EMBRYONIC AND FETAL DEVELOPMENT IN THE DONKEY

A Capstone Experience Manuscript

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Abstract

The donkey, which is the common name for the species Equinis asinus and a member of the Equidae family along with horses and mules, is a species that has relatively little information available regarding its reproductive physiology. In the United States, donkeys are used primarily as pets, with some using them for farming purposes, guard animals, and as therapy animals. In this study four females were observed from the time of conception to parturition. Two out of the four have carried their foals to term. Ultrasonography was used to observe embryonic and fetal growth. For most of the examinations a 5 MHz linear transducer was used to perform transrectal examinations. The jennets were examined three times a week until approximately 240 days of gestation when the frequency was reduced to twice a week. Previous studies have indicated that there are no major differences between the horse and the donkey pregnancies. The goal of this study is to provide a resource for donkey breeders/owners and veterinarians that is more comprehensive than what is currently available. It also provides preliminary information that can be eventually compared to the horse pregnancy. For further study, more donkeys should be included in the project, including mammoth sized donkeys, so that all sizes can be represented.
Introduction

The donkey, which is the common name for the species *Equinis* asinus and a member of the *Equidae* family along with horses and mules, is a species that has relatively little information available regarding its reproductive physiology. The donkey is found all over the world and is considered a highly valuable and important animal in many countries. The donkey is the work animal of most third world nations. They rely on the donkey for transportation, packing, and plowing, and they can also act as guard animals for livestock. Figure 1 shows donkeys being used as pack animals in the Nuñoa District, Peru. In the United States, donkeys are used primarily as pets, with some using them for farming purposes, guard animals, and therapy animals. Female donkeys are called jennets, while male donkeys are called jacks.

![Image of donkeys in Peru](image)

**Figure 1: Donkeys being used as pack animals in the Nuñoa District, Peru**

Reproduction is an important part of donkey stewardship whether in the United States or around the world. With the world population on the rise and the need for more food production worldwide, the importance of the donkey as a work animal in previously mentioned third world countries should not be overlooked.
There have been three previous research projects which followed donkey pregnancies which should be acknowledged:


The text Ultrasonic Imaging and Animal Reproduction: Horses was used as a template for the experimental design of this project. It was also used to compare the results that we found in the donkey to the results of the horse. Each of the papers has followed the donkey pregnancy from conception to Day 60. However, none has followed the pregnancy any further. Also, all of these papers have compared their results to that of a horse and have not found any major differences between jennies and mares. Therefore, in the research conducted at the University of Massachusetts, our goal was to follow the pregnancies from conception all the way to parturition so that more data could be gathered with the long term goal of obtaining enough data to fully compare a donkey pregnancy to that of the horse. The other goal of this project is to collect data to provide a resource for donkey breeders/owners and veterinarians that is more comprehensive than what is currently available.
Methodology

In this study 4 females were observed throughout their pregnancy (Table 1). 2 jennets have carried their foals to term. 2 of the jennets observed were considered standard size (36 - 54 inches in height at the withers) and can be seen in Figures 3 and 4. The other 2 were of miniature size (36 inches or less) and can be seen in Figures 2 and 5. 2 were known to have had offspring before, while the other two were first time breeders. The first pregnancy was started in the fall of 2010. The same jack (Figure 6) was used to breed all 4 jennets.
The project began by ultrasounding the jennets three days a week with at least one day in between. Examinations were conducted using an Aloka SSD-500V Ultrasound Console with either a 5MHz or a 7.5 MHz probe transrectally. Behavior testing was conducted whenever a large follicle was present. If a large follicle (≥ 25 mm diameters) was present and the female was receptive, hand breeding was used. Behavior testing was performed by bringing the female on a halter over to the jack’s pen. If the female showed signs of receptivity it was considered a positive result. Signs of receptivity include: jawing with ears pointing backwards, pawing, urinating, and light to moderate kicking. Following a positive behavior test the female was prepared for breeding. She was tied in a pen in an area that sloped slightly downward so that the jack could mount more easily. The jack was kept on a halter with a chain lead at all times for the safety of the jennet and the handlers. The act of breeding lasted from 4 to 50 minutes. If the jack tried to mount without having an erection he was pulled off and was not allowed to mount until he had an erection. After ejaculation the jack would pull out and a dismount sample of semen was collected from the penis. This sample was evaluated for estimated and actual sperm concentration, estimated sperm motility, and percent live sperm and sperm morphology using eosin-nigrosin stained slides. Jennets were bred every other day until no longer receptive to the jack’s advances. After the final breeding the female was not examined for approximately 10 days. At 10 days after the last breeding she was returned to the 3 days a week ultrasound.

<table>
<thead>
<tr>
<th>Jennets</th>
<th>Age (yrs)</th>
<th>Height (inches)</th>
<th>Weight (lbs)</th>
<th>Previous Pregnancy</th>
<th>Size</th>
<th>Dates of Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Anna)</td>
<td>8</td>
<td>32</td>
<td>186</td>
<td>Yes</td>
<td>Miniature</td>
<td>9/29/10-11/10/11</td>
</tr>
<tr>
<td>2 (Charise)</td>
<td>7</td>
<td>46.8</td>
<td>381</td>
<td>No</td>
<td>Standard</td>
<td>4/1/11-4/11/12</td>
</tr>
<tr>
<td>3 (Gigi)</td>
<td>8</td>
<td>45.5</td>
<td>505</td>
<td>Yes</td>
<td>Standard</td>
<td>4/1/11-present</td>
</tr>
<tr>
<td>4 (Mojita)</td>
<td>4</td>
<td>35.5</td>
<td>238</td>
<td>No</td>
<td>Miniature</td>
<td>9/18/11-present</td>
</tr>
<tr>
<td>Jack</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Standard</td>
<td>n/a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Taco</th>
<th>16</th>
<th>36.5</th>
<th>238</th>
<th>n/a</th>
<th>Standard</th>
<th>n/a</th>
</tr>
</thead>
</table>

Table 1: Study animals
schedule. This was done to allow the jennet to have a period of rest and because the embryo would not appear on ultrasound that early in pregnancy.

Figure 6: Taco breeding Gigi (Jennet #3)

Each ultrasound examination was conducted in the following manner. The jennet was brought in from her pen and led into the laboratory. The donkey was then walked into a chute and tied so that she could not back out. While in the chute, the jennet was offered several different types of food to keep her entertained. Types of food included: grain, hay stretcher, hay, apples, peppermints, and other treats. When the jennet was properly positioned in the chute the handler would use a gloved hand and lube to excavate feces from the caudal rectum. This was done so that there would be room for the probe to safely enter the rectum. Once the rectum was free of excess feces, 60 ml of water soluble lubricant was placed into the rectum to act as a lubricant and as a conduction medium. Next either the 7.5MHz or the 5.0 MHz linear probe was inserted in the rectum. Usually the 5.0 MHz probe was used. The probes were connected to an extension which was created out of PVC pipe and then wrapped in duct tape. This was done because when ultrasounding miniature donkeys and even standard donkeys it is neither practical,
comfortable, nor safe for most technicians to perform a transrectal ultrasound by inserting the probe and arm into the donkey rectum. Therefore, the extension was attached to the probe and that was inserted into the fully lubricated rectum. While the advantages of the extended probe allowed us to look deeper than possible with a gloved hand, there were certainly disadvantages. Use of the extended probe does not allow one to palpate the fetus or the reproductive tract. It also limits the ability to manipulate the fetus while ultrasounding. Essentially this means that with the extension we can only see what is “in position” for that particular day. We cannot feel around in the rectum and manually manipulate the fetus into a position for better viewing. Also using the 7.5MHz probe, the depth of viewing was limited to 60 mm, while the 5.0 MHz probe provided imaging as deep as 140 mm. Another limitation of transrectal ultrasound noted throughout this project was that between Days 70-98 the fetus dropped out of sight of the probe; the fetus was deeper that 140mm. However after the fetus completed its “drop” it would resurface usually by Day 100 with the head visualized first, and it would continue to change position until the whole body was able to be visualized. However the head was not able to be viewed at the same time as the body after the fetus resurfaced. It is suspected that when the fetus resurfaces the head is in one horn while the body is in the other. The donkey embryo was referred to as an embryo up until Day 50, after which it was referred to as a fetus. During the actual examinations, photos and video were recorded of several different structures including: overall fetus length, head, heart, stomach, eye socket, etc. Video was used to record fetal movements and heartbeat.
Results

Unlike Ginther’s research in the horse, the donkey pregnancies that we followed did not all follow the embryo decent patterns described in his text. In two of our animals the embryo proper first appeared at the top of the embryonic vesicle and descended to the bottom before forming the umbilicus. This can be seen in Figures 42 and 45. This is directly opposite to Ginther’s description of embryo decent in the horse.

In this study we tracked the development of the following major structures of the donkey embryo and fetus: the head, the heart, the overall body length and width, the eye, and the stomach. Each of these structures was measured and graphed with a trend line illustrating the rate of growth. In several cases the trend lines of the four different donkeys used in this study were graphed together to compare the overall growth rate for that specific structure among the four jennets.

The timeline of events for the four jennets that we followed (Table 2) did not correspond exactly with previous studies between 0-60 days. In previous studies all structures that we followed were found at earlier times than in our study. As seen in Table 2, the timeline of all four animals in this study is delayed compared to those in the previous studies of Meira and Gastal. Our timeline is also delayed compared to what Ginther found in his monitoring of horse fetal development.

When examining the growth rate of the overall length and width of the embryo we found no significant difference in the growth rate between standard and miniature jennet in the first 70 Days. However there was a slight difference between the growth of embryos expected to be males (jacks) and those expected to be female (jennets) this can be seen in Figure 41.
To determine the sex of the embryo the genital tubercle and its proximity to the posterior end was examined. Also the appearance of testicles and or the penis on the ultrasound was used to determine fetal gender. This can be seen in Figures 46-52.

Growth of the embryo proper for each animal is displayed in Figures 8-11. Figure 7 shows how the measurements were taken for the embryo proper. The growth of the embryo proper was followed from Day 24 when it was first visible to Day 70 when the fetus became too large to measure in one screen on the ultrasound machine. Gaps in the measurements may be due to the embryo proper not being in a viewable position during the examination. For all four of the animals followed the length of the embryo proper appears to grow in an exponential fashion.

Figure 7: Measurements of the embryo proper
Figure 8: Growth of embryo proper for Anna.
Figure 9: Growth of embryo proper for Charise
Figure 10: Growth of embryo proper for Gigi
Figure 11: Growth of embryo proper for Mojita
The growth of the head for each animal is displayed in Figures 13-16. Figure 12 shows how the measurements of the head were obtained. The growth of the head was followed from Day 35 when it was first observed and measured to Day 156 when it became too big to fit on the ultrasound screen to be measured or it was no longer visible due to a change in the positioning of the embryo or fetus. For all four animals the head appeared to grow in a linear fashion.

Figure 12: Measurements of the head
Figure 13: Growth of the head for Anna
Figure 14: Growth of the head for Charise
Figure 15: Growth of the head for Gigi
Figure 16: Growth of the head for Mojita
The growth of the heart for each animal is displayed in Figures 18-21. Figure 17 shows how measurements for the heart were obtained. The growth of the heart was followed from Day 31 when it first appeared and was measured to Day 254 where the positioning of the fetus no longer allowed the heart to be viewed on the ultrasound screen. For all four animals the heart appears to grow in a linear fashion when examining just the length or the width.

Figure 17: Measurements of the heart
Figure 18: Growth of the heart for Anna
Figure 19: Growth of the heart for Charise
Figure 20: Growth of the heart for Gigi
Figure 21: Growth of the heart for Mojita
The growth of the stomach for each animal is displayed in Figures 23-26. Figure 22 shows how measurements for the stomach were obtained. The growth of the stomach was followed from Day 65 when it was first visible to Day 226 when the fetus no longer was in a position so that it could be viewed. For all four animals followed the stomach appears to grow in a linear fashion.
Figure 23: Growth of the stomach for Anna
Figure 24: Growth of stomach for Charise
Figure 25: Growth of the stomach for Gigi
Figure 26: Growth of the stomach for Mojita
The growth of the eye for each animal is displayed in Figures 28-31. Figure 27 shows how measurements for the eye were obtained. The growth of the eye was followed from Day 67 when it was first observed to approximately Day 365 or the end of pregnancy. The period of examination depended upon the positioning of each individual fetus. For the four animals observed the growth of the eye appears to grow in a linear fashion.
Figure 28: Growth of the eye for Anna
Figure 29: Growth of the eye for Charise
Figure 30: Growth of the eye for Gigi
Figure 31: Growth of the eye for Mojita
The growth of the overall area of the eye for each animal is displayed in Figures 32-35. The area of growth was calculated by using the data of length and width of the eye and using the formula: Area = (Length x 0.5) x (Width x 0.5) x 3.14. This was then graphed for each animal. For all four animals the overall area of the eye appears to grow in a linear fashion.

![Area of Eye Growth](image)

**Figure 32: Growth of the area of the eye for Anna**
Figure 33: Growth of the area of the eye for Charise
Figure 34: Growth of the area of the eye for Gigi
Figure 35: Growth of the area of the eye for Mojita
The growth of the overall area of the heart for each animal is displayed in Figures 36-39. The area of growth was calculated by using data of length and width of the heart. This data was used in the formula: \[ \text{Area} = (\text{Length} \times 0.5) \times (\text{Width} \times 0.5) \times 3.14 \] to calculate the overall area of the heart. This was graphed for each animal. When this data was graphed the overall area of the heart appears to grow in an exponential fashion for all four animals.

Figure 36: Growth of heart area for Anna
Figure 37: Growth of heart area for Charise
Figure 38: Growth of heart area for Gigi

\[ y = 30.546 e^{0.012x} \]

\[ R^2 = 0.9923 \]
Figure 39: Growth of heart area for Mojita
The comparison of the growth of heart for all animals can be seen in Figure 40. By examining this graph it can be seen that there is no significant difference between the growth rates of the four different animals.

Figure 40: Growth of the heart for all animals
The comparison of the growth of eye for all animals can be seen in Figure 41. All donkeys appear to be growing at approximately the same rates except for the donkey Charise. She is a standard donkey and was carrying a jack foal at the time of observation.

Figure 41: Growth of the eye area for all animals
The comparison of the growth of the embryo proper for all animals can be seen in Figure 42. It is interesting to note here that the growth of the embryo proper in the first 70 days showed little difference between Anna, Charise, and Mojita. However Gigi who is carrying a jennet foal is shown to be growing at a slower rate.

Figure 42: Growth of the embryo proper for all animals
Embryo travel in Anna (Donkey #1)

Anna’s embryo proper first appeared at the top of the embryonic vesicle on Day 24. From there it began its descent. It very clearly appears at the top of the vesicle and travels to the bottom by Day 37. After Day 37 it develops the umbilicus until Days 51 and 56 where it can be seen that the fetus is attached to the bottom of the vesicle.

Figure 43: Embryo travel for Anna
Embryo travel in Charise (Donkey #2)

Charise’s embryo proper first appears in the lower left of the embryonic vesicle on Day 24. From there it begins its ascent to the upper right corner of the vesicle as seen on Day 37. By Day 53 the umbilicus can clearly be identified.

Figure 44: Embryo travel for Charise
Embryo travel in Gigi (Donkey #3)

Gigi’s embryo proper first appears in the bottom center of the embryonic vesicle on Day 24. It then ascends upward until Day 38 when it reaches the top of the embryonic vesicle. By Day 52 the umbilicus can be identified.

Figure 45: Embryo travel for Gigi
Embryo travel in Mojita (Donkey #4)

Mojita’s embryo proper first appears in the top of the embryonic vesicle on Day 25. From there it descends until Day 39 when it reaches the bottom of the embryonic vesicle. By Day 48 the umbilicus can be identified.

Figure 46: Embryo travel for Mojita
Timeline for Embryonic and Fetal Structures:

The timeline of first detection for the major events of development (embryonic vesicle, loss of spherical shape, embryo, heartbeat, allantoic sac, umbilicus, and head) are displayed in Table 2.

Table 2: Timeline of first appearance of major structures

<table>
<thead>
<tr>
<th>Feature</th>
<th>1st Detection (Days)</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anna</td>
<td>Charise</td>
<td>Gigi</td>
</tr>
<tr>
<td>Embryonic Vesicle</td>
<td>15</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>Loss of spherical shape</td>
<td>21</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>Sighting of embryo</td>
<td>24</td>
<td>22</td>
<td>28</td>
</tr>
<tr>
<td>Heartbeat</td>
<td>24</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Allantoic sac</td>
<td>26</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Umbilicus</td>
<td>51</td>
<td>53</td>
<td>52</td>
</tr>
<tr>
<td>Head</td>
<td>35</td>
<td>44</td>
<td>52</td>
</tr>
</tbody>
</table>
Fetal Sexing:

The Fetuses were all sexed by examining either the genital tubercle or by observing the testicles or the penis. In Figure 47 Anna’s fetus is sexed as a jack by observing the head of the penis located between the thighs on Day 133.
In Figure 48 and 49 Charise’s fetus was sexed as a jack by observing the testicles at Day 196 and the head of the penis, Day 214 (Figure 46) between the thighs. Figure 49 is a clear photo of the scrotum and testicles of the fetus.

Figure 48: Charise male fetus testicles
Figure 49 Charise male fetus testicles and penis
In Figure 50 and 51 Gigi’s fetus was sexed as a jennet. This was done by observing the genital tubercle and examining its position. Because of the proximity of the genital tubercle on Day 73 to the posterior of the fetus it was determined that the fetus would be a jennet. On Day 103 the genital tubercle was located again toward the rear of the fetus; therefore the original hypothesis of a jennet is reinforced.

Figure 50: Gigi female fetus genital tubercle
Figure 51: Gigi female fetus genital tubercle
In Figure 52 and 53 Mojita’s fetus was sexed as a jack. This was determined by observing the genital tubercle and examining its positioning in relation to the center of the body. By observing the genital tubercle on Day 69 and Day 72 it was determined that the genital tubercle was closer to the center of the fetus as opposed to the rear. Thus the fetus is expected to be a jack.

Figure 52: Mojita male fetus genital tubercle
Figure 53: Mojita male fetus genital tubercle
Birth

Jennet #1, Anna, gave birth on Day 370 to the foal shown in Figure 54. Jennet #2 gave birth on Day 366; her foal can be seen in Figure 55.

Figure 54: Anna's foal
Figure 55: Charise's foal
Conclusions

There were several different variables that were discovered during the course of this project. For example, the largest variable encountered was the fact that we were not able to alter the position of the embryo or fetus and were reliant upon whatever position that the embryo or fetus was in during that particular exam period. Another variable was the skill of the operator. This could have affected what structures were seen during the exam. Other variables worth noting are that we had two different sizes of donkeys in this study, both miniatures and standards. Finally the animals used in this study all had different reproductive backgrounds, some had foals previously, others had been attempted to be bred, and others were never bred before.

As previously mentioned, this study found that unlike Ginther’s research regarding the developing horse embryo, 50% of the time the donkey embryo appeared at the top of the embryonic vesicle as opposed to the bottom. Also this study’s timeline was consistently 3-4 Days behind the timelines developed in Meria’s and Gastal’s research. Another conclusion reached from this project can be seen in Figure 38, the comparison of the growth of the embryo proper. This study has found that the jennet carrying the female fetus grows at a slower rate during the first 70 Days than the other three jennets carrying male fetuses. However this study only examined four different animals; before strong correlations can be made more study is needed. The reappearance of the fetus in ultrasound examinations is something that was found across all four jennets and occurred at approximately the same time. This data suggests that at around Day 70 the embryo will drop out deeper than 140mm into the uterus and will reappear within 2 weeks in a different position, with the head in one horn and the body in another.
Finally the sexing of the fetuses was conducted using two different methods, one the visual observation of reproductive organs and two the positioning of the genital tubercle. Both visual observations were proven to be correct upon birth of the foal.

**Future Study:**

The overall goal of this project was to gather data so that more information could be available for eventual comparison to the horse and to provide a source of reproductive information for veterinarians, breeders, and owners. Several of our conclusions including the differences in embryo travel, and the differences in growth of the embryo proper and fetus need more data before they can be considered statistically significant. For future study I would recommend several things. First, ultrasounds should be conducted sooner after conception, and provided that there is no health hazard to the animal the ultrasounds should continue daily so that more data can be gathered with less gaps between data points. Also a 3.0 MHz probe should be used after approximately Day 175. This is when the fetus is deep into the abdomen and it is quite large. Having a probe that could view a wider and deeper range would be extremely helpful. In addition to the different probe, more measurements should be taken during the ultrasound exams. This would make the overall exam time longer. As long as the animal tolerates it well it would provide more data. Along with taking more measurements, video of each exam should be recorded and saved for future reference in case of missing measurements or discrepancies in the data. If video of the ultrasound could be recorded while recording a voiceover of the examiner and what he or she was viewing or trying to view this would prove to be extremely helpful. Furthermore, the organization of data should be established and written down in precise protocols. This would prevent data from being missed, and data being confusing
or jumbled. Finally the most significant improvement to this project would be the addition of more donkeys, both jacks and jennets. The addition of new donkeys would provide more data for comparison. Mammoth donkeys should also be added to the project so that all sizes could be represented in the study. With the addition of mammoth donkeys a rectal ultrasound conducted by hand becomes a possibility, therefore increasing the ability to position the probe. This could be useful in gathering more pertinent data.

Finally, this project is simply a start, more donkeys must be observed all the way from conception to parturition so that a more complete library of records can be developed and eventually compared to the horse development.
References


