumental insemination requires practice and patience. The first attempts may seem awkward and frustrating. In learning the procedure, it is not unusual to experience high mortality of queens. Accuracy and efficiency will be gained with practice. It is also important to establish a routine and maintain highly sanitary conditions throughout the insemination procedure to ensure success. To learn the technique, it is advisable to train with someone who has experience.

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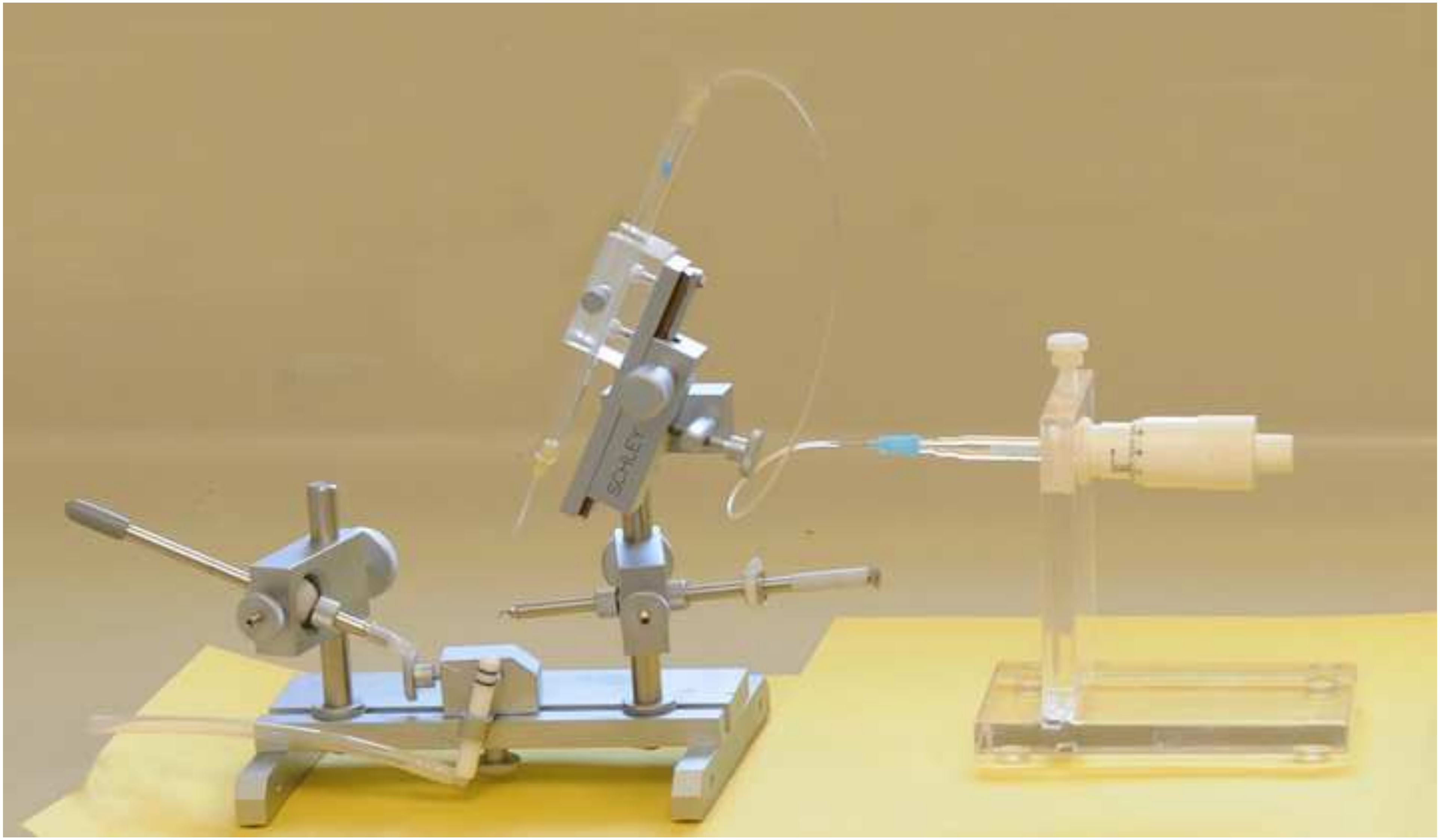
Figure/Photographs



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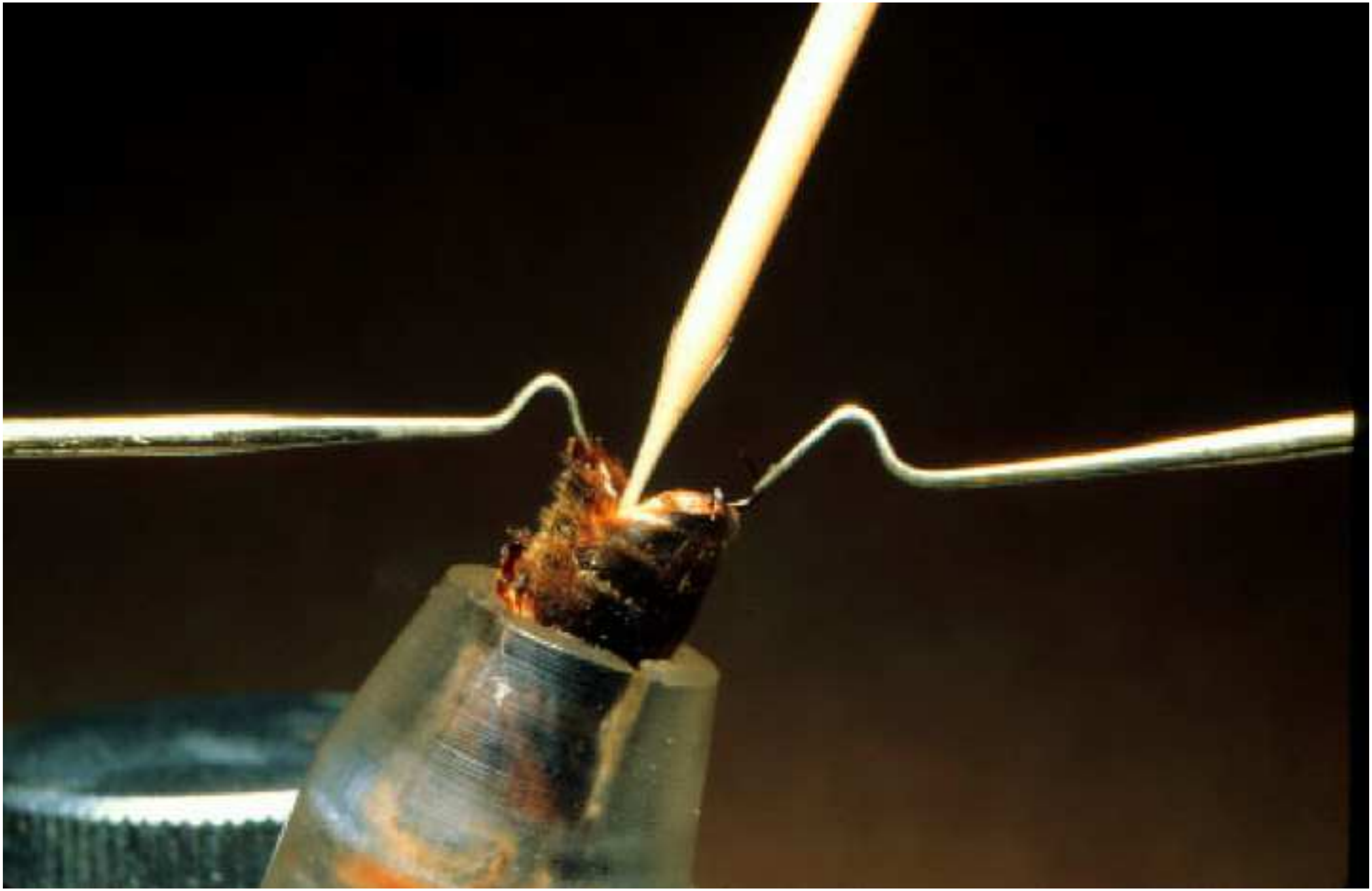

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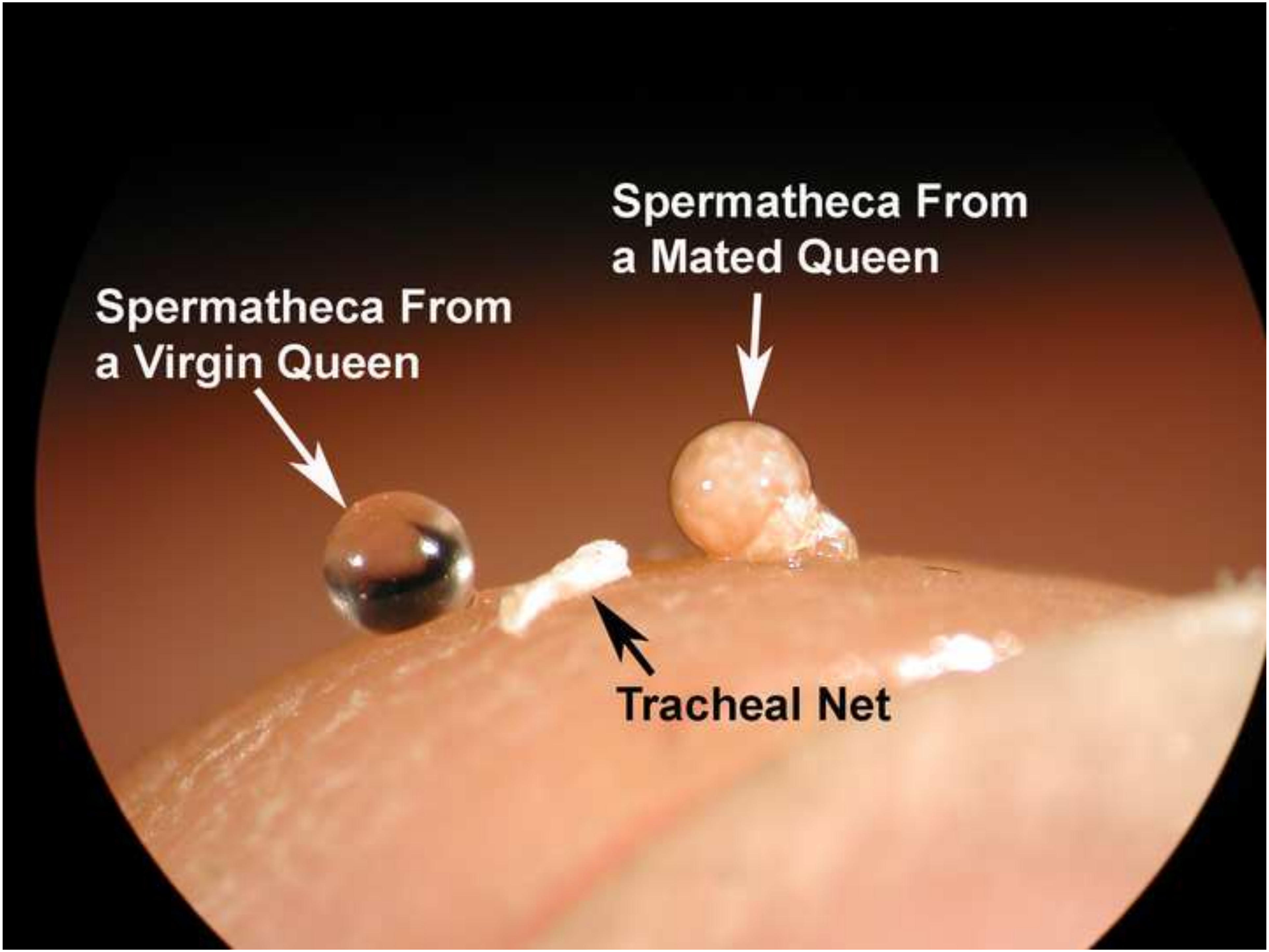

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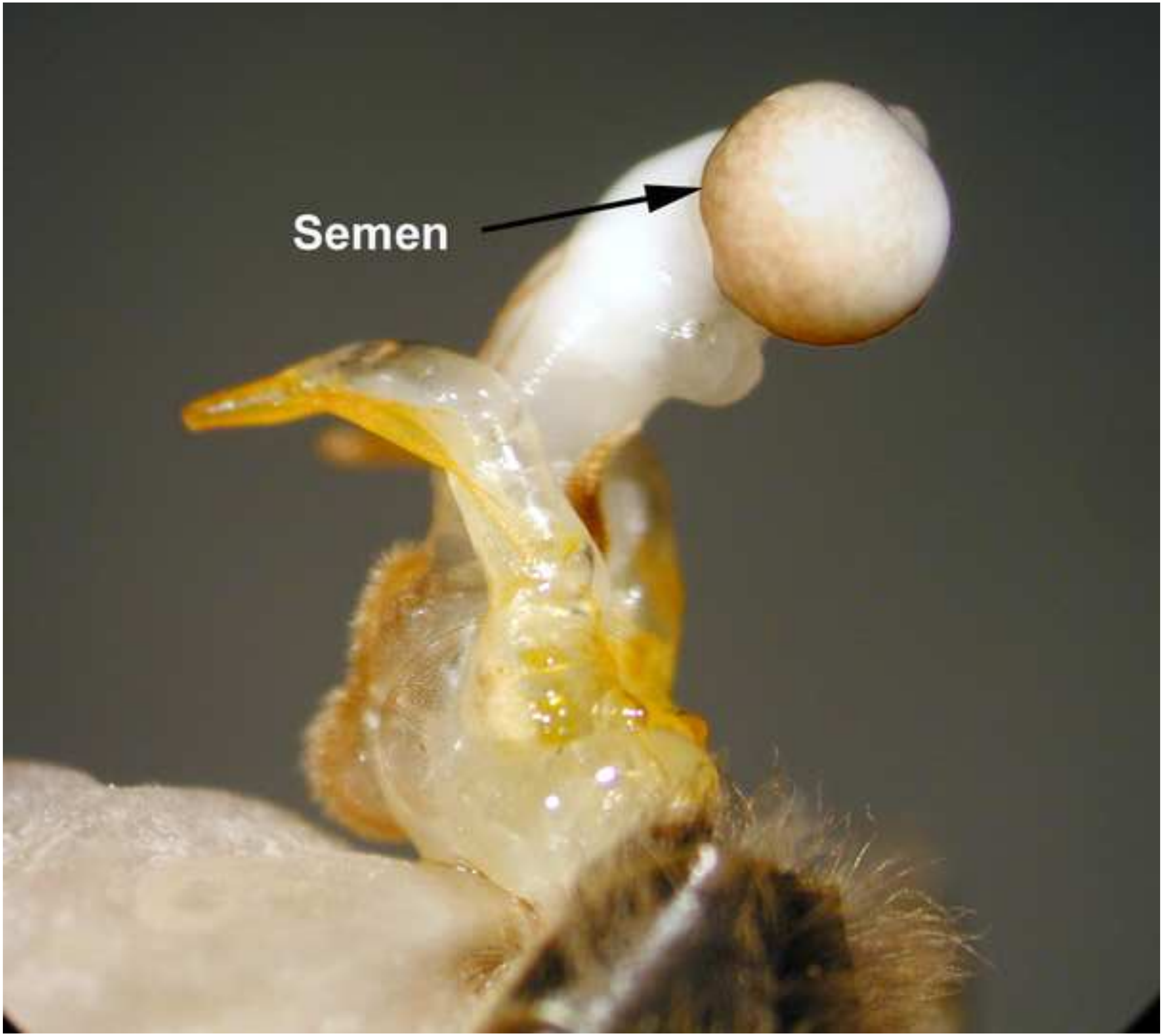

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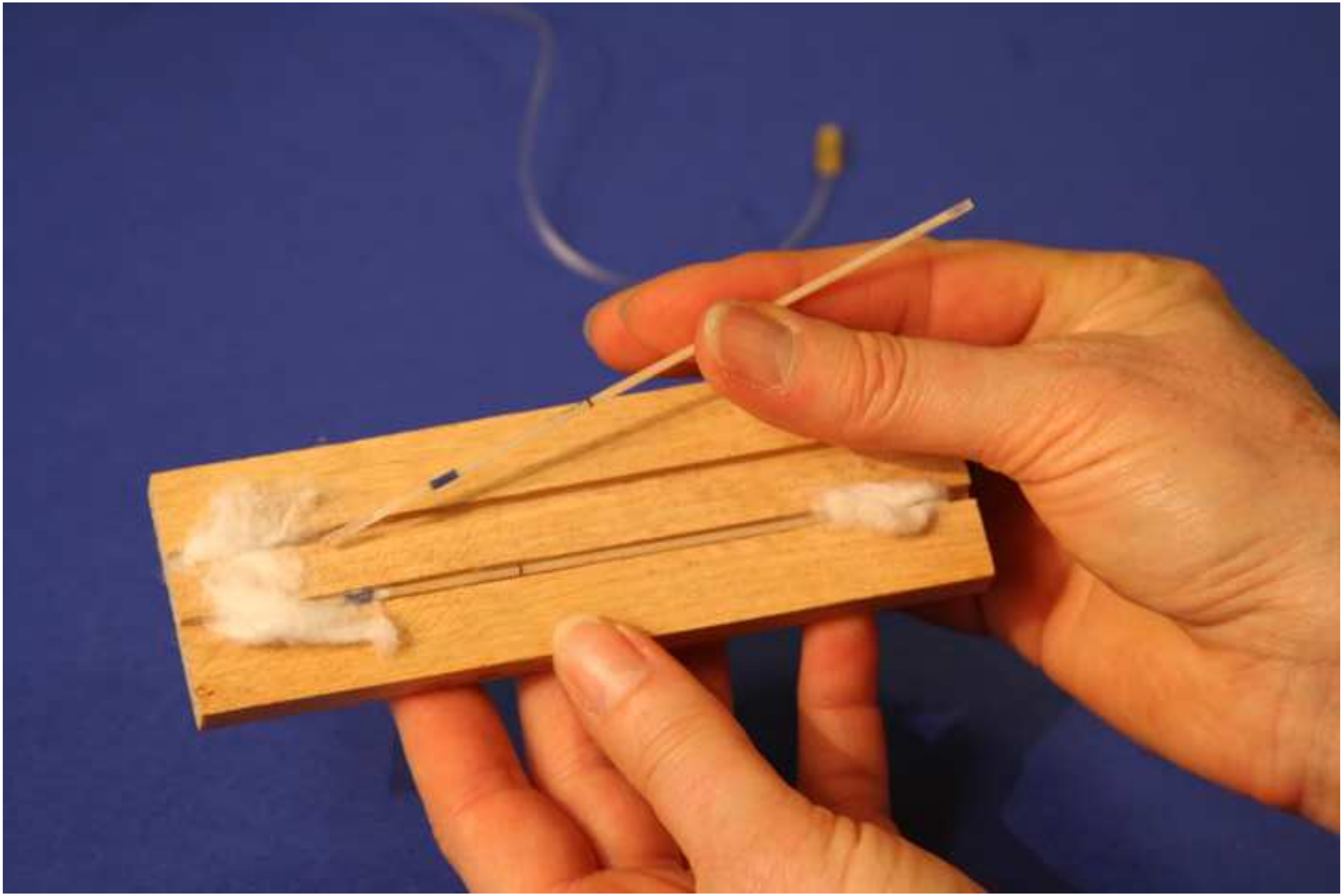

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Photo Captions

1. Schley Instrument with Harbo large capacity syringe.
2. Insemination of the queen.
3. Queen spermathecas. The virgin queen's spermatheca is crystal clear and the mated queen's is a marbled "café au lait" color.
4. Full eversion of the drone endophallus exposing semen.
5. Capillary tubes of semen prepared for shipment.

Photos by S. Cobey