Advanced techniques in rabbit reproduction
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Abstract
The use of assisted reproductive technologies in rabbits, especially the use of artificial insemination (AI) techniques with extended liquid refrigerated semen, is a common practice in European countries such as Italy, France and Spain where there are well-established rabbit meat production industries due to public interest and demand in consuming rabbit meat as part of their daily diets. In the USA and Canada, the rabbit industry is in its infancy and for producers interested in improving their genetic lines, the use and application of AI will be an excellent tool to be considered. This article reviews all the steps involved in the use of AI techniques in rabbits.

Keywords: Artificial insemination, sperm, semen, rabbit, rabbit doe, rabbit buck.

Introduction
The intensive production of rabbit meat is relatively new in comparison with the pork, chicken and beef industries. The industrial production of rabbit meat started in the late 1970s. The 25 countries that are members of the European Union produce 520,000 tons of rabbit meat, a number that represents 50% of the rabbit meat world production. Italy with 215,000 tons, Spain with 120,000 tons and France with 75,000 tons together produce together 80% of the European rabbit meat.1

The estimated number of rabbit AIs happening per year in Italy is 5 million; in France 4 million/year and Spain 3.5 million/year are reported (Guy Delhomme, personal communication). For the year 2009, France estimated its rabbit carcass production obtained by AI as 45,800 tons.2

In Canada and the USA, rabbit meat production is in its infancy and the consumption of rabbit meat is extremely low. In Canada, the consumption of rabbit meat between 2008 and 2011 fluctuated between 18.7 and 22 grams per capita.3

The market for rabbit meat in the USA is small. However, rabbit meat is lower in fat, cholesterol, and calories, and higher in protein than beef, chicken, turkey, or pork, and these ideal nutritional attributes hopefully will increase demand. Rabbit production in the USA grew from being predominantly for home consumption to a large-scale commercial operation of about 200,000 rabbit producers in 2004. Researchers estimate that about 8 million rabbits are produced each year and that between 8 and 10 million pounds of meat are consumed annually.4 Hopefully this healthier source of animal protein will continue to attract more American and Canadian people to consume rabbit meat, and a real growth and development of the rabbit meat industry can become a reality similar to what happened in Italy, Spain and France.

This article is a summary of the basic steps involved in use of AI as an assisted reproductive technology in the rabbit industry.

Reproductive anatomy of the rabbit doe
The rabbit doe reproductive system has the following organs: two ovaries, two oviducts and two uterine horns. These two uterine horns are separated from the vagina by the presence of two uterine cervixes. Each rabbit doe ovary is approximately 1 cm wide by 2 cm long. On each ovary follicles are observed in different stages of maturation and during pregnancy it is possible to see the corpora lutea that secrete progesterone. The weight of each ovary varies depending on their physiological stage. The oviducts are tubular sinusoidal structures that can be 10-16 cm long and have the following three components: 1) the infundibulum that partially covers the ovary and functions to receive the ovum at the moment of ovulation; 2) the ampulla where the fecundation process happens and which is internally covered by ciliated cells that facilitate gametes transit of gametes; and 3) the isthmus which is thin and covered by mucus, secretory cells, and a higher number of ciliated cells. The uterus has two uterine horns where the gestation process occurs. Each horn is cylindrical, measures 10-12 cm long and has three
circumvolutions. The uterine horns will receive the embryos that will become implanted in the endometrium. The two horns are connected independently to the common vagina through two individual uterine cervices of approximately 2 cm long each. The presence of bicornual cervices in rabbits does not allow embryo migration to occur from one horn to the other. The vagina is 4-8 cm long. It is possible to see the urethral orifice caudally. The vagina continues caudally with the vestibule that measures approximately 2-3 cm. The vestibule ends at the external vulva. The vulvar lips change color depending on the sexual receptivity of the rabbit doe. The clitoris is well-developed and is found on the ventral commissure of the vulva.5

**Follicular growth in rabbit does**

Oogenesis is completed in rabbit does during the first two weeks of life with simultaneous growth of primordial follicles. The ovaries of four to eight week-old rabbit does already show follicles at early stages of development. It is well-known that rabbit follicles can produce polyovular structures containing two to three oocytes that can develop depending on their intrafollicular position. Follicular peripheral oocytes can have less opportunity of resuming meiosis when compared to centrally localized oocytes. For this reason, not all the oocytes present inside one follicle can be fertilized.6

Age at puberty in rabbit does depends on the breed, nutrition and management conditions. Rabbit does of small breeds can reach puberty at 3-4 months, medium breeds at 4-5 months, and large breeds at 8-9 months of age.7

It is recommended to breed primiparous rabbit does when they have reached 80% of their adult body weight.8 Table 1 shows the recommended age and a weight for primiparous rabbit does, according to breed size:

<table>
<thead>
<tr>
<th>Breed</th>
<th>First breeding age (weeks)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small breeds</td>
<td>20</td>
<td>2.8</td>
</tr>
<tr>
<td>Medium breeds</td>
<td>23</td>
<td>3.6</td>
</tr>
<tr>
<td>Large breeds</td>
<td>27</td>
<td>4.8</td>
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</table>

After puberty, rabbit does do not have a well-defined estrous cycle. They grow waves of follicles that continuously develop to the antral stage under the influence of follicle stimulating hormone (FSH). The presence of large antral ovarian follicles that increase plasma estrogen concentration will initiate sexual receptivity in rabbit does that lasts for several days (erroneously considered to be permanently in estrus).6 During this period of receptive behavior, rabbit does can be mounted by the rabbit buck and become pregnant.7 The follicular waves will regress at approximately 7-10 day intervals.6

In receptive multiparous rabbit does, their fertility is higher when the vulva color is pink or red; this color change is associated with elevated estrogen concentration. Fertility rates are improved by up to 10% if rabbit does are inseminated when the vulva is red, turgescent pink or turgescent purple. The number of rabbit does that kindled following AI was significantly higher when the vulva color was red (55.2%) or pink (51.5%) than when the vulva color was white (33.3%). However, failure to conceive in does with pale or white vulva has been observed even after natural mating.9

It is well known today that rabbit does that are showing receptive behavior at the time of AI will determine the fertility, ovulation frequency, fertilization rate and prolificacy results, facts that will result from the ovulation rate, embryo and fetal survival. For instance, the productivity of primiparous receptive does (6.3 weaned rabbits/AI) is higher than in non-receptive does (1.6 weaned rabbits/AI). The same is observed in multiparous receptive and non-receptive does: 7.8 vs. 2.9 rabbits/AI respectively.6
Induced ovulators
Rabbit does are induced ovulators, as was discovered in 1905 by Heape. Ovulation is induced by mating with the rabbit buck and happens 10-12 hours post-mating. The neuro-hormonal response of induction of ovulation in rabbits has two pathways.\(^\text{10}\)

- A neural pathway that after mating activates several sensory areas whose evoked signals are transmitted via neural afferent pathways along the spinal cord, in the brainstem and hypothalamus.\(^\text{6}\)
- A hormonal pathway, that sends the signal from the central nervous system to the ovum by producing the ovulation per se.\(^\text{10}\) The hormonal mechanism after copulation is the release of gonadotropin releasing hormone (GnRH) from the hypothalamus that induces the release of luteinizing hormone (LH) from the pituitary gland, with respective ovulation of follicles.\(^\text{7}\) It has been demonstrated from direct sampling of portal blood from the pituitary stalk of rabbits that GnRH increases rapidly after coital stimulation, with peak secretion within 1-2 hours. Plasma LH levels start to rise within 3 min after mating and reach a plateau within 15 to 75 min.\(^\text{11}\) The LH surge preceded by the GnRH rise has a maximal release 60-90 minutes after mating and gradually decreases within the next 4-6 hours post-mating.\(^\text{6}\)

Fertilization, gestation and pseudopregnancy
The success of fertilization requires coordination between the time of transportation and duration of viability of the gametes. Ovulation will occur 10-12 hours after mating. Ova will be fertilized 2-6 hours after ovulation or 12-18 hours after mating. Spermatozoa should reach the fertilization location before ova arrive, and during this waiting time each spermatozoon should undergo a series of modifications (sperm capacitation) that will allow penetration of the ovum for fertilization. The duration of pregnancy in rabbit does is 30-31 days, with a range of 28 to 35 days.\(^\text{5}\)

If for any reason the released ova are not fertilized after ovulation, the rabbit doe will begin a stage of pseudopregnancy that lasts between 15 and 18 days with development of corpora lutea and uterine changes similar to those of a pregnant animal. During any pseudopregnant period the rabbit doe is not fertile. Pseudopregnant animals can be identified because towards the end of the stage (around 16 days after mating) the level of progesterone decreases due to the involution of the corpora lutea, triggering the development of maternal and nesting behaviors. In pregnant does, the maternal and nesting behaviors begins after 25 days of pregnancy.\(^\text{7,8}\)

Rabbit bucks
Mature rabbit bucks are fertile during the whole year, although they may show decreased fertility during the summer due to the variation in photoperiod and increase in environmental heat.\(^\text{8}\)

The majority of rabbit bucks will try mating a rabbit doe a few seconds after she is introduced into the cage where the buck regularly lives. Natural mounts will happen very quickly with intense pelvic thrusting by the buck. Natural breeding lasts approximately 70 seconds, ranging from 5 to 300 seconds and may be repeated several times.\(^\text{8}\)

Although the first spermatozoa could be observed around 110 days of age in rabbit buck ejaculates, bucks should be used for the first time for reproductive purposes when they reach five months of age by introducing them progressively from one breeding per week to a maximum of six to eight breedings per week by the time they reach eight to ten months of age. At this age, breeding can be done every second day.\(^\text{8}\)

In ideal conditions for AI purposes, the room where the rabbit bucks are living must have controlled lighting, temperature, humidity, and ventilation. The photoperiod should be of 16 hours of daylight. The ideal room temperature for rabbit bucks should be at 21°C. The ideal range of controlled relative humidity must be between 50-60%. Air ventilation should be between 20-40 cm/second.\(^\text{1}\)

Rabbit bucks that are two to five months old should be placed in replacement cages. When they reach 4.5 months of age, they should have an exhaustive physical evaluation and those who pass it should
be moved to production cages. Between five to seven months of age, the rabbit bucks should be trained to be collected and to ejaculate into an artificial vagina (AV). This period of time will also be used to evaluate the semen quality of the bucks. Approximately 10-30% of rabbit bucks are rejected and will not be used for AI purposes due to lack of adaptation to the AV or poor production and low quality of semen.¹

**Preparation of semen doses for artificial insemination**

Semen is a mixture of spermatozoa, produced by the two testicles, and seminal plasma secreted at different sites by the accessories glands and by the epididymides; these are combined at the time of ejaculation. Seminal plasma can also contain seminal granules other particles of different size produced by the accessory glands which can affect the spermatozoa behavior during the transit along the rabbit doe reproductive tract.¹²

**Rabbit buck semen collection**

In the rabbit industry, ejaculates should be collected with the most hygienic conditions and in the most efficient way. For this reason it is ideal to have at least the same number of AVs and collection tubes than the number of ejaculates needed per day. If each ejaculate is collected with a different AV, it will prevent any potential contact between animals, promoting optimal sanitary conditions. It is also important to send samples to an external laboratory for periodic microbiological culture and evaluation of ejaculates according to veterinary instructions.¹

The most popular method of semen collection from rabbit bucks is use of an AV. The most popular AV model has a semi-rigid outer case and an internal liner that can be made of rubber or latex (Figure 1). The bottom of the semi-rigid case has two openings of different sizes. The largest opening is to place the ejaculate collection tube and the smaller opening is to fill the AV with hot water.¹ The ideal AV temperature for rabbit buck semen collection is 45-50ºC.⁷,¹³ In order to reach this temperature range, the AVs are filled with hot water and kept inside an incubator before they are used for the collection. If the AV temperature is too hot, the buck could contaminate the ejaculate with urine or could burn the penile mucosa producing balanitis, or the high temperature could cause damage to the spermatozoa collected.¹,⁷,¹³ Rabbit bucks respond to appropriate stimulation created by the AV pressure and temperature so it is important to make sure the water pressure and temperature between the outer case and the rubber liner are appropriate.¹,⁷,¹³ If the temperature is too cold (below 40ºC), the buck will refuse to jump.¹,⁷

Semen collection normally happens inside the rabbit buck’s cage. For this purpose a decoy animal (rabbit buck or doe),¹,⁷,¹³ or the arm of the semen collector covered with rabbit skin that simulates the decoy rabbit are used (author’s personal experience). When a decoy animal is placed inside the cage, the rabbit buck will get closer and try to jump it. At this moment the hand holding the AV has to be directed to the rabbit buck’s penis to perform the collection.⁷ When using the arm covered with rabbit skin, the buck will jump into the arm and start thrusting. At this moment the AV has to be directed to the rabbit buck’s penis to perform the collection (author’s personal experience; Figure 2).

In the rabbit AI industry, the recommended collection frequency is two ejaculates per rabbit buck per week with a time interval of 15-20 minutes between collections.¹,¹³ In high demand bucks, it is possible to perform up to four extractions per week divided into two days per week and two collections per day.¹

The collection tubes containing the ejaculates should enter the laboratory through a pass-through window located between the rabbit buck’s room and the laboratory.¹ A pass-through window contributes to clearly separate the dusty and non-sterile environment where the bucks are collected from a clean and disinfected laboratory environment where the samples should be evaluated and processed making a great contribution to biosecurity, especially when processed extended semen samples will be shipped to other farms to inseminate other animals (author’s personal experience).

**Rabbit buck semen evaluation**
When using AI, an ejaculate from one rabbit buck can be used to inseminate a large number of rabbit does. In the rabbit industry, a single ejaculate can be divided into 20-50 doses for insemination of rabbit does.\textsuperscript{14}

Variation in the seminal characteristics of rabbit bucks is known to be affected by many factors such as genetic strain, feeding, health status, rearing condition, season, age and collection frequency. Substantial differences among laboratories may increase variability in the evaluation of sperm parameters (sperm counts, motility and morphology).\textsuperscript{12}

After the collection tubes containing the ejaculates enter the laboratory, they should be placed in a water bath at 37°C in order to prevent thermal shock.\textsuperscript{1}

Semen macroscopic evaluation

A visual observation is done in order to determine the semen color, odor and volume.\textsuperscript{1}

\textit{Appearance.} The ejaculate could contain a mucus plug, urine, calcium carbonate crystals or blood.\textsuperscript{1} If a mucus plug that is produced by the accessory sex glands is present, it should be removed because it causes sperm agglutination.\textsuperscript{7}

\textit{Color.} Evaluation of color helps to determine the ejaculate density. Different color spectra that can be observed in ejaculates include: ivory (ideal), white milky, semi-transparent white, or cream.

\textit{Odor.} It should not be unpleasant.

\textit{Volume.} It should be measured in the graduated tube after removing the mucus plug.\textsuperscript{1}

Semen dilution

After visual evaluation, the ejaculate should be diluted 1:5 by adding warm extender to the semen collection tube. There are different commercial semen extenders available in the market. Liquid, powder and gel extenders are available. Liquid and gel extenders should be kept refrigerated while powder extenders should stay in a cool place and away from direct light. It is important to make sure that the semen extender is prepared, warmed and kept at 37°C before the semen evaluation has started.\textsuperscript{1} Examples of commercial rabbit semen liquid extenders are Cudil\textsuperscript{TM} manufactured by Magapor from Spain\textsuperscript{1} and Galap\textsuperscript{TM} manufactured by IMV Technologies from France.\textsuperscript{15}

Microscopic evaluation

The ejaculates of rabbit bucks contain seminal granules. These particles are secreted by the prostate gland, mainly in the first lobe, called pro-prostate. These semen granules are not homogeneous and are composed by different populations of vesicles. They are of different sizes (0.5-6 mm diameter; Figure 3) and are generally surrounded by a bilaminar membrane containing a scarcely organized electron dense material. Researchers have suggested that these semen particles modulate the capacitation process and acrosome reaction of spermatozoa, their kinetics, the immune-response of female tracts, and the transit of spermatozoa in the female tract. In the rabbit species these granules are mainly involved in the control of capacitation and the acrosome reaction. It has been shown that the presence of seminal granules significantly reduces the response of spermatozoa to \textit{in vitro} inducers of the acrosome reaction and as a result the level of capacitated spermatozoa is almost equal to zero. In contrast, when granules are removed by using Percoll\textsuperscript{®} centrifugation the decapacitative effect is virtually eliminated. As commented earlier, ovulation in rabbit does occurs about 10-16 hours after mating and during this lag-phase rabbit spermatozoa must avoid premature capacitation and acrosome reaction, and the seminal particles contribute to delaying this process.\textsuperscript{12}

Semen should be evaluated with a microscope that has a 37°C warming stage, and a warming plate that will also be used to keep the microscope slides and cover slips warm at 37°C.\textsuperscript{1}

The following are the microscopic characteristics to be evaluated:

1. Individual motility observing the progressive motility of spermatozoa. This evaluation can be done with a phase-contrast microscope and a magnification between 200X to 400X (author’s personal experience). The individual progressive motility can be done as a subjective evaluation (Table 2) or as an objective evaluation with a Computer Assisted Sperm Analysis system.\textsuperscript{1} It is necessary to have ejaculates
with individual progressive motility equal to or greater than 70% in order to be included in pooled extended semen doses.23

Table 2. Subjective mark scale and equivalency in progressive motility

<table>
<thead>
<tr>
<th>Subjective Mark scale</th>
<th>Progressive motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>1-10%</td>
</tr>
<tr>
<td>1</td>
<td>11-20%</td>
</tr>
<tr>
<td>1.5</td>
<td>21-30%</td>
</tr>
<tr>
<td>2</td>
<td>31-40%</td>
</tr>
<tr>
<td>2.5</td>
<td>41-50%</td>
</tr>
<tr>
<td>3</td>
<td>51-60%</td>
</tr>
<tr>
<td>3.5</td>
<td>61-70%</td>
</tr>
<tr>
<td>4</td>
<td>71-80%</td>
</tr>
<tr>
<td>4.5</td>
<td>81-90%</td>
</tr>
<tr>
<td>5</td>
<td>91-100%</td>
</tr>
</tbody>
</table>

2. Sperm concentration. After performing a dilution of the sample, the sperm concentration can be calculated with help of counting systems such as the Newbauer chamber, the Bürker chamber,1,7 or the Nucleo Counter SP100.1

3. Sperm morphology. The percentage of normal spermatozoa can be evaluated by placing a drop of semen on a glass slide and staining it with eosin-nigrosin. An eosin-nigrosin stained semen smear is made, allowed to dry and observed under the microscope at 1000X magnification7 with oil immersion (author’s personal experience). An ejaculate will be accepted if it has more than 70% morphologically normal spermatozoa.7

4. Other semen microscopic characteristics. Depending on the laboratory, additional characteristics can be evaluated and scored such as the presence of sperm agglutination, presence of seminal granules, the presence of dead spermatozoa, cytoplasmic droplets and others.1

The most common semen parameters found in rabbit bucks for AI purposes are:

Ejaculate volume: 0.3 to 0.8 ml.8 On some occasions, volumes of 3 to 5 ml could be collected.7 Spermatozoa concentration: 150-300 million/ml with a range of 50 to 500 million spermatozoa per ml of semen.8

Some laboratories give a score to each semen characteristic evaluated in the ejaculates collected. The ejaculates that have a final passing score will be added to the semen pool that will be used to prepare the insemination doses.1

Definitive dilution and storage

The most accepted volume to inseminate a rabbit doe is 0.5 ml of extended liquid semen that should be stored at 18°C. When planning to use extended fresh semen doses within the next 12 hours, each 0.5 ml dose must have 6 to 8 million spermatozoa.23 When planning to use extended fresh semen doses 12 to 36 hours after collection, each semen dose should have a minimum of 12-16 million spermatozoa.23 Some companies use as a final target 15 million spermatozoa per insemination dose.1

When the volume and concentration of an extended semen pool is known, it is possible to dilute the concentration to 30 million spermatozoa per ml of extended semen.1

Calculation to prepare pooled semen doses:

- Pool volume (ml) X Pool concentration = Final Volume X Target concentration of 30X10^6 (spz/ml)
- Final Volume = Pool volume (ml) X Pool concentration/30X10^6 (spz/ml)
The final extender volume that is needed will be the result of subtracting the final volume from the pool volume.

- Final volume $X 2$ = Number of doses produced

Calculation example: Pool volume is 250 ml with a final concentration of $60 \times 10^6$ (spz/ml). The target final concentration is $30 \times 10^6$ (spz/ml)

Final Volume = $(250 \times 60 \times 10^6) / (30 \times 10^6) = 500$ ml

Extender volume to be added: $500 - 250 = 250$ ml

Total number of doses produced: $500 \text{ ml} \times 2 = 1,000$ doses

The extender will be slowly added to the pool and must have the same temperature as the pooled semen. Once the pool has been fully diluted to the final concentration, a sample should be taken to evaluate the individual motility of the cells present in the pool. After the final dilution, the pooled semen should be refrigerated at 18°C until use.1

Methods to synchronize estrus and sexual receptivity in rabbit does

The extensive and wide application of AI in European rabbit farms has evolved to current use of methods of production such as “cycled production” in which all the does in a batch must be inseminated on the same day. Researchers found a strong antagonism between lactation and reproductive functions in non-receptive rabbit does because at the moment of AI these lactating and non-receptive does had poor reproductive performance. This difficulty was a serious problem in intensive rabbit production because does had to be inseminated during the first days of lactation, from 0 to 11 days post-partum. It is important to clarify that with natural mating this situation would not be happening because in general non-receptive does refuse to mate. As was explained earlier, sexual receptive behavior in does is correlated with the presence of more pre-ovulatory follicles on the rabbit ovary and higher concentration of circulating estradiol. High, regular and intensive production levels require the use of techniques to induce and synchronize estrus and receptivity in lactating does.16

A very common system of production in commercial rabbit farms is to use batch management systems that will create groups of animals (batches) that are in the same physiological stage of production such as insemination, kindling, weaning, etc. One of the most common production systems is the 42-day batch management system that requires the rabbit producer to perform AI 11 days after kindling. In the 42-day batch management system the does’ reproduction rhythm is based on the time interval that happens between two inseminations performed on the same animal. After the first insemination there are 31 days of gestation, then parturition, and the second AI is done 11 days later in the same lactating post-partum doe, for a total of 42 days between inseminations.17

In order to synchronize estrus and induce receptive behavior in lactating does 11 days post-partum, it is very common to use a hormonal treatment 48 hours before AI that consists of g 20 IU equine chorionic gonadotropin (eCG) intramuscularly.18 The same hormonal protocol can be used in primiparous does.17

Additional biostimulation programs have been developed as alternatives to stimulate rabbit does for AI programs. Practical examples of biostimulation are increased lighting from 8 to 16 hours per day in does prior to insemination, separation of the lactating mother from the litter for 24-48 hours before AI, and nutritional flushing.17,19

Artificial insemination

There are several restraining techniques that can be used in order to perform AI in rabbit does:

- Insolation with rabbit doe in vertical position. The animal is restrained by the cervical area with one hand and by the inguinal zone with the other hand, as if performing an abdominal palpation. This technique requires two people.1

- Insolation with rabbit doe in inverted position. The main advantage of this restraint technique is that the doe does not adopt a defensive position because the insemination will be done quickly. This technique requires two people.1
Cannon restraining device. In 1990, a system was created in Italy that permits only one operator to perform AI. The system is basically a cylindrical restraining device that is 24 cm long and has an internal diameter of 15 cm. The system has a 23 degree slope and the bottom part is 12 cm longer than the top area in order to hold the rabbit’s abdomen or rump, leaving the rump exposed for AI. The inseminator can work hands-free and inseminate 80-100 does/hour without excessive doe manipulation.\textsuperscript{7}

Rabbit does that will be inseminated should be in excellent health and be sexually receptive, which can be confirmed by observing the red color of the vulvar lips.\textsuperscript{1}

There are different instruments that can be used to perform AI in rabbits:

- A pipette made of plastic or glass that is approximately 16 cm long and has an external diameter of 6 to 7 mm. This pipette is slightly curved on the tip, approximately 15 degrees. The other end should be connected to a syringe in order to allow the aspiration of the semen dose into the pipette (Figure 4).\textsuperscript{7} The pipette should be carefully introduced with the curvature directed towards the dorsal area of the vagina. During intromission the pipette will rotate spontaneously and penetration will become easier. The pipette should be introduced inside the vagina as far as possible. As soon as it stops, the pipette should be slightly withdrawn and the syringe plunger pushed to inject the semen (Figure 5). The pipette should enter an average of 15 cm inside the rabbit doe, depending on the age and size of the animal. In order to remove the pipette, it has to be pulled slowly. Rotation will occur and it will come out without any difficulties. After the pipette has been removed, it is important to look for the presence of pus or blood on the pipette tip, elements that will suggest infection or laceration.

- 0.5 ml semen straws can also be filled with liquid extended semen. The straw should be placed inside a metallic AI gun. A specific plastic sheath that perfectly fits the tip of the semen straw will then be used to cover the metallic insemination gun (Figure 6; author’s personal experience). The insemination technique should be done using the same steps explained with the plastic/glass pipette.

**Induction of ovulation after artificial insemination**

In order to induce the ovulation of follicles after AI is performed, it is necessary to administer GnRH (20 \( \mu g \)/animal intramuscularly). In an effort to induce ovulation after insemination, researchers have added GnRH analogs such as ethylamide to the semen dose (25 \( \mu g \)/semen dose intravaginally) which resulted in similar fertility and prolificacy and provides an alternative to the intramuscular administration of GnRH or its analogs.\textsuperscript{20} Similarly, another research group added the synthetic GnRH analogs buserelin (10 \( \mu g \)/ml of semen extender) or triptorelin (10 \( \mu g \)/ml of semen extender) in order to be absorbed by the vaginal mucosa, and obtained acceptable fertility and prolificacy.\textsuperscript{21} A different method that has also been investigated is the use of vasectomized bucks AI, which produces results similar to natural breeding. This technique has the disadvantage of increased time to perform the procedure, plus the necessity of having a group of vasectomized males on the farm and for these reasons this method is not very popular.\textsuperscript{18}

**Pregnancy diagnosis**

Pregnancy can be diagnosed by transabdominal ultrasonography as early as seven days after ovulation when early embryonic vesicles can be observed when using the appropriate transducer and unit equipment.\textsuperscript{22}

Producers working in batching management systems could organize the schedule to perform pregnancy diagnosis by transabdominal palpation or ultrasonography at 14 days post-ovulation.\textsuperscript{17}

**References**


Figure 1. Left: Rabbit AV; semi-rigid body with the rubber inner liner already in place. Right: Assembled rabbit AV showing the semen collection tube and a protective case to cover the ejaculate during collection. (Pictures courtesy of Guy Delhomme, IMV Technologies, France).

Figure 2. Left: Arm covered with rabbit skin and holding an AV ready for semen collection from a rabbit buck. Right: Rabbit buck semen collection. The rabbit buck has jumped the rabbit skin covered arm and is already thrusting inside the AV held by the collector (Author’s personal pictures).

Figure 3. Rabbit buck semen stained with eosin-nigrosin and observed under light microscopy at 1,000X magnification. Notice the presence of seminal granules of different sizes around the white spermatozoa. (Author’s personal pictures).
Figure 4. Disposable blue pipette with curved tip for doing AI in rabbit does. It can be connected to a syringe (Picture courtesy of Guy Delhomme, IMV Technologies, France).

Figure 5. Artificial insemination of a rabbit doe that is being restrained by an operator. The inseminator is using the disposable blue pipette to perform the insemination and injecting the semen with a syringe. (Picture courtesy of Guy Delhomme, IMV Technologies, France).

Figure 6. Metallic AI gun for rabbits that can be used with 0.5 ml straws. Notice the disposable blue sheath that will cover the metallic gun and fits perfectly well with the straw tip. (Picture courtesy of Guy Delhomme, IMV Technologies, France).

(Editor’s note: The photographs in this paper are available in color in the online edition of Clinical Theriogenology.)