Correlating Female Alpaca Behavioral Receptivity with Cervical Relaxation and Ovarian Follicle Growth

Caitlin Donovan May 2011

Introduction

The alpaca - a member of the Camelidae family along with the Old World bactrian and dromedary camels and the New World guanacos, vicunas, and llamas - is a species that has little information available regarding its reproductive physiology. Although the alpaca is traditionally found in the Andes Mountains of South America at high elevations, used primarily for fleece and meat, the popularity of the species has spread around the world. Specifically, in the United States, the alpaca industry primarily revolves around reproduction, so it is essential that breeders have a firm grasp on the physiology of camelid reproduction in order to maximize profit.

Camelids are induced ovulators - therefore, the act of copulation is what stimulates the LH surge that results in ovulation, unlike many other livestock species that have regular estrous cycles without the need for copulation. Furthermore, the act of a natural mating is solely dictated on the behavioral response of the female (Bravo and Sumar, 1989). When the female is receptive, she assumes sternal recumbency, also known as kushing, where she drops to the ground in order to allow the male to penetrate. In order to be receptive, it is assumed that the female has an ovarian follicle of at least 6-7 mm in diameter to produce enough estrogen to result in receptivity (Vaughan et al., 2003), but sexual receptivity in the female does not always mean that there is an ovarian follicle present that contains an oocyte with high fertilization potential (Bravo et al., 1991). Therefore, being able to predict the time of optimal fertility in the female would improve mating efficiency significantly.
Previous studies on camelid follicular development have the common conclusion of follicular growth and regression occurring in waves. For example, in a study done by J.B. Sumar (2000), it was determined that ovarian follicles grow and wane in a 12 day period with a range of 9-17 days, given that there is no act of copulation.

Figure 1: Unmated female showing follicular phase or wave, where follicles grow and wane in a 12 day wave-like fashion

Another study on llamas (Bravo et al., 1991) and yet another on the dromedary camel (Skidmore et al., 1996) showed similar results with follicular development divided into three phases: a growth phase, a mature phase, and a regression phase, even though Skidmore noted specifically that wave patterns in the camel “varied considerably between individual camels.” Finally, a study done by J.L Vaughan (2003) on alpacas concluded that follicular wave intervals were observed of 12, 16, 18, 20, and 22 day duration, resulting in the conclusion that the “use of a mean inter-wave interval should be avoided” because “a mean inter-wave interval may not accurately describe what is occurring in an individual animal.” This conclusion is in stark contrast to Sumar’s rigid 12 day wave period, and therefore, the goal of this research conducted at the University of Massachusetts was to determine whether follicular development can truly be defined in concrete wave periods of growth and regression. Furthermore, other elements that contribute to receptivity were observed in relation to each other, including relaxation of the
cervix, uterine horn echogenicity, and behavior in the presence of a male. This research will hopefully be able to determine the optimal time for fertility and breeding, thus resulting in an increased number of pregnancies in a shorter duration of time.

Methodology

In this study, 14 females were observed over a period of four semesters: fall 2008, fall 2009, spring 2010, and fall 2010. 11 females were proven with at least one previous cria, or baby alpaca, and three were maidens. All females were considered to be of mature breeding age and free from any obvious flaws that could hinder receptive behavior. Multiple intact males were used to test for behavioral receptivity.

<table>
<thead>
<tr>
<th>Female</th>
<th>Age</th>
<th>Number of Crias</th>
<th>Time Since Last Cria</th>
<th>Date of Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>2</td>
<td>12 months</td>
<td>9/23-10/25 2008</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>3</td>
<td>1 month</td>
<td>10/28 - 11/25 2008</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1</td>
<td>24 months</td>
<td>9/21-10/16 2009</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>4</td>
<td>12 months</td>
<td>9/21-10/16 2009</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
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<td>N/A</td>
<td>10/21-11/16 2009</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
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<td>N/A</td>
<td>10/21-11/16 2009</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>1</td>
<td>5 months</td>
<td>1/25-4/30 2010</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>1</td>
<td>5 months</td>
<td>1/25-4/30 2010</td>
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<td>1/25-4/30 2010</td>
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<td>9/13-12/10 2010</td>
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<td>12</td>
<td>3</td>
<td>1</td>
<td>12 months</td>
<td>9/13-12/10 2010</td>
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<tr>
<td>13</td>
<td>4</td>
<td>2</td>
<td>6 months</td>
<td>9/13-12/10 2010</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>None</td>
<td>N/A</td>
<td>9/13-12/10 2010</td>
</tr>
</tbody>
</table>

Table 1: Female information

For the first two semesters, each group of animals (females 1-6) were observed for a four week duration, which, according to previous research stated above, would have been ample time
to establish at least one wave of follicular growth and regression. However, based on findings with these animals, the females used for the next two semesters (females 7-14) were observed exclusively for the entire semester in order to attempt to further establish a pattern in terms of follicular activity. All females were studied for three days a week with at least one day in between observations.

During observations, females were first tested for behavioral receptivity. Females were kept together loose in a pasture, and the male was haltered and brought to them. The handler would then bring the male towards the female of interest, and her resulting behavior would then determine her receptivity. The scale used to grade her behavioral receptivity is as follows:

<table>
<thead>
<tr>
<th>Receptivity Grade</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not receptive. Ran away, spit, kicked, did not allow the male to mount.</td>
</tr>
<tr>
<td>2</td>
<td>Not receptive. Allowed the male to mount but did not assume the kush position.</td>
</tr>
<tr>
<td>3</td>
<td>Receptive. Assumed the kush position after the male mounted.</td>
</tr>
</tbody>
</table>

Table 2: Behavioral Receptivity Scale

Figure 2: An example of female behavioral receptivity. The white female is displaying grade 2 receptivity, and the black female is displaying grade 3 receptivity.
After the first male tested all the females necessary, a second male was brought in and the process was repeated. Some females have a preference towards a male’s style, whether it be more aggressive or more passive, so it was important to use at least two males throughout the study to allot for female preference. If a female kushed for one male and not the other, it was assumed she was behaviorally receptive.

After all females were behavior tested, they were individually brought into the lab for a reproductive examination. They were restrained in a chute and their tails were wrapped and tied to the side. After the vulva was scrubbed with iodine, a vaginal speculum was inserted to allow for viewing of the vagina and cervix. When copulation occurs in camelids, the penis penetrates the cervix and deposits semen in the uterine horns. Therefore, when a female is receptive, it is expected that the cervix would be moderately or fully relaxed to allow for penetration of the penis to occur without injury. Contrarily, if the female is not receptive or is pregnant, it is expected that the cervix would be closed. Cervical appearance was graded on a 1 to 4 scale, depicted below:

![Figure 3: Cervical Relaxation Scale](image-url)
After cervical examination, the female’s uterine horns and ovaries were observed. The female was ultrasounded transrectally using a 7.5 MHz linear transducer. To prepare for ultrasounding, any manure in the rectum was removed and approximately 60 cc’s of lubricant was inserted in the anus to allow for good contact to be achieved. During the ultrasound examination, still photos were taken of each uterine horn and of each ovary. The uterine horns were measured widthwise and given a grade based on echogenicity. A darker, fluid-filled uterus was given a grade of 1, expected when a female is receptive, and a light-colored uterus was given a grade of 3, expected when the female was not receptive. A color in-between was given a grade of 2.

![Figure 4: Uterine horn echogenicity scale](image)

Each ovary was also located and observed for follicular development. Multiple small follicles seen on an ovary, of size no greater than 3mm, were recorded as ‘MSF.’ Any larger follicles were measured and the size was recorded. The dominant follicle was considered to be the largest follicle present that was of significant size, 6mm or greater. During the study, there were
instances where there was no defined dominant follicle, and there were also instances where there was more than one dominant follicle observed.

Figure 5: Ovarian follicles

Results

What trends were found?

Unlike Sumar’s research that depicts a 12 day wave cycle, no such wave was found. Follicular growth varied considerably between females – sometimes growth of a specific follicle could be tracked, and other times a large dominant follicle would be found when there were only multiple small follicles found the previous observation. There was a similar discovery as far as follicle regression. Sometimes, the follicle could be followed as it regressed, and other times, it regressed so quickly that the follicle would be present for one observation and nonexistent the next. So therefore, in some instances, there were no periods of growth or regression whatsoever.
Furthermore, there was not always the establishment of one dominant follicle, which concurred with research done by Vaughan et al. with the alpaca in 2003, who observed that “the simultaneous presence of more than one follicle of greater than 7mm in diameter was observed 5 times [after approximately 50 observations].” There were similar findings of two dominant follicles being present in a few of the females observed, which is unexpected because the occurrence of twins in the camelid species is so rare. Therefore, in chart comparisons, the combined follicle size was used when applicable so both large follicles were taken into consideration.

A trend that was found throughout the study, as discovered with previous research (Bravo et al., 1991; Vaughan et al., 2003) is that there is an inverse relationship between the amount of follicles on an ovary and the size of the dominant follicle. For example, if there is no dominant follicle on an ovary, it will have many small follicles across the surface. However, if a dominant follicle grows, the amount of follicles on that ovary decreases, even though there is the possibility of there still being other follicles on the ovary besides the dominant one. Furthermore, this relationship is isolated to the individual ovary – so even if the left ovary has a dominant follicle and few others, the right ovary can still have multiple small follicles.

In terms of behavioral receptivity, there was a broad range of differences among females. Some were not receptive, even if they did have a follicle of significant size. Some were always receptive, even if there was no significant follicular growth. But some females did kush when they had a dominant follicle present, as would be expected. There are a lot of different variables that could affect the behavioral receptivity of the female, including weather, the specific male used, the behavior of other females in the vicinity, and overall pasture conditions, and these variables would need to be examined closer for further study.
In general, when the female had a follicle of significant size (6mm or greater) present, the cervix would be at least moderately relaxed, which is what was expected – if the female is receptive and producing estrogen, the cervix has to be relaxed in order for the penis to penetrate. However, there were a few instances where the female would kush for the male and the cervix would be closed, which is perplexing because penetration of a closed cervix can result in a much higher rate of injury.

Uterine echogenicity did not vary too much between observations – most females were nearly always a grade 2 with an occasional grade 1 or grade 3 observed that did not seem to be linked to any specific ovarian activity.

Analysis of Individual Females

**Female 1: Observed for four weeks**

**Behavioral Receptivity:** Grade one for observations 6, 7, 10, 11, 12

Grade two for observations 2, 4, 8

Grade three for observations 1, 3, 5, 9

**Cervical Relaxation:** Grade one for observations 1-3, 6, 8-10

Grade two for observations 4, 5, 7, 11, 12

Cervix was never observed as a grade three or four

**Uterine Echogenicity:** not observed
Ovarian Follicles:

**Figure 6: Ovarian follicular size chart, female 1**

Comparisons between behavioral receptivity, cervical relaxation, and follicle size:

**Figure 7: Follicle diameter in comparison to behavioral receptivity, female 1**
Female 1 showed little correlation between follicle size, cervical relaxation, and behavioral receptivity. She was receptive four observations out of the total twelve: observations 1, 3, 5, and 9. For observation 1, her cervix was closed and she had no significant follicular growth. For observation 2, her cervix was closed, but she had a large 9mm follicle on her right ovary and a combined follicle diameter of 12mm, which did correlate to her behavioral receptivity. For observation 5, her cervix was slightly relaxed, but there was no significant follicular growth. For observation 9, her cervix was again closed and there was a 4mm follicle on each ovary – individually, the follicle was not considered large enough to result in receptivity, but the combined size of 8mm is assumed to produce enough estrogen that could result in receptive behavior. What question remains is if two 4mm follicles would be enough to result in pregnancy if the female had been bred. Finally, for observation 8, this female had two large follicles present: a 6mm follicle on her left ovary and a 16mm follicle on her right. Although she did not display receptivity that day, she displayed receptivity for the next observation. This could
potentially indicate that there is a delay in estrogen taking effect, but further research is needed to confirm this.

Overall, female 1’s results did not support the hypothesis that the combination of a relaxed cervix and a follicle of 6mm diameter or greater results in behavioral receptivity, and her follicular development did not form a consistent wave throughout the twelve observations. There was one large spike with no growth period beforehand and an incredibly short regression period afterwards, and there was no consistency as to how long a large follicle remained present.

**Female 2: Observed for four weeks**

Behavioral Receptivity: Grade one for observations 2, 3, 5-12  
Grade two for observations 1, 4  
Grade three for no observations

Cervical Relaxation: Grade one for no observations  
Grade two for observations 2, 6, 7, 12  
Grade three for observations 1, 4, 5, 10, 11  
Grade four for observations 3, 8, 9

Uterine Echogenicity: not observed
Ovarian Follicles:

Figure 9: Ovarian follicular size chart, female 2

Comparisons between behavioral receptivity, cervical relaxation, and follicle size:

Figure 10: Follicle diameter in comparison to behavioral receptivity, female 2
Female 2 never displayed receptivity throughout the four weeks that she was observed. However, her cervix was never closed - scored as a 2, 3, or 4 throughout the study – and she did develop follicles of significant size at certain points. She had individual large follicles on observation 7 and 9, and she had a large combined follicle size on observations 3 and 8. Interestingly, her cervix was completely relaxed, with a grade of 4, for observations 3, 8, and 9, which does correlate to follicular development on that specific day, even though she was not behaviorally receptive.
Female 3: Observed for four weeks

Behavioral Receptivity: Grade one for no observations

Grade two for observation 1

Grade three for observations 2-12

Cervical Relaxation: Grade one for observation 12

Grade two for observations 5, 8-11

Grade three for observations 2, 6, 7

Grade four for observations 1, 3, 4

Uterine Echogenicity: Grade one for observation 4

Grade two for observations 1, 3, 5-10, 12

Grade three for observation 11

Ovarian Follicles:

Figure 12: Ovarian follicular size chart, female 3
Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

Figure 13: Follicle diameter in comparison to behavioral receptivity, female 3

Figure 14: Cervical relaxation in comparison to behavioral receptivity, female 3
Female 3, in stark contrast to female 2, was behaviorally receptive for 11 out of the 12 observations. She was not receptive for the first observation, regardless of a relaxed cervix and significant follicle sizes – this can potentially be attributed towards her adjusting to being away from her home farm. For all other observations, she was behaviorally receptive, even though follicular size and cervical relaxation fluctuated. However, a trend was apparent with follicle size and cervical relaxation for the first half of the study. For observations 1, 3, 4, 7, 8, 9, 10, 11, and 12, there were individual follicles of 6mm or greater measured, and for observations 2 and 6, the combined follicle size was at least 6mm. Concurrently, for observations 1, 2, 3, 4, 6, and 7, the cervix was a grade 3 or 4, relaxed enough for penetration. For the second half of the study, on the other hand, there was a change in cervical relaxation for observations 8-12, where the cervix was only slightly relaxed or closed, which does not agree with the hypothesis that relaxation increases with follicle size.

An important observation made with female 3 was the lack of growth and regression periods with some follicle developments. For example, for observation 10, the right ovary had
only multiple small follicles. For observation 11, an 8mm follicle on the right ovary was
detected, which is a significant spike! And finally, on day 12, the right ovary only had multiple
small follicles again. This sudden appearance and then disappearance of a large follicle occurred
a total of four times throughout the four weeks of study.

There was no correlation in terms of behavioral receptivity and uterine horn echogenicity.
Because the female was behaviorally receptive for eleven observations, it would be expected that
the horns would be of low echogenicity. This was not the case – the horn was nearly always a
grade of 2 with a few variations.

**Female 4: Observed for four weeks**

**Behavioral Receptivity:** Grade one for observation 1

Grade two for observations 2, 7-12

Grade three for observations 3-6

**Cervical Relaxation:** Grade one for no observations

Grade two for observations 2, 7, 8

Grade three for observations 4-6, 9-11

Grade four for observation 3

**Uterine Echogenicity:** Grade one for observations 3, 9

Grade two for observations 2, 4, 5, 7, 8, 11, 12

Grade three for observation 1, 10
Ovarian Follicles:

**Figure 16: Ovarian follicular size chart, female 4**

Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

**Figure 17: Follicle diameter in comparison to behavioral receptivity, female 4**
Female 4’s behavioral receptivity appears to reflect subsequent follicle size fairly accurately, but due to some missing data due to either poor contact or a fidgety animal, it is hard to make definitive conclusions. Observations 1, 2, 3, 6, and 10 are missing either one ovary observation or both ovary observations, and this leaves gaps in the data too large to draw definitive conclusions from.
There was a correlation determined between cervical relaxation and behavioral receptivity, however. When the female was receptive, observations 3-6, the cervix was relaxed. When the female was not receptive, the cervix was either slightly relaxed or moderately relaxed. Although moderate relaxation is expected with receptive behavior, the female did allow the male to mount her during behavior testing, and being tolerant towards the male can be attributed to some estrogen production that can result in some cervical relaxation, even if it is not enough estrogen to result in kushing.

The inverse relationship observed during observations 1-3 between uterine horn echogenicity and behavioral receptivity matches the hypothesis exactly, but otherwise, the remaining results show no correlation - echogenicity was a grade 2 except for two observations.

Female 5: Observed for four weeks

Behavioral Receptivity: Grade one for observation 1
Grade two for observations 2-12
Grade three for no observations

Cervical Relaxation: Grade one for observations 1-3, 6, 8-12
Grade two for observations 4-7
Cervix was never observed as a grade three or four

Uterine Echogenicity: Grade one for no observations
Grade two for observations 1-3, 6, 7, 11, 12
Grade three for observations 4, 9, 10
Ovarian Follicles:

**Figure 20: Ovarian follicle chart, female 5**

Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

**Figure 21: Follicle diameter in comparison to behavioral receptivity, female 5**
Female 5 had only one follicle of 6mm or greater for one observation, was not receptive, had a nearly consistent uterine echogenicity of 2, and had a closed or slightly relaxed cervix the entire duration of the study. Although she was of breeding age, the fact that she is a maiden could have been the primary factor as to why she showed no interest in breeding behaviorally, especially if she has had no exposure to seeing the act of copulation amongst other alpacas.
previously. And because she did not display any significant follicular activity, the fact that she may be less mature than other females her age is something that should be taken into consideration. Regardless of the female’s history, her results match the hypothesis that behavior, follicle size, and cervical relaxation correlate – she was not receptive and consequently had no follicular growth or cervical relaxation.

**Female 6: Observed for four weeks**

Behavioral Receptivity: Grade one for observations 1, 8
Grade two for observations 2-7, 9-12
Grade three for no observations

Cervical Relaxation: Grade one for observations 1, 2, 6, 7, 9, 10
Grade two for observations 3-5, 8, 11
Grade three for observations 12
Grade four for no observations

Uterine Echogenicity: Grade one for observation 2
Grade two for observations 1, 3, 5-12
Grade three for observation 4
Ovarian Follicles:

**Figure 24: Ovarian follicular size chart, female 6**

Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

**Figure 25: Follicle diameter in comparison to behavioral receptivity, female 6**
Just like female 5, female 6 was a 2 year old maiden that was consecutively not receptive, had very little follicular activity with only two observations showing large follicles, had grade 2 echogenicity almost consistently, and had a closed or slightly relaxed cervix throughout the duration of the study. Again, based on her behavior and the lack of follicular activity, there is a chance that she is still immature and just delayed in terms of sexual development, even though
she is of breeding age. Again, female 6 supports the hypothesis because her lack of receptivity correlated with no significant follicle growth for all but two observations as well as a cervix that was not relaxed.

**Female 7: Observed for 12 weeks**

Behavioral Receptivity: Grade one for observations 25-34

Grade two for observations 1, 2, 4, 6, 8-12, 14,

Grade three for observations 3, 5, 7, 13, 15-24

Cervical Relaxation: Grade one for observations 1, 2, 9, 14, 22, 26-34

Grade two for observations 3, 4, 6, 8, 10-13, 15, 20, 23-25

Grade three for observations 5, 7, 16-19, 21

Grade four for no observations

Uterine Echogenicity: Grade one for observations 16

Grade two for observations 1-10, 12-15, 18-34

Grade three for observation 11, 17
Ovarian Follicles:

**Figure 28: Ovarian follicular size chart, female 7**

Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

**Figure 29: Follicle diameter in comparison to behavioral receptivity, female 7**
After collecting data that was scattered in all directions for females 1-6, it was decided to start studying specific females for longer periods of time in order to see if trends over a 12 week period could be established as opposed to a 4 week period.

Female 7’s follicular growth established a wave pattern. When viewing figure 29, the combined follicle diameter grows and regresses over a period of approximately every three observations, which would be a 7 day period. However, her behavioral receptivity does not seem to correlate with follicular activity towards the end of the study. For the first 9 observations, her behavior reflects follicular development accurately. However, her behavior plateaus at a grade 3
for observations 15-25, even though follicles continue to grow and regress. Her behavior then
takes a drastic change and plateaus at a grade 1 – but again, follicular activity still resumes.

Cervical relaxation correlates with behavioral receptivity with the exception of
observation 22. When female 7 shows behavioral receptivity, her cervix is a grade 2 or 3 – so
there is relaxation. When female 7 plateaued at the end of the observation at a grade 1 for
receptivity, her cervix was consistently closed.

Female 7’s uterine horn echogenicity was nearly always a grade 2, with only three
observations of the total 34 being a 1 or a 3. Therefore, Female 7’s echogenicity is not indicative
of her behavioral receptivity.

**Female 8: Observed for twelve weeks**

**Behavioral Receptivity:** Grade one for observations 23, 30, 31, 34

Grade two for observations 1-14, 16, 18, 22, 24, 28, 29

Grade three for observations 15, 17, 19-21, 25-27, 32, 33

**Cervical Relaxation:** Grade one for observations 10, 22, 23, 28, 30

Grade two for observations 1-9, 11-16, 18-20, 24, 26, 29, 31, 32, 34

Grade three for observations 17, 21, 25, 27, 33

Grade four for no observations

**Uterine Echogenicity:** Grade one for observation 34

Grade two for observations 3, 5-8, 10, 11, 13-33

Grade three for observation 1, 2, 9, 12
Ovarian Follicles:

**Figure 32: Ovarian follicle size chart, female 8**

Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

**Figure 33: Follicle diameter in comparison to behavioral receptivity, female 8**
Although female 8 has definable rises and falls in terms of follicular activity, it is much more sporadic in comparison to female 7 and in no definable pattern.

Female 8 was not receptive for the first 14 observations, and although there was follicular activity occurring, there were not any of significant sizes. At observation 15, she had a sudden
spike of follicle growth and this appears to have prompted receptive behavior. Also, from observations 23-34, behavioral receptivity reflects follicle size accurately.

In terms of cervical relaxation, female 8’s cervix remained as stagnant as her grade 2 receptive behavior for the first observations, observed as slightly relaxed with one instance of it being closed. When she started to show receptivity, cervical relaxation started showing activity with spikes of grade 3’s and grade 1’s that, except for a few exceptions, correlated with her behavior.

As seen with other females, no correlation between receptivity and uterine horn echogenicity was observed. Echogenicity was almost consistently a grade 2 with only four fluctuations that did not seem to correlate with any follicular activity or behavioral receptivity.

**Female 9: Observed for twelve weeks**

Behavioral Receptivity: Grade one for observations 19, 20, 23, 24,  
Grade two for observations 1, 2, 5, 6, 10-18, 21, 22, 25-30, 32, 33, 37  
Grade three for observations 3, 4, 7-9, 31, 34-36

Cervical Relaxation: Grade one for observations 6, 7, 9, 12, 16, 18-23  
Grade two for observations 2-5, 8, 10, 11, 13-15, 17, 24-28, 30-32, 34-37  
Grade three for observations 1, 29, 33  
Grade four for no observations
Uterine Echogenicity: Grade one for observations 7

Grade two for observations 1, 2, 5, 6, 10, 13, 14, 17, 18, 20-25, 31-36

Grade three for observations 3, 4, 8, 9, 11, 12, 15, 16, 19, 26-30, 37

Ovarian Follicles:

Figure 36: Ovarian follicular chart, female 9
Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

**Figure 37: Follicle diameter in comparison to behavioral receptivity, female 9**

**Figure 38: Cervical relaxation in comparison to behavioral receptivity, female 9**
Figure 39: Uterine echogenicity in comparison to behavioral receptivity, female 9

The most obvious difference in female 9 compared to all other females is the fact that she had individual follicles of significant size, 6mm or greater, for every single observation, even though her behavioral receptivity did not reflect that. The largest difference in terms of history between her and the other females used in this study was the number of crias she has had – she has had a lot of crias and she’s an older female. However, the hypothesis that the amount of offspring could correlate to follicle size and estrogen production would need to be tested further in order to have any validity.
Female 9 has no correlation between behavioral receptivity and follicle size. Except for observations 29 and 33, she should have been receptive for every observation, but she was not – she was primarily not receptive except for the very beginning and very end of the study.

There is correlation between cervical relaxation and behavioral receptivity. The cervical grade spiked from grade 2 to grade 3 at points of receptivity, and it stayed at grade 2 or moved to a grade 1 at times of non-receptivity.

There is no uterine echogenicity correlation. There should be an inverse relationship between uterine grade and receptivity grade, but Figure 39, which compares the two, does not show this.

Female 10: Observed for twelve weeks

Behavioral Receptivity: Grade one for observations 14, 15, 24, 31, 32, 35, 36
Grade two for observations 20, 21, 25
Grade three for observations 1-13, 16-19, 22, 23, 26-30, 33, 34, 37

Cervical Relaxation: Grade one for observation 31
Grade two for observations 9, 11, 13, 14, 16-26, 28, 30, 32, 35, 36
Grade three for observations 1-6, 8, 10, 12, 15, 27, 29, 33, 34, 37
Grade four for no observations

Uterine Echogenicity: Grade one for observations 4, 7, 17, 18, 22, 31, 33, 35
Grade two for observations 1-3, 5, 6, 9-16, 21, 23-28, 30, 32, 34, 36, 37
Grade three for observations 8, 19, 20, 29, 31
Ovarian Follicles:

Figure 40: Ovarian follicular growth chart, female 10

Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

Figure 41: Follicle diameter in comparison to behavioral receptivity, female 10
Figure 42: Cervical relaxation in comparison to behavioral receptivity, female 10

Figure 43: Uterine echogenicity in comparison to behavioral receptivity, female 10
Female 10, like female 9, had large individual follicles large enough to expect receptivity for all but three observations, and like female 9, she is older with a history of having many crias. However, unlike female 9, she was almost always receptive for her observations, which does correlate to her large follicles present. She had five spikes of non-receptivity, and three spikes correlated to the three observations of no significant follicle growth – this matches our hypothesis, but the two spikes of non-receptivity that do not correlate with decreased follicles size does not agree with our hypothesis.

Female 10 was nearly always receptive, and her cervix was never once observed as closed – it showed grade of slight and moderate relaxation, which correlates with her receptivity. However, there was one observation where she tested not receptive behaviorally but still had a cervical grade of 3 – but because she had such short periods of being not-receptive, but follicles were still very large and therefore producing estrogen that is expected to relax the cervix.

Allowing for a few exceptions, female 10 showed an inverse relationship between behavioral receptivity and uterine echogenicity. She had a lot more echogenicity variation than other females in this study did, and she showed multiple clear spikes where she would either be receptive and have a uterine grade of 1 or 2 or be non-receptive and have a uterine grade of 3 or 2.

**Female 11: Observed for eight weeks before pregnancy study**

Behavioral Receptivity: Grade one for observations 9

Grade two for observations 10, 13-16

Grade three for observations 1-8, 11, 12, 17-25
Cervical Relaxation: Grade one for observations 13-16
Grade two for observations 5-7, 10, 18
Grade three for observations 1, 3, 4, 8, 9,
Grade four for observations 2, 11, 12, 19-25

Uterine Echogenicity: Grade one for observations 13, 14
Grade two for observations 1-12, 15-25
Grade three for no observations

*Note: Female 11 was confirmed pregnant from observations 13-16. She was given an injection of prostaglandin after observation 16 and was confirmed open on observation 17. Therefore, even though these observations are included in the graphs, they are not taken into consideration into the discussion because the pregnancy dictated behavior, echogenicity, cervical relaxation, and follicular development.

Ovarian Follicles:

Figure 44: Ovarian follicular size chart, female 11
Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

**Figure 45:** Follicle diameter in comparison to behavioral receptivity, female 11

**Figure 46:** Cervical relaxation in comparison to behavioral receptivity, female 11
Female 11 did not show any correlation between follicular activity and behavioral receptivity because, except for observation 9 and when she was pregnant, female 11 was always receptive. Follicle growth was sporadic and not in any wave-like pattern – however, the graph does show that, for this animal, dominant follicle growth alternated between ovaries – so if a follicle grew and regressed on the left ovary, the next follicle to grow to a significant size would be on the right ovary.

Female 11’s cervix was nearly always moderately or fully relaxed, which correlates to her grade three receptivity. There were only three observations where the cervix was a grade 2, and it was never observed as being closed except when she was confirmed pregnant.

Uterine echogenicity and behavioral receptivity both remained consistent, with the uterus being a grade 2 for the duration of the study (except the pregnancy) and the female being receptive. However, it would be expected that the uterus would be a grade 1, fluid filled, for a receptive animal, so these results do not match the hypothesis.

**Figure 47: Uterine echogenicity in comparison to receptivity, female 11**
Female 12: Observed for twelve weeks

Behavioral Receptivity: Grade one for observations 8, 11, 20-22,

Grade two for observations 10, 12-14, 17-19, 23-25, 31

Grade three for observations 1-7, 9, 15, 16, 26-30, 32-34

Cervical Relaxation: Grade one for observations 6, 10, 13, 20

Grade two for observations 7, 11, 12, 18, 21-24,

Grade three for observations 2, 3, 5, 8, 9, 14, 17, 25, 27, 29

Grade four for observations 1, 4, 15, 16, 19, 26, 28, 30-34

Uterine Echogenicity: Grade one for observation 11

Grade two for observations 1-10, 12-34

Grade three for no observations

Ovarian Follicles:

Figure 48: Ovarian follicular growth, female 12
Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

**Figure 49**: Follicle diameter in comparison to behavioral receptivity, female 12

**Figure 50**: Cervical relaxation in comparison to behavioral receptivity, female 12

**Figure 51**: Uterine echogenicity in comparison to behavioral receptivity, female 13
Female 12 had a much different follicular growth pattern than the other females studied. Except for observations 12 and 26, the left ovary was pretty stagnant throughout the entire study. On the other hand, a dominant follicle would grow on the right ovary, grow larger than the standard dominant follicle (the largest measured was 17mm), and sit for a few observations before regressing slightly then growing large again. Female 12’s behavior was also strange – at the very beginning of the study, when there were no significant follicles, she tested receptive. From then on, when follicles were growing, she would be receptive – but when the follicle would reach its peak, she would test non-receptive until the next growth period.

There were two exceptions, but otherwise, female 12’s cervix was moderately or fully relaxed when she was receptive and closed or slightly relaxed when she was not, which is what we hypothesized.

Female 12’s uterine echogenicity, like other females, was always a grade 2 except for one observation. Because her behavior fluctuated between receptive and not receptive throughout the study, there seems to be no correlation between the two.

A point of interest about female 12 is that a large follicle was present a longer than normal amount of time (nearly 15 observations!) on her right ovary. She was not always receptive when the follicle was present, so that could mean that there is a limit as to how large the follicle can be in order to omit the estrogen required for the animal to be receptive. Also, there is the possibility that a present follicle can stop being effective at producing estrogen after ‘x’ amount of time – so if female 12 had been bred, would the large and long-term follicle result in pregnancy?
Female 13: Observed for twelve weeks

Behavioral Receptivity: Grade one for observations 1-32

Grade two for no observations

Grade three for no observations

Cervical Relaxation: Grade one for observations 2, 15, 17, 19-22, 33

Grade two for observations 1, 3-5, 7-12, 14, 16, 18, 23-32

Grade three for observations 6, 13

Grade four for no observations

Uterine Echogenicity: Grade one for observations 1, 5, 8, 9, 11, 13-20, 22-24,

Grade two for observations 2-4, 10, 12, 27, 29,

Grade three for observations 6, 7, 21, 25, 26, 30-33

Ovarian Follicles:
Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

Figure 52: Ovarian follicular size chart, female 13

Figure 53: Follicle diameter in comparison to behavioral receptivity, female 13
Female 13 never showed receptivity throughout the semester, regardless of follicle size or cervical relaxation, so therefore, no correlation existed whatsoever. Her complete lack of interest towards any males was perplexing, and considering she has been bred successfully before, her behavior was most likely indicative of a reproductive issue. Multiple techniques were tried to bring her into receptivity after confirming that she was in good health and not pregnant – she was given a prostaglandin injection in the event that a spontaneous ovulation resulted in a retained corpus luteum, she was moved to a different pasture with a different set of females, and she was behavior tested with a multitude of different males. However, she did not respond to any attempts made to help her become receptive for the twelve week duration of the study.

**Female 14: Observed for eight weeks, replaced unusable female in the study**

Behavioral Receptivity: Grade one for observations 22, 23

Grade two for observations 20, 21
Grade three for observations 1-19

Cervical Relaxation: Grade one for no observations

Grade two for observations 1, 3, 4, 23

Grade three for observations 5, 7, 8, 13, 21, 22

Grade four for observations 2, 6, 9-12, 14-16, 18-20

Uterine Echogenicity: Grade one for observations 3, 4, 6, 9, 13, 16

Grade two for observations 1, 2, 5, 7, 8, 10-12, 14, 15, 17-22

Grade three for observation 23

Ovarian Follicles:

Figure 56: Ovarian follicular chart, female 14
Comparisons between behavioral receptivity, cervical relaxation, uterine horn echogenicity, and follicle size:

**Figure 57: Follicle diameter in comparison to behavioral receptivity, female 14**

**Figure 58: Cervical relaxation in comparison to behavioral receptivity, female 14**
Even though female 14 was a two year old maiden in this study, she differed greatly from the other two maidens looked at previously. Female 14, based on the data collected, was sexually mature and ready to be bred. She was a grade 3 in terms of receptivity for almost every observation, but she also showed continuous significant follicular activity that warrants receptivity. On observation 20, follicle size plummeted significantly to the lowest it had been throughout the entire study, and this potentially attributed to the sudden lack of receptivity in female 14 from observation 20 through 23, even though follicle size started increasing again after observation 20. Therefore, female 14’s behavior correlates with follicular activity.

Except for a strange grade 1 on observation 17, cervical relaxation correlates with behavioral receptivity. Female 14 was consistently receptive, and her cervix always showed some degree of relaxation. When she began showing non-receptive behavior, her open cervix moved from a grade 4 to a grade 2, as to be expected.
With a steady receptivity grade of 3, it would be expected that female 14 would have steady uterine echogenicity of grade 1 or 2. This was not the case - observations alternated between grades 1 and 2. Therefore, there was no correlation found between female 14’s behavior and her uterine echogenicity.

**Conclusion**

This research project resulted in a lot of data that pointed in a lot of different directions, and even though there is no concrete conclusion made yet as to when the optimal fertility and breeding time is for the female camelid, a lot was learned during the course of this study that allows the project to keep evolving in a way that will lead to results.

**Variability**

To begin, there were many different variables that would surface during the course of the observations that were not thought of, and this was what caused the research project to branch off in so many directions. A significant variable was the age and sexual experience of the females being studied. For example, females 9 and 10 were the oldest and had been bred the most compared to the other females studied, and they had follicular growth that was much larger and much more consistent than any of the other females. This could be due to age and amount of offspring, but it could potentially be due to other factors that researchers weren’t aware of.

There were many possible influences that could have affected behavioral receptivity, and these need to be taken into consideration as well. From the ground being too muddy to the weather being uncomfortable, there are many elements out of the researcher’s control that can change how a female will react to a male on a given day. Another factor is fellow pasture-mates
the female is with – dominant non-receptive female pasture-mates can get in the way of a male attempting to breed via spitting and kicking at the male, and receptive female pasture-mates kushing next to the male while he is trying to breed another could potentially affect the subject female’s resulting behavior. However, separating the subject female from the herd could result in stress that could have just as big of an effect on behavioral receptivity.

Finally, the females used in this study came from multiple farms with different management practices, and this is likely to affect results. Even though females were all housed and fed the same way during their time at the Hadley Farm, different nutrition from their home farms as well as exposure to different things prior to beginning research are likely to have an effect on the animal. For example, female 14, a maiden, is a University of Massachusetts animal, so her history from birth is well known. She was frequently exposed to behavior testing and breeding from a young age, and consequently, when she became of age to be studied reproductively, she knew how to behave for a male. On the other hand, the two other maidens used in this study had most likely not been exposed to a lot of breeding because they were so intimidated by a male presence when behavior testing first began. Fortunately, they made progress as the study progressed, even though they never displayed grade 3 receptivity.

An interesting concept for further study would be to see if the manipulation of these variables could thus manipulate behavior and/or follicular development. There are a lot of different studies that can arise from this, from feeding different diets to housing animals in different ways – close to males, far away from males, pasture versus barn, amount of animals in a group, etc. However, a study that could better female reproductive knowledge is studying females from birth until reproductive age. Is it truly an advantage to be exposed to males breeding other females from a young age, and does female pasture-mate behavior affect the
behavior of the developing cria? It can be hypothesized, based on the very small sample size studied for this research project, that female crias not exposed to males prior to breeding age will be later in terms of maturity and behavioral receptivity, and females in the presence of other receptive females will be more likely to be receptive than females in the presence of non-receptive females. Furthermore, does the mother’s behavior affect that of her cria? And finally, does all this attempted manipulation of behavior manipulate follicular growth as well? This study, although an extensive one with the potential to branch in a lot of directions, could help alpaca breeders better determine how to create the best possible environment for female crias that would hopefully maximize behavioral receptivity and thus pregnancies as well.

Correlations

No definitive follicular wave pattern was found during this study, unlike previous research done by Bravo and Sumar. Because so many different variables can affect reproductive tendencies in the female, it is unlikely that a concrete pattern of follicular growth and regression exists. All females are individuals, and this needs to be taken into account when looking for a general theme amongst them.

A correlation that did exist amongst nearly all the females was that cervical relaxation increased with follicle size. This makes sense because follicles produce the estrogen needed that essentially cues the cervix to relax and the female to be receptive, but there were exceptions to this found during the study.

There was no correlation found for uterine echogenicity, but the results collected here can be attributed to individual researcher interpretation. Distinguishing between shades of gray can
be difficult, especially when the uterus isn’t one consistent shade in the first place, and different researchers can interpret a color in different ways.

Follicular growth patterns can vary so significantly that a particular growth cycle would beg the question as to whether it would be enough for a female to get pregnant. For example, female 12 had a persistent follicle on one ovary that was larger than normal and was present for weeks — would something like this be a hindrance to her getting pregnant? What about female 1’s two 4mm follicles? Are those follicles large enough to result in pregnancy, and would twins result? The constant question from female to female helped develop a new project for further study, which is currently going on at the University of Massachusetts. Females are behavior tested, and if they are receptive, they are ultrasounded for follicular developments. If they have a follicle of 6mm or greater present on an ovary, they are bred and then ultrasounded to confirm the pregnancy. The pregnancy is then followed for ‘x’ amount of days to mark important developmental milestones such as sac development, conceptus development, and implantation of the embryo. Not only will this study help researchers determine what can possibly prevent a female from getting pregnant, it will also help develop a concrete timeline of camelid embryonic development.

Finally, for other further studies, measuring estrogen levels in females would be extremely beneficial in order to determine peak times of fertility. Measuring estrogen would also help determine whether multiple large follicles play the same role as one dominant follicle — do estrogen levels increase based on multiple follicle amounts and sizes? How do estrogen levels vary amongst different sizes of follicles? These questions are extremely relevant to determining optimal times to breed, and measuring estrogen levels alongside ultrasounding would be the next progressive step of this study in order to answer these questions.
Sources


