Practical Aspects of Donkey Breeding Management

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1. Introduction

Donkeys are bred worldwide for a variety of uses. From the production standpoint, donkey milk has become a delicacy particularly useful for infants and those allergic to cow or goat milk and the cosmetic industry.1 In Asia, the renewed interest in donkeys for ejiao, a product of traditional Chinese medicine, has generated a higher demand for the production of donkeys.1 However, in many countries, donkeys are bred primarily to produce hybrids with horses (i.e., mules and hinnies).2 Hybrid animals are used for agricultural work, pleasure, Western sports, and herding cattle worldwide.2 In North and South America, mule showing and pleasure rides attract thousands of enthusiasts to specific events such as the Mule Day at the Shawnee National Forest in Herod, Illinois, and Mule Days in Bishop, California. Production of mules for showing, pleasure riding, and racing are reasons practitioners are contacted to breed horse mares with jack semen. In addition, practitioners are typically asked to breed seedstock jennies in the American continent to obtain quality jacks, and less commonly for pets or conservation efforts. Donkeys are classified as miniature, small standard, standard, and large breeds. Therefore, it is paramount that equine practitioners be prepared to provide reproductive services for donkey and mule breeders. Thus, this manuscript discusses recent advances in donkey breeding management and the authors' clinical experiences with the species.

2. Female (Jenny) Features

Estrous Cycle and Sexual Behavior

The interovulatory interval is longer in donkeys (23–26 days) than in mares (~21 days; Table 1).2 Duration of the estrous cycle varies with breeds, body condition scores (BCS), geographical location, and time of the year.2 Estrus usually lasts about 6 days, but it varies from 4 to 10 days, and diestrus lasts from 15 to 19.8–6 Ovulation takes place 1 day before ceasing signs of estrus.7,8 In the spring and summer, the estrus is shorter than in the fall and winter.6,9,10 Well-fed donkeys do not display seasonal anestrus; under tundra weather, some jennies experience one prolonged interovulatory interval of 40 to 50 days during the deep winter and then resume regular interovulatory intervals.2 Also, a recent study described that older jennies display longer interovulatory intervals, a slower growth rate of the dominant follicle.6 In addition, the BCS affects the duration of the interovulatory intervals.6 Specifically, higher BCS (>6/9) lengthened the interovulatory intervals...
due to longer diestrus; however, the follicle growth rate and the dominant follicle diameter were independent of the BCS in the same study.6 Multiple ovulation was positively associated with BCS and had no effect on the interovulatory interval.5 Signs of estrus in jennies include standing to be mounted, a lowered head with ears back against the neck, clitoral winking, urination, mouth clapping, and tail raising (Fig. 1).11 It is worth noting that the mouth clapping is the most typical and also the first and last estrus sign in jennies.12 Jennies kept on pastures will congregate into a sexually active group similar to cows.11 In the sexually active group, the female in estrus is mounted by other females coming in or out of estrus. Inhaling urogenital secretions from one another followed by the Flehmen’s response is a typical feature of sexually active groups.11 This jack-like behavior is a normal species-specific behavior in jennies, and it should not be confused with intersex and those with granulosa-theca cell tumors;2 jennies in diestrus typically do not stand to be mounted and may kick at the jack.13

Folliculogenesis and Reproductive Hormones in Jennies

Follicular deviation (i.e., diameter when the dominant follicle starts growing faster than subordinate follicles) occurs 8 to 9 days before ovulation at an approximately 18-mm follicle for small-frame breeds5 and ~20 mm for large donkey breeds.6,14 Generally, jennies have one or two follicular waves per estrous cycle.3,5,14 In small-frame donkeys, the dominant follicle grows 2 to 3 mm/day from deviation to ovulation,5,8 while in large donkey breeds, the dominant follicle grows up to 4 mm/day.14 Thus, the diameter of the periovulatory follicle seems to be associated with the jenny’s body frame, and follicle size varies from 30 to 48 mm.2 Concentrations of estradiol-17β increase from 10 pg/mL during early estrus to peak around 40 to 60 pg/mL at 24 hours before ovulation. After ovulation, progesterone concentrations slowly increase for 4 to 6 days, reaching a peak by 10 days and a plateau by 14 to 16 days postovulation.2 Luteolysis starts by 14 to 15 days postovulation, lasting up to 2 to 3 days for progesterone concentration to nadir (<1 ng/mL; Fig. 2). Multiple ovulations seem to be higher in donkeys than in mares; it varies from 5% to 70% in jennies across studies.14–17 Some breeds have a high incidence of multiple ovulations, such as the Portuguese breed Miranda (48%), the Chinese black donkey Dezhou (13%), the Spanish breed Catalan (42%), and the American Mammoth (61%).6,14 The high incidence of multiple ovulations in donkeys makes a twin pregnancy more likely in jennies than in mares.1 At the same time, donkeys have an extremely efficient placenta rendering a high probability for the delivery of live twins.2 However, twin pregnancies have an increased risk for pregnancy loss, and live donkey twins are of a smaller frame and weaker than singletons; also, there is a high risk of dystocia (authors’ unpublished observations). Therefore, it is still advisable to perform manual reduction (as done in horses) by transrectally crushing one of the embryonic vesicles around 14 to 17 days postovulation.

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**Table 1. Duration of Estrous Cycles, Estrus, and Diestrus in Various Donkey Breeds**

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Country</th>
<th>Estrous Cycles (n)</th>
<th>Estrus (d)</th>
<th>Diestrus (d)</th>
<th>IOI (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miranda</td>
<td>Portugal</td>
<td>33</td>
<td>6.6 ± 0.3</td>
<td>17.9 ± 0.5</td>
<td>23.8 ± 0.5</td>
</tr>
<tr>
<td>Pega</td>
<td>Brazil</td>
<td>13</td>
<td>8.0 ± 2.5</td>
<td>18.0 ± 2.3</td>
<td>26.0 ± 2.7</td>
</tr>
<tr>
<td>Burro Mexicano</td>
<td>Mexico</td>
<td>27</td>
<td>6.9 ± 0.7</td>
<td>17.9 ± 0.5</td>
<td>23.8 ± 0.4</td>
</tr>
<tr>
<td>Crossbreed</td>
<td>USA</td>
<td>19</td>
<td>6.4 ± 0.6</td>
<td>19.3 ± 0.6</td>
<td>25.7 ± 0.7</td>
</tr>
<tr>
<td>Catalonian</td>
<td>Spain</td>
<td>10</td>
<td>5.6 ± 0.2</td>
<td>20.0 ± 0.4</td>
<td>25.0 ± 0.3</td>
</tr>
</tbody>
</table>

IOI, interovulatory interval. Adapted from a review by Canisso et al. (2019)2 and numerous authors cited in the table.5,6,12–14

**Fig. 1.** Signs of estrus in jennies. A, Baudi du Poitou displaying mouth clapping and ears lowered back while being teased by a mature jack. B, Tail lifting, clitoral eversion, and urination in a Northeast Brazilian jenny while being teased to a mature jack (not shown).
Estrous Cycle Monitoring

Transrectal palpation and ultrasonography are the most widely useful approaches for the breeding management of jennies. To safely perform this examination, jennies can be restrained in horse stocks, placed in a doorway of a stall, or have a lip twitch placed and palpated with no minimal restraining (Fig. 3). Sedation is rarely necessary to palpate jennies. While the reproductive tract is proportionally larger in jennies than mares, rectally palpating small-frame jennies can be challenging due to the reduced anus and rectum sizes. In addition, some jennies may strain excessively during transrectal palpation and ultrasonography. Lidocaine mixed with lubricant (20 mL of 2% lidocaine in 80 mL of carboxymethylcellulose) alone or in combination with NB-Butylscopolammonium bromide (10–20 mg/animal, IV) may be necessary to safely and successfully perform a transrectal examination in jennies. Miniature donkeys cannot typically be palpated transrectally without significant risk for rectal tears. The ultrasonographic appearance of jennies’ reproductive tract resembles mares; however, jennies are well-known to present a less pronounced endometrial edema, particularly in fat donkeys (Fig. 4). Therefore, if the clinician waits to induce ovulation or breed jennies using the mare endometrial edema scores, ovulations will be missed. Close to ovulation, the periovulatory follicle changes from spherical to an oval or ellipsoid shape, and the follicular wall becomes irregular and thicker. Of interest, the vascularization beneath the follicular wall increases up to 24 hours before ovulation (Fig. 5). After ovulation, jennies can present homogeneous appearance and two other distinct corpus luteum (CL) echogenic appearances. One appearance has a white central hyperechoic line leading up to luteolysis; the second morphologic type has a nonechogenic central lacuna that gradually reduces in size until luteolysis (Fig. 6). On the first day of diestrus, the size of CL represents 77% of preovulatory follicle diameter. The vascularization of the CL rises gradually until 4 to 6 days after ovulation, peaks around 11 days, and starts to decline 2 to 3 days after luteolysis (14-15 days postovulation; Fig. 7).

Hormone Therapy for Jenny Estrus Cycle Manipulation

Prostaglandin F2alpha (PGF2α) analogs can be used to bring jennies back to estrus. Jennies typically return to estrus between 2 and 5 days after luteolytic agent administration (Table 2). Side effects such as sweating, abdominal pain, and loose manure can be seen with all luteolytic drugs. For small-frame donkeys, dinoprost tromethamine should be reduced to 2.5 mg to minimize the side effects caused by standard doses used in the horse (i.e., 5-7.5 mg). In general, luteolytic drugs are used >5 days after ovulation; however, some authors suggest giving a luteolytic agent 3 days after ovulation, with the efficacy varying from 17% to 100% (Table 2). The authors’ preference is to use dinoprost (2.5-5 mg) or cloprostenol (0.075 mg) at least 5 days postovulation. Baudet du Poitou jennies may take up to 10 to 12 days to return into estrus after standard PGF2α administration 5 days postovulation. Induction of ovulation in jennies is typically carried out with human chorionic gonadotropin (hCG) or gonadotropin-releasing hormones (GnRH) analogs (e.g., lecirelin, buserelin, histrelin acetate, deslorelin acetate). The dose, route, size of the dominant follicle, and ovulation rates for each

Fig. 2. Progesterone profile during the estrus cycle in three jennies (authors’ unpublished results).

Fig. 3. Transrectal palpation and ultrasonography in a mature Mammoth jenny.

Fig. 4. The ultrasonographic appearance of jennies’ reproductive tract resembles mares; however, jennies are well-known to present a less pronounced endometrial edema, particularly in fat donkeys.

Fig. 5. After ovulation, jennies can present homogeneous appearance and two other distinct corpus luteum (CL) echogenic appearances.
therapy are highlighted in Table 3. The ideal time for induction of ovulation in jennies varies with BCS, age, and body size. As previously described, jennies do not consistently present a marked endometrial pattern before ovulation as mares do (Fig. 4)\textsuperscript{10,18} therefore, the endometrial pattern should not be considered

![Fig. 4. Endometrial edema of jennies assessed via transrectal ultrasonography. A, Absent; B, Mild; C, Moderate; D, Pronounced.](image)

![Fig. 5. Sequential B-mode and power doppler ultrasonograms of a preovulatory follicle at 36 hours (A and D), 24 hours (B and E), and 1 hour (C and F) before ovulation. Over time, there is an increase in an anechoic line (asterisks) beneath the follicular wall, a loss of the spherical shape, and development of the stigma (apical area with brackets), and a thickening of the granulosa layer (arrows) can be noticed upon impending ovulation. Also, there is an increased vascularization beneath the preovulatory follicle. A-C, Power Doppler; D-F, B-mode.](image)
the sole criteria to induce ovulation in jennies.\textsuperscript{2} Small-size jennies (200–250 lb body weight) usually can be induced when periovulatory follicles reach 28 to 32 mm in diameter. Small standard and standard jennies (300-400 lb body weight) can be induced when follicles measure 35 to 40 mm, and larger breeds (500-800 lb body weight) such as Mammoth and Baudet du Poitou follicles are induced when attaining 40 to 45 mm in diameter.\textsuperscript{a} Well-fed animals tend to grow follicles more rapidly and ovulate follicles with a smaller diameter for the expected body frame.

Estrus Synchronization Protocols

Hormones commonly administered to jennies to synchronize estrus include PGF2\(_\alpha\),\textsuperscript{15} progesterone or progestin,\textsuperscript{15,28} hCG, and GnRH analogs (Fig. 8).\textsuperscript{15,28,29} Single or double doses of PGF2\(_\alpha\) with 16-day intervals can be used. Administration of GnRH 7 days before the first PGF2\(_\alpha\) ensures ovulation, creating a CL that will be responsive to the second PGF2\(_\alpha\) administration.\textsuperscript{29} Progesterone (intravaginal device or intramuscular) coupled with PGF2\(_\alpha\) can also be used in jennies. Combining progesterone with estrogen for 10 days provides better synchrony and synchronous ovulation in 73% (8/11) of jennies; however, it is still not as efficient as double PGF injections 16 days apart (100%, 10/10).\textsuperscript{15}

Artificial Insemination

Artificial insemination (AI) in jennies is performed similarly to mares (Fig. 9).\textsuperscript{2} However, jennies have a narrowed and tortuous cervix, with the vaginal portion of the cervix assuming variable appearance from straight to C, D, and V appearance.\textsuperscript{2} Thus, particularly for some jennies, trans-passing the cervix for routine intrauterine procedures can be a daunting task.\textsuperscript{2} In addition, some jennies present excessive straining during intravaginal procedures, and the reduced lumen of the vagina and vulva can further complicate routine procedures. Thus, in this case, the authors may perform caudal epidural anesthesia with 2 to 4 mL of lidocaine 2%. Alternatively, transcervical AI can be performed using Wilsher and Allen’s forceps described for the embryo transfer technique (Fig. 10).\textsuperscript{30} A vaginal speculum is introduced, and the cervix is then grasped with the forceps and retracted caudally to facilitate the passage of the AI pipette through the cervix. This reduces the effects of manipulation and the risk of introducing contamination during AI. Semen can be deposited into the uterine body or guided to the uterine horn ipsilateral to the preovulatory follicle/ovulation. Deep-horn AIs are appropriated for small semen volumes (2-4 mL) to avoid reflux of semen, while larger volumes can be deposited into the uterine body. Of interest, a higher sperm dose should be used to breed jennies. Jennies inseminated with at least 1 billion motile sperm achieve similar pregnancy rates compared with mares inseminated with 500 million motile sperm.\textsuperscript{31} Overall, jennies and mares have satisfactory fertility when bred with raw or freshly extended donkey semen.\textsuperscript{2} Although frozen donkey semen typically presents excellent post-thaw quality and achieves satisfactory fertility rates in mares (~50%),\textsuperscript{32} it does not necessarily translate into satisfactory fertility in jennies.
Historically, pregnancy rates in jennies inseminated with frozen-thawed donkey semen varied from 0 to 30%.2 Replacement of the cryoprotectant such as glycerol to other cryoprotectants such as dimethyl sulfoxide (DMSO), glutamine, dimethylformamide, and dimethylacetamide or rediluting the semen after thawing with extender containing no glycerol improved post-thaw semen quality; however, the conception rates remained low.34,35 Since jennies have a more pronounced postbreeding inflammatory response than mares, an association between this acute inflammatory response and the poor conception rates when frozen donkey semen is used may be speculated in jennies.36 Dexamethasone is a widely used therapy to lessen the postbreeding inflammatory response in susceptible mares.37 However, many donkeys are insulin-resistant animals as an adaptive response to the lack of energy; therefore, donkeys can be more susceptible to laminitis, and corticosteroids should be used with caution in these animals. The authors typically use 10 mg/jenny before or after breeding with frozen semen. Authors have proposed uterine lavage and infusion with donkey seminal plasma (v:v, 1:2, frozen-thawed semen: seminal plasma).36 The addition of donkey seminal plasma seems to reduce the endometrial inflammatory response;38,39 the authors suggested that post-thawed semen resuspended in seminal plasma tended to increase the conception rates.36 Seminal plasma reduces the postbreeding endometrial inflammatory response and the polymorphonuclear cells bound sperm.40 A recent study concluded that donkey seminal plasma, not sperm-intrinsic factors, are able to trigger NETosis (the process that culminates in the release of neutrophil extracellular traps) in both a time- and sperm-concentration-dependent manner and suggests that NETosis could represent a mechanism by which the female reproductive tract selects sperm.41 Uterine lavage after insemination (at least 4 hours after insemination) can also be used to mitigate the postbreeding uterine reaction in jennies. The authors of the present report recommend that increasing the breeding dose coupled with deep horn insemination and uterine lavage 4 to 6 hours after breeding can be used to enhance pregnancy rates in jennies. A recent study out of China, utilizing a timed-AI protocol to synchronize ovulation in jennies, obtained >70% of conception rate on deep horn insemination with frozen-thawed jack semen (500 × 10⁶) at 28 and 40 hours after hormone (luteinizing hormone [LH] or hCG) injection.42 This study warrants testing in practice to see if these results can be consistently achieved. Also, the LH used in that study is not available in the United States. The authors use ecblolics extensively postbreeding in jennies; doses vary from 10 to 20 units/jenny, q6-12h. 

### Table 2. Induction of Luteolysis in Jennies Using Prostaglandin F2 Analogs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (IM, mg)</th>
<th>Cycles (n)</th>
<th>Days Postovulation at PGF₂α</th>
<th>Estrus Detection</th>
<th>Interval to Estrus (d) or to Progesterone Decline (&lt; 1 ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinoprostone15</td>
<td>5</td>
<td>58</td>
<td>–</td>
<td>44/58 (76%)</td>
<td>4.4 ± 1.6</td>
</tr>
<tr>
<td>Cloprosteno122</td>
<td>0.075</td>
<td>10</td>
<td>3</td>
<td>10/10 (100%)</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>5/5 (100%)</td>
<td>3.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>5/5 (100%)</td>
<td>4.2 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9</td>
<td>5/5 (100%)</td>
<td>4.6 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Cloprosteno23</td>
<td>0.075</td>
<td>22</td>
<td>3</td>
<td>21/22 (96%)</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>Luprosti24</td>
<td>7.5</td>
<td>169</td>
<td>Diestrus</td>
<td>NS</td>
<td>5</td>
</tr>
<tr>
<td>Alphaprostol25</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>1/6 (17%)</td>
<td>2a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6</td>
<td>6/6 (100%)</td>
<td>4a</td>
<td></td>
</tr>
</tbody>
</table>

IM, intramuscularly; NS, not specified. Content adapted from review by Canisso et al. 201915 and numerous other authors cited above.15,22–25

### Table 3. Induction of Ovulation in Jennies Using hCG and GnRH Analogs

<table>
<thead>
<tr>
<th>Hormones</th>
<th>N of Cycles</th>
<th>Dose (route)</th>
<th>Preovulatory Follicle Size (mm)</th>
<th>Ovulation Rates at 48 h after Induction</th>
<th>Induction-Ovulation Interval (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG26</td>
<td>12</td>
<td>2500 IU (IV)</td>
<td>30–35</td>
<td>11/12 (91.7%)</td>
<td>4.2 ± 13</td>
</tr>
<tr>
<td>hCG26</td>
<td>15</td>
<td>2500 IU (IV)</td>
<td>36–40</td>
<td>15/15 (100%)</td>
<td>4.2 ± 13</td>
</tr>
<tr>
<td>Lecirelin26</td>
<td>23</td>
<td>100 ug (IV)</td>
<td>30–35</td>
<td>19/23 (82.6%)</td>
<td>42.8 ± 14</td>
</tr>
<tr>
<td>Lecirelin26</td>
<td>20</td>
<td>100 ug (IV)</td>
<td>36–40</td>
<td>16/20 (80.0%)</td>
<td>42.8 ± 14</td>
</tr>
<tr>
<td>Buserelin27</td>
<td>103</td>
<td>3.3-0.4 mg (SC)</td>
<td>32–34</td>
<td>72/103 (69.9%)</td>
<td>49.1 ± 25.9</td>
</tr>
<tr>
<td>Histrelin18</td>
<td>10</td>
<td>250 ug (IM)</td>
<td>25–28</td>
<td>50% (5/10)</td>
<td>209.8 ± 48</td>
</tr>
<tr>
<td>Histrelin18</td>
<td>10</td>
<td>250 ug (IM)</td>
<td>29–32</td>
<td>100% (10/10)</td>
<td>145.2 ± 34.6</td>
</tr>
<tr>
<td>Histrelin18</td>
<td>10</td>
<td>250 ug (IM)</td>
<td>33–36</td>
<td>60% (6/10)</td>
<td>183.3 ± 33.9</td>
</tr>
</tbody>
</table>

GnRH, gonadotropin-releasing hormones; hCG, human chorionic gonadotropin; IM, intramuscularly; IV, intravenously; SC, subcutaneously; ND, nondisclosure. Adapted from a review by Canisso et al. (2019).17
Fig. 8. Estrus synchronization protocols for jennies. A, Double PGF2α doses 16 days apart.15  B, Progesterone and 17-ß estradiol preceded by PGF2α 10 days apart from the steroid administration.15  C, Double PGF2α 17 days apart.15  D, Serial PGF2α administrations.60  E, Continual progesterone administration for 10 days with PGF2α on day 8.60  F, Double PGF2α-GnRH and timed artificial insemination.29  G, Consecutive PGF2α-GnRH-PGF2α-GnRH and timed artificial insemination (AI).29  H, GnRH-PGF2α-GnRH and timed AI.29
is our impression that oxytocin works as effectively as it does in mares. However, up to date, there are no studies assessing the efficacy of oxytocin in jennies. Guidelines for treating uterine fluid in jennies are currently lacking; however, the authors have used equine guidelines to determine when to flush jennies. For instance, jennies with a large amount of anechoic or moderate to large amount of intrauterine fluid accumulation (Fig. 11) are typically flushed with a crystalloid solution such as lactated Ringer’s solution.

Pregnancy Diagnosis

Although transrectal palpation can be used as a diagnostic tool in jennies, this method requires experience and skill for a reliable diagnosis, and feasibility is determined by the type and size of the jenny along with the stage of gestation. Therefore, pregnancy diagnosis by transrectal ultrasonography minimizes the risk of false-negative diagnosis and aids in the identification and management of twins. Pregnancy diagnosis can be performed by transrectal ultrasonography 9 to 10 days postovulation, when the embryo vesicle is $2.3 \pm 1.1 \text{ mm}$ in diameter (Fig. 12A); however, the accuracy of detecting an embryonic vesicle pregnancy this early in gestation is only $25\%$. The first pregnancy diagnosis is ideally performed between 12 and 15 days after ovulation when the embryonic vesicle measures between $6 \pm 2.3 \text{ mm}$ and $17.9 \pm 3.8 \text{ mm}$ (Figs. 12B and C). The early embryonic features in jennies are highlighted in Table 4. The embryonic vesicle retains its spherical shape from the first detection (10 days) to fixation (18.5 $\pm$ 1.4 days), and the vesicle grows continually at 3.1 $\pm$ 2.7 mm/day. After fixation, the embryonic vesicle becomes irregularly shaped. The embryo proper appears at the ventral pole of the embryonic vesicle around days 19 to 21 postovulation (Fig. 12D), beginning the transition from the yolk sac to the allantoic sac phase (Fig. 12E). By day 24 to 25 of pregnancy, an embryo heartbeat can be detected.

3. Male (Jack) Features

Jack Sexual Behavior and Semen Collection

Jacks are generally regarded as challenging to collect semen using estrus jennies, mares, or a dummy mount. Strategies to optimize semen collection are described elsewhere in detail. Except for a few high-libido donkeys, most jacks can take up to 60 minutes to collect semen or may not collect unless conditions are optimized. Jacks are attracted to jennies, and some can be conditioned to mares; unfortunately, most mares are not receptive to jacks (60%), and several react violently to jacks during teasing or mounting. Thus, in mares not conditioned to jacks, the authors elect to sedate the mare heavily with alpha-2-agonists, place breeding hobbles, and lip twitch (Fig. 13). Additionally, breeding stocks with or without a pit in the center can aid in restraining the mounting mare being used for live cover or semen collection (Fig. 13). Mares exposed to donkeys tend to become used to jacks, and after repeated uses, they become more receptive to them when in estrus. Some jacks present extremely low libido in the presence of an estrus mare; patience and incorporating mimicked elements of donkey courtship with jennies while working with mares can be extremely helpful. In equids, washing the penis immediately before collection, particularly in sexually rested animals, is paramount to decrease bacterial contamination and debris in semen. Teasing by proximity to the teaser or allowing the jack to interact with the mare or jenny should result in penile exposure and erection (Fig. 14). While washing, untrained

![Fig. 9. Artificial insemination in a Baudet du Poitou jenny.](image)

![Fig. 10. Transcervical AI of jenny using the Wilsher and Allen’s forceps to retract the cervix of a small-frame jenny.](image)
donkeys can benefit from cleaning the penis with wet cotton rather than throwing water with a cup as water splashing might be enough negative stimuli for a donkey to lose an erection. Anecdotally, PGF2α analogs (dinoprost 2.5–5 mg/animal, IM, or sodium cloprostenol 125–250 mg, IM) have been used to enhance semen collection of donkeys failing to attain an erection in clinical practice. A recent study demonstrated that administration of cloprostenol reduces the interval for semen collection without affecting semen quality in donkeys. In the authors’ practice, some donkeys receive PGF2α analog throughout the breeding season three times/week without any apparent detrimental effect, and jacks do not seem to become refractory. Jacks can have semen collected in an artificial vagina while mounting an estrus mare, jenny, or a dummy mount (Fig. 15). All artificial vagina (AV) models (e.g., Missouri, Botucatu, and Hanover) used for horses can be successfully used to collect semen in donkeys. Despite donkeys having a penis significantly longer than stallions, a medium-size AV (e.g., regular length 22 inches) can be used to collect jack semen (Fig. 15). The water temperature of the AV is similar to horses and ranges from 49 to 55°C while filling the AV. To filter the semen, practitioners may use horse filters or well-folded gauze. Filters can be coupled with the collection bottle, or semen filtration can be carried out right after collection. The gel fraction is absent in most ejaculates, and when it is present, it will be in a much smaller proportion than typically seen in stallions. Since some jacks may take a while to attain a sustained erection, the AV can be kept in a Styrofoam box or a warm incubator once ready. Currently, protocols used for chemical induction of ejaculation do not work in donkeys. One study administered oral imipramine (3 mg/kg) followed by xylazine (0.66 mg/kg, IV) or a single dose of butorphanol (0.02 mg/kg, IV) and xylazine (0.33 mg/kg, IV), which resulted in no ejaculation. A second study, combining...
imipramine (2 or 3 mg/kg, orally) and xylazine (0.44, 0.66, or 0.7 mg/kg) 1 or 2 hours later, resulted in only one animal ejaculating, even though 74.5% (41/55) of the animals displayed erection.2,50

Semen Processing and Evaluation

Donkey semen is processed similarly to stallions.2 It is worth noting that donkey semen has colors varying from white, light grey, to yellow,1 and the latter should not be confused with urospermia (Fig. 16). Gel-free semen volume can be assessed by direct measurement or more accurately by determining the weight of the semen (1 g = 1 mL). Sperm concentration can be evaluated with a hemocytometer, spectrophotometerb or nucleocounterc with horse settings.2 Donkeys are well-known to have larger testes, a shortened length of spermatogenesis, and the highest spermatogenic efficiency of domesticated animals per gram of testicular parenchyma.51,52 Therefore, concentration and the total number of sperm are greater in donkeys than horses, and younger donkeys produce ejaculates with lower volume and greater concentration than older jacks.47 Young donkeys tend to produce gel-free ejaculates of 30 to 50 mL, and older donkeys tend to produce gel-free ejaculates of 60 to 90 mL.2,47 Typically, sperm concentrations for donkeys vary from 250 to 500 million sperm/mL in donkeys collected two to three times/week.2,47 The typical total number of sperm ejaculates vary from 20 to 40 billion in donkeys collected once or twice a week.5,47,48 Donkey motility parameters, including velocity and progressive motility, are also higher than stallions.53 Jacks often have ejaculates with 80% to 90% of total and progressive sperm motility. Morphology is rarely associated with infertility in donkeys, with most donkeys presenting < 15% of morphologic defects.54 Therefore, sperm morphology evaluation is not typically carried out for the breeding unless there is a history of subfertility or poor semen quality in serial semen collections.

Semen Cooling and Shipping

Cooled-shipped semen in donkeys can be carried out using horse recommendations, with some exceptions.2 Passive cooling semen containers, such as those used for horses44 can keep donkey semen refrigerated at 5 to 8°C.2,55 Noncentrifuged semen should target 25 to 50 million sperm/mL, and it needs to be extended at least one part of semen to three parts of extender.2 Milk-based extender alone results in poor motility and conception rates.55 If a milk-based extender is used to ship donkey semen, the seminal plasma should be removed via centrifugation or semen filter8 or the milk-based extender should be enriched with a source of cholesterol such as egg yolk to maintain motility and fertility.2 Alternatively, milk-based extenders containing cholesterol loaded in cyclodextrinh,i result in better semen cooling and fertility than milk-based extenders with no additional source of cholesterol.55,56 Commercially available semen-freezing egg-yolk-based extendersj can be used to cool donkey semen up to 24 hours at 5°C with excellent semen parameters and fertility in horse mares.32,34,36 However, up to now, the fertility of cooled donkey semen extended in egg-yolk based extendersl has not been tested in jennies. Satisfactory pregnancy rates (73%) can be obtained when jennies are bred with at least 1 billion progressively motile fresh sperm.31 In the authors’ practice, mares and jennies are bred with at least 2 billion progressively motile sperm, the uterus is flushed 6 hours

Table 4. Early Embryo Features in 8 Jenny Conceptuses

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Days of Gestation</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection of EV</td>
<td>11.8 ± 1.3</td>
<td>10–14</td>
<td>6.5 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Fixation of EV</td>
<td>18.5 ± 1.4</td>
<td>16–20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of spherical shape</td>
<td>18.8 ± 1</td>
<td>18–20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detection of embryo</td>
<td>22 ± 1.1</td>
<td>20–24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detection of HB</td>
<td>25 ± 1.1</td>
<td>24–26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detection of AS</td>
<td>27.3 ± 1</td>
<td>26–28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV, embryonic vesicle; HB, heartbeat; AS, allantois sac. Data obtained from Crisci et al. (2014).54</td>
<td></td>
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</tr>
</tbody>
</table>

EV, embryonic vesicle; HB, heartbeat; AS, allantois sac. Data obtained from Crisci et al. (2014).43

Fig. 13. Restraining mounting females for donkey semen collection. A, Breeding hobbles and twitch used to restrain a jenny in estrus while being teased by a donkey. B, Estrus mare with breeding hobbles in place while heavily sedated with detomidine before donkey semen collection.
postbreeding, and eccholics are given to aid uterine evacuation if needed. Barren mares or jennies with signs of endometritis (intrauterine fluid accumulation or infertility despite optimal breeding management with fertile semen) may require additional therapy as described elsewhere.

Semen Freezing

Protocols used to process donkey semen for freezing have been adapted from stallions. After collection, semen is extended 1:1 in a temperature-matched cooling semen extender, centrifuged at 600 × g for 10 minutes, or cushion-centrifuged for 1000 × g for 20 minutes. Thereafter, the supernatant is discarded, and the pellet is resuspended in a horse semen-freezing extender (or homemade extender). The semen is extended at 100 to 200 million sperm/mL and then loaded in 0.5 mL straws. Cooling and stabilizing semen can be carried out in a freezing control machine or a domestic refrigerator for 20 to 60 minutes, depending on the type of extender. Thereafter, the semen can be kept in the freezing machine or transferred 3 to 6 cm over liquid nitrogen vapor for 15 to 20 minutes and then plunged into liquid nitrogen. Semen frozen in 0.5-mL straws are typically thawed at 37 to 38°C for 30 to 60 seconds. The straws can be used for AI via deep-horn insemination with a flexible pipette coupled with a stylet or a couple with an inner catheter. If the practitioner is not familiar with deep-horn AI, semen can be thawed and deposited in the uterine body using a standard AI pipette. Pregnancy rates with frozen semen vary drastically with the protocol used and between species (mares vs. jennies). Mares bred with donkey frozen-thawed semen present pregnancy rates around 50% to 70% in commercial breeding programs, while jennies have much lower pregnancy rates, averaging 20% to 30% per cycle. Results are jack dependent, with some males demonstrating satisfactory pregnancy rates, while others obtain extremely poor pregnancy rates despite apparently satisfactory sperm motility.

Donkey Epididymal Transport and Epididymal Sperm Recovery and Processing

Upon unexpected death, castration, or euthanasia, donkey epididymal sperm can be harvested and cryopreserved using similar techniques described for stallions. The testes and epididymides should be removed in-block, and ductus deferens should be ligated to prevent semen wastage. For

Fig. 14. Washing (A) and drying off (B) the penis of a Mammoth jack.

Fig. 15. Semen collection using a Missouri artificial vagina of jack donkey mounting a dummy mount (A) and using a rigid Botucatu model artificial vagina of a jack mounting an estrus jenny (B).
transportation, the epididymis or the whole testis should be submerged in some sort of liquid (i.e., lactated Ringer’s solution, 0.9% saline solution, or a semen-cooling extender) and stored in a passive cooling system\(^{57}\) at 4 to 5°C.\(^{59}\) Upon arrival in the laboratory, either immediately after removal from the live animal or postmortem, or after transportation, the epididymides need to be rinsed with room-temperature sterile lactated Ringer’s solution and wiped with gauze to remove any contamination from blood or debris. Then, the epididymides are dissected from the testes.\(^{58,59}\) Dissection should be performed well enough to be able to stretch the epididymis and allow for a good sperm recovery.\(^{58,59}\) Following dissection, semen is harvested by retrograde flushing or flotation/float-up technique.\(^{58,59}\) For retrograde flushing, the tail of the epididymis is transected with a straight-bladed Mayo scissor, and the ductus deferens is catheterized with an intravenous catheter, a Tomcat, or less ideally with a needle.\(^{58,59}\) The epididymis is then flushed with semen extender until the complete distension of the tail of the epididymis, and then it is cut at its most distal portion with a straight-bladed Mayo scissor, and the fluid containing the semen is allowed to outflow into a 50-mL conical tube.\(^{58,59}\) The epididymis can be flushed with milk-based cooling extenders\(^{58,59}\) with volumes from 5 to 30 mL\(^{58,59}\) or can be flushed with a semen-freezing extender\(^{58,59}\) with volumes from 2 to 10 mL\(^{58,59}\) In the authors’ clinical practice, the preferred technique is directly flushing the epididymides with freezing extender.\(^{58,59}\) Then, concentration is adjusted to 200 million sperm/mL, and semen is cryopreserved as described above. If poor recovery is obtained with the retrograde flushing, or if the ductus deferens or epididymis are inadvertently perforated, the float-up technique can be used. Sperm yield is approximately 40% to 60% less with the float-up technique than retrograde flushing. Up to now, the fertility of donkey epididymal frozen-thawed sperm has not been tested in mares or jennies.

4. Conclusion

In conclusion, reproductive features and protocols available to manage donkeys in breeding programs have been described. It is worth noting that despite the similarities between donkeys and horses, some reproductive features differ remarkably. Therefore, not all horse guidelines are useful for the donkey species. Knowledge of the reproductive features of the donkey is useful to implement strategies to improve the reproductive efficiency of this species.

Acknowledgments

Declaration of Ethics

The Authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors have no conflicts of interest.

References and Footnotes


aCanisso IF, personal observation, 2021.
bEquine Densimeter, ARS, Ontario, CA 91761.
cChemoMetic, Lillerød, Denmark.
dEquitainer II, Hamilton Research, Inc., Ipswich, MA 01938.
eEquine Express II, Exodus Breeder Supply, York, PA 17406.
fBotuFlex, Botupharma, Scottsdale, AZ 85050.
gSpermFilter, Botupharma, Scottsdale, AZ 85050.
hBotuSemen Special, Botupharma, Scottsdale, AZ 85050.
iBotuSemen Gold, Botupharma, Scottsdale, AZ 85050.
jBotuCrio, Botupharma, Scottsdale, AZ 85050.
kINRA Freeze, IMV, Maple Grove, MN 55369.
lE-Z Freezing, ARS, Ontario, CA 91761.
mINRA96, IMV, Maple Grove, MN 55369.
nEquiPlus, Minitube, Verona, WI 53593.
oEZ-Mixin, ARS, Ontario, CA 91761.