An informational article:

The “flexible” insemination technique

Choosing Insemination Techniques and Instruments

Instrumental Insemination, as a tool to control mating has been widely used by the honey bee research community. A very specialized technique, this has been slow to be adopted by the beekeeping industry. Yet, today the current bee crisis has increased interest in this technique as a means to enable selection for honey bees expressing mite and disease tolerance.

One aspect impeding the use of instrumental insemination is the confusion in the variety of techniques and instruments available. There is instrumentation that increases the ease and repeatability of the technique, as well as instruments that make the procedure difficult to preform. To the beginner this presents a mystique and confusion in what to purchase. Too often the initial trails with an inadequate instrument result in frustration, abandonment and a perception the technique is difficult to master.

The procedure requires precision and attention to detail, thought is not necessarily difficult. An instrument that provides accuracy in fine movements and proper alignment is essential. Pricing can be a factor in choice, although one must realize the value in quality. Instruments offering precision in micromanipulation increase the ease of use. The low demand for Instruments has kept the pricing for quality instruments high and resulted in a scarcity of sources. This often makes a more economical model attractive. The lack of success with an instrument that does not offer precision results in frustration and failed attempts, and propagates the mystique of this procedure.

Many practices have been tried to perfect the technique of instrumental insemination. Of the numerous trials and suggestions, few have been put into practice. A major improvement providing precision, ease of use and repeatability is the use of micromanipulators to control fine movements of the hook and syringe.

Another improvement is the variety of sting hooks used to open the sting chamber of the queen. The introduction of my perforated dorsal hook, designed in 1988, in which the sting is threaded and lifted provided a advantage. This sting structure is lifted and the vaginal chamber exposed in manner to ease bypassing the valvefold and delivery of semen into the oviduct. The use of hand held forceps to grasp and lift the sting also accomplishes this.

The hand held forceps technique, initially used by Dr. Lloyd Watson in the 1920's, was reintroduced with the Kühnert – Laidlaw instrument design. Ms. M. Kühnert, an experienced insemination technician at the Oberursel honey bee research institute in Germany, dedicated herself to this idea. She perfected the technique in 2004 and published this method as the "flexible insemination method" in the bee journal "Apidologie". This new method worked well and was intended to be used with a simplified, economical instrument beekeepers that could build themselves. Simplification was intended to promote the use of instrumental insemination.

The Kühnert – Laidlaw instrument is used without dorsal hook (sting hook) and with a fixed queen holder. The queen bee is placed in a loose, open cylindrical tube, without a tapered cone to steady her position. A hand is placed on the edge of the instrument with the fingers up against the queen holding tube while manipulating the sting with the other hand holding the forceps. This technique requires steady hands and precision in the syringe manipulation. This instrument was not commercially produced as the difficult was providing a precision manipulator for the syringe.

The hand held forceps method was adopted by Joseph Latshaw, of Ohio, USA. The initial prototype of the Latshaw instrument design used finger controlled micromanipulators. Unfortunately this instrument is no longer available. The new Mini Latshaw instrument, designed to be economical, is disappointing in the lack of precision and misleading claim of semen mixing in the large capacity syringe. The syringe is also difficult to assemble and the glass tip pops off with little pressure.

The excessive play in the syringe manipulation makes the procedure extremely unsteady and difficult, especially with the use of hand held forceps. To gain stability during the procedure, it is suggested to use the thumb screw to tighten and secure the syringe barrel before
delivering the semen. This is difficult and cumbersome to do one handed, while using a hand help pair of forceps in the other hand. Unsteady movement of the syringe risks injury to the queen and make the technique extremely difficult.

For demonstration purposes, Latshaw shows a video clip using the hand held forceps technique on his website in which the opening is stained blue. In the video, this technique appears straightforward and easy.

From years of experience in teaching this technique, we know beginners have difficulty with such unfamiliar, delicate handling. The hand movements must be practiced. Not everybody has such skill for detail and patience. This is where a comparison of the various techniques and instruments are warranted. Micromanipulators offering precision in fine movements and control make the procedure easier to learn and preform with consistency.

The introduction of my precision manipulator drive system for the syringe and hook holders greatly increased the ease, precision and high rate of repeatability

of the technique. The addition of the perforated hook increases the ease of opening the queen's vaginal cavity and ease of semen insertion. This has further been improved by the design of the forceps pressure grip sting manipulator. This provides the option of using the forceps method with fine control. The precision and accuracy of fine movements makes the technique easier to learn and use, especially for those challenged by the use of handheld forceps. This also offers high repeatability, especially for production work.

There is personal choice and flexibility in the method of sting manipulation used; perforated hook or forceps, etc.. However, the manipulation of the syringe is critical. Placement of the syringe tip into the oviduct and delivery of semen requires precision. Micromanipulators provide the precision needed, and this is the most expensive part of the instrumentation. This will make the difference between frustration in learning the technique and the success and ease of repeatability.

The photo below shows a queen being prepared for insemination using the hand held forceps method, rather than the use of a supported dorsal hook (sting hook). The syringe-manipulator, not shown, must guide the insemination syringe to position the cannula (glass tip) while viewed under the microscope, and a measured amount of semen administered. The left hand must be used to hold the sting chamber open during this process, while the right hand guides and operates the insemination syringe.

Anybody interested in this method and curious should try it. The hand held forceps method can be tried with any instrument design. No specific instrument is required.

An area of concern is the claim of the Latshaw syringe design for homogenizing or mixing of honey bee semen. In the large syringe receptacle, semen is held over time and claimed to self-mix. Latshaw demonstrates a dye moving through semen in saline to indicate the mixing of semen. This is actually the movement of a fluid (blue ink) from an area of high concentration to an area of lower concentration (saline) of two different liquids. It is an example of diffusion, not mixing.

Honey bee semen is very viscous and dense and the sperm tightly clumped. Mixing requires mechanical shear.
Mechanical shear is required to mix two viscous liquids or a viscous liquid and water. A method of mechanical mixing is essential in this situation. The high density and tendency of semen to clump requires that semen be diluted and subject to mechanical movement to be mixed. In this situation, progeny testing is needed to verify the level of mixing.

Under natural mating conditions the sperm are subject to specialized fluids and muscular contractions of the oviducts and abdominal muscles of the queen to transport the sperm into the spermatheca. The active movement of the queen

after mating also promotes sperm migration. Research has shown that when the muscles of the queen are paralyzed, few sperm reach the spermatheca.

Methods involving dilution and centrifuging to mix semen have been somewhat successful. The very long sperm tails are easily damaged. During the process of diluting & reconstituting semen, some essential components of the seminal fluid are washed away. As a research tool the dilution and centrifuge method is adequate, as high losses of viability can be tolerated verses the need for high productivity at the commercial level.

In choosing equipment, talk with an experienced inseminator and learn about the various options. It is best to purchase the right set up for yourself initially, so that you get a good start and build confidence in mastering the technique.

Literature:

KÜHNERT, M.; LAIDLAW H. H. (1994): Vereinfachter Apparat zur instrumentellen Besamung von Bienenköniginnen mit der "Flexiblen Besamungsmethode".
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