ARTIFICIAL INSEMINATION FOR MINIATURE DONKEYS

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INTRODUCTION

Artificial insemination is a viable technique for use in all equine species, including donkeys. Standard equine equipment may be used to collect, evaluate, transport, and inseminate semen. Limited experience exists with fresh cooled semen and immediate post-collection insemination in miniature donkeys.

SEMEN COLLECTION

Standard equine collection equipment can be used in donkeys of all sizes, including miniature donkeys. I have exclusively used the 16" Missouri artificial vagina (AV), which is available from various sources. Collection may also be performed with the Roanoke AV (Roanoke AI Laboratories, Roanoke, VA, 540-774-0676 or www.roanokeai.com).

It is easy to train a jack for semen collection, even one that is only used to field breeding, by using a jump jennet that is in estrus. Proper handling is important – the handlers and collector must be patient and allow for the normal, cautious behavior of donkeys when confronted with new situations. As always, grain is an excellent motivator to entice a donkey into an unfamiliar area. The collection process with donkeys is different from horses due to the different reproductive behavior of the donkey. A jack may take as long as 30 minutes to achieve a full erection (Fig. 2). As the jack approaches a jennet in estrus, she will "jaw" (open and close her mouth) (Fig. 7). The jack should not be allowed to bite the jennet, or to stay on her back until he has achieved a full erection. He should be handled with a chain lead shank over the nose when first training him for collection to make him pay attention to the handler. The chain soon becomes unnecessary in most jacks as they readily take to the collection procedure.

In this description of collection, the 16" Missouri AV (artificial vagina) is used (Fig. 1), along with a sterile disposable liner with attached gel filter. The jack initially teases the jennet to verify that she is in a strong, standing heat. Note that she will kick the jack in the chest on approach and that this seems to be necessary for him to achieve a full erection. The jack is kept in sight of the jennet, but not in close physical contact while the AV is prepared. A disposable liner with an in-line gel filter is inserted into the apparatus and taped over the opening for the penis (Figs. 2A, 2B). The rubber AV liner is filled with tap water at a temperature of approximately 115 °F. Temperature may be verified by filling a small insulated, plastic thermos bottle with water and measuring with a thermometer (Fig. 3). The AV liner is completely filled, all air expelled, and then the leather cover (Fig. 4) is placed over it. Non-spermicidal, sterile lubricant is applied to a long, plastic OB sleeve on the collector's arm, which is inserted into the AV to carry it and confirm water temperature (Fig. 5), an important feature in cold weather collections.

Figure 1: Full Size Missouri AV Used to Collect Miniature Donkey Jacks.
A = liner; B = leather cover/carrier.
Figure 2A: Inserting the Disposable, Sterile AV Liner into the Water Filled AV Liner.

Figure 2B: Fixing the Gel Filter in Place
A = collection end of disposable, sterile, plastic AV liner;
B = in-line semen gel filter.
Figure 3: Insulated Thermos with Thermometer Inserted Being Filled with Water at Proper Temperature to Fill Rubber AV Liner.

Figure 4: Filling the Rubber AV Liner by Pouring 115 °F Water into a Funnel (A) Attached to the Liner (B).
The jennet may be tied in a corner or be controlled by a handler in an aisle that provides good footing for both donkeys. The jack handler should tease the jack until he is fully erect. Only then should he be allowed to mount and stay on the jennet (Fig. 6 and 7). It is helpful to learn the individual breeding characteristics of the animals involved. After the fully erect jack is allowed to mount, the collector handling the AV quickly directs the jack’s penis into the AV (Fig. 7). The other hand is placed at the base of the penis to feel for ejaculation. The collector should strip the penis into the AV after ejaculation when the jack dismounts.

The next procedures are performed in rapid succession to preserve the maximum amount of viable semen. Water is drained from the AV to allow collected semen to drain through the filter and into the collection receptacle tip, which is opened with scissors. Semen is drawn into a preheated (stored in a 100 °F incubator), all plastic syringe and all syringes should remain in an incubator until used for insemination. The collected dose may be split if needed for more than one jennet, depending on the semen quality of the collected jack. Other collection methods are under investigation, including use of a phantom jennet and ground collection without a jennet or phantom.
Figure 7: Directing the Erect Jack Penis into a Missouri AV after the Jack Mounts the Receptive Jennet.

SEMEN EVALUATION
Semen can be collected for evaluation after hand-breeding, natural cover by grasping the jack penis immediately after dismount and letting semen drip into a warmed plastic tube. The evaluation procedures for donkeys used by the author are those used for horses.

Parameters to evaluate include:
- Semen volume (ml)
- Sperm concentration (sperm x 10^6 per ml)
- Progressive motility (%)
- Morphology (form or shape) of sperm (%)
- Total number useful sperm in the ejaculate (x10^6)

Semen volume is measured after removing it from the AV collection reservoir tip with the plastic syringes. Sperm concentration may be measured inexpensively using the Unopette ® WBC and hemocytometer method. Motility and morphology are determined using a standard microscope, and morphology is further evaluated using a nigrosin-eosin stain. The total number of useful sperm in the ejaculate is determined by multiplying the first four parameters together. At the present time, I have not determined the minimal number of sperm to be used in donkey insemination. It seems reasonable to use the numbers recommended for horses until further studies have been completed.

Semen collected from miniature donkeys by myself and Brian Ramsey, DVM of Tyler, TX has been evaluated and characteristics are tabulated in Table 1 with comparisons shown to full-size and miniature horses. (Miniature horse values are courtesy of Ron Emond, DVM, Bridgewater, CT.)

<table>
<thead>
<tr>
<th>Miniature Donkeys</th>
<th>Average</th>
<th>Range</th>
<th>Horse Normals</th>
<th>Miniature Horse Normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen Volume (ml)</td>
<td>25 - 50</td>
<td>21 - 115</td>
<td>60 - 80</td>
<td>20 – 45</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>80 - 90</td>
<td>65 - 90</td>
<td>70 - 90</td>
<td>&gt;75%</td>
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<tr>
<td>Sperm Concentration (x 10^6)</td>
<td>200 - 500</td>
<td>75 - 877</td>
<td>60 - 150</td>
<td>250 – 350</td>
</tr>
<tr>
<td>Normal Morphology (%)</td>
<td>80 - 90</td>
<td>69 - 92</td>
<td>Similar</td>
<td>Similar</td>
</tr>
<tr>
<td>Useful Sperm per Ejaculate (x 10^6)</td>
<td>5,000 - 11,000</td>
<td>3,307 - 18,000</td>
<td>Less</td>
<td>6,000 - 10,000</td>
</tr>
</tbody>
</table>

Table 1: Miniature Donkey Semen Characteristics (16 Animals)

Summary of miniature donkey semen parameter data as compared to horses:
- Volume is lower than full-sized horses
- Motility is comparable to horses
- Sperm concentration is generally higher than horses
- Total number of useful sperm is generally higher per ejaculate

In general, mini-donkey semen has parameters equal to or better than horse semen when collected for artificial insemination!
SHIPPING FRESH COOLED MINIATURE DONKEY SEMEN

Limited experience and data are available with regard to the efficacy of shipping miniature donkey semen, however the experiences of myself and Dr. Ramsey indicate that it can be done. More work is needed to establish standard protocols. Scarcity of data is largely due to reluctance of donkey owners, particularly stud owners, to use artificial insemination as a breeding technique. This is fairly normal for any animal species when a novel reproductive technique is first introduced.

Semen shipping containers used with success are the Equitainer (Hamilton Thorne Research, Inc., South Hamilton, MA (800) 367-0266, www.equitainer.com/home) and the Equine Express II (Exodus Breeders Supply, York, PA (877) 396-3874, www.exodusbreeders.com). Semen extenders in current use contain antibiotics and nutrients to help maintain semen viability. Examples include Kenney's with ticarcillin or EZ Mixin containing amikacin, ticarcillin, polymixin B and potassium penicillin.

MANAGEMENT OF THE FEMALE MINIATURE DONKEY

The goal for AI when using transported semen is to breed only once per cycle. If a stud is available to tease the female, the process is easier since he helps determine the timing for semen to be ordered from the stud owner. If a stud is not locally available, the timing of insemination may be selected by using hormonal control with a prostaglandin injection to induce estrus, as is done in the mare. Ultrasound evaluation of the ovaries and uterus and the changes associated with estrus and ovulation are also followed with transrectal ultrasound. A vaginal speculum exam tracks cervical changes as in the mare and this procedure corroborates information gained from the transrectal ultrasound exam. The use of HCG (human chorionic gonadotropin) to induce ovulation in miniature donkeys needs investigation.

ARTIFICIAL INSEMINATION TECHNIQUE

The technique of artificial insemination is similar in all equine species. Semen is stored in an incubator for on-farm insemination or in the cooled semen transport container until it is ready to be used. The jennet’s tail is wrapped or covered with a taped on, inverted plastic sleeve for cleanliness, and the tail is tied out of the way. Her vulva and the surrounding area are cleaned with soap and water followed by thorough rinsing to prevent soap toxicity to sperm, and then allowed to dry. A long-sleeved, sterile glove, a plastic pipette, non-spermicidal lubricant, and an all-plastic syringe are used for insemination. The inseminator’s gloved and lubricated hand carries the sterile pipette into the vagina. One finger is inserted into the cervix and the pipette is passed into the uterine body, aiming slightly toward the horn on the side of the ovary with the largest follicle. The semen-filled syringe(s) is attached to the pipette to deliver semen with a gentle push. The cervix is "feathered" with a finger before withdrawing the gloved hand from the vagina. If the jennet’s vagina is too small to insert a hand, the technique becomes more difficult to perform; it can be challenging to pass a pipette into a donkey cervix, which tends to be located on the floor of the vagina, pointing slightly upwards. Alternate techniques for insemination are being researched, including use of cervical forceps to hold the cervix rigidly in position, and the use of a fiberoptic endoscope.

PREGNANCY DETERMINATION

Transrectal ultrasound can be safely and accurately performed at 16 plus days to visualize the conceptus. Failure of a jennet to return to estrus only means that a corpus luteum is present and not necessarily that a pregnancy has been established. Behavioral estrus may not be possible to observe unless a stud is available for teasing. Hormone analyses using estrone sulfate or pregnant mare serum gonadotropin (PMSG) have not been used extensively in donkeys.

FROZEN SEMEN IN DONKEYS

Presently, limited data and experience are available regarding the use of frozen semen in miniature donkeys. Donkey semen can be frozen and thawed successfully (Purdy/Ballard 2001), with approximately 30 % motility following thawing; as yet, I have no experience using frozen semen for donkey AI. This technique has the potential benefit of sharing genetic material around the world. More work is needed to explore this technique and to evaluate its effectiveness.

SUMMARY

Artificial insemination is a viable technique for use in donkeys. Standard equine equipment may be used to collect, evaluate, transport, and inseminate semen in donkeys. Limited experience exists with fresh cooled and immediate post-collection semen insemination in donkeys. AI with frozen semen has the potential to be a valuable breeding technique. Timing of insemination for females may be successfully managed using serial ultrasound exams and hormonal control with prostaglandin and HCG injections. Pregnancy determination is also safe and accurate using standard equine ultrasound equipment with a probe extender as needed.

REFERENCES: