U.S. Geological Survey
Fort Collins Science Center
2150 Centre Avenue, Building C
Fort Collins, Colorado 80526-8118

November 24, 2015

Dean Bolstad
Acting Division Chief,
Wild Horse and Burro Program
Bureau of Land Management, WO-260
20 M Street,
Washington, DC 20003

Dear Mr. Bolstad,

Attached please find a summary table and notes resulting from expert panel discussions on September 24, 2015, exploring several alternative methods for wild horse spaying. In addition to veterinary and equine experts, several USGS, BLM, USDA-APHIS, and Colorado State University staff also observed and contributed to discussions.

The materials reflect professional opinions about the current state of understanding of four spay methods currently used on domestic horses, as represented and discussed by panel members during and after the day-long meeting. These materials do not provide BLM with a recommendation, but hopefully provide useful information for BLM to consider.

Sincerely,

Zack Bowen
Branch Chief, Ecosystem Dynamics

Attachments.

Sarah R.B. King, Ph.D.
Research Scientist
Coordinator of the Equid Red List Authority, IUCN

Department of Ecosystem Science & Sustainability
A242 NESB - Campus Delivery 1476
Colorado State University, Fort Collins, CO 80523
Phone: (720) 587-9890; Fax: (970) 491-1965
Email: sarah.king@colostate.edu

November 24, 2014

Dean Bolstad
Acting Division Chief
Wild Horse and Burro Program
Bureau of Land Management, WO-260
20 M Street
Washington, DC 20003

Dear Mr. Bolstad,

Attached please find a summary table and notes resulting from expert panel discussions on September 24, 2015, exploring several alternative methods for wild horse spaying. In addition to veterinary and academic equine experts, several USGS, BLM, USDA-APHIS, and Colorado State University staff also observed and contributed to discussions.
These materials do not provide BLM with recommendations, but provide information for BLM to consider. The materials reflect the professional opinions on the current state of understanding about the pros and cons of four spay methods currently used on domestic horses, as represented and discussed by panel members during and after the day-long meeting.

Sincerely,

Sarah R. B. King
Research Scientist, CSU

Attachments.

Assessment of spay techniques for mares in field conditions

Panel meeting held at USGS Fort Collins Science Center

September 24, 2015

Summary of panel expert responses on four potential spay methods

	Colpotomy	**Ventral midline**	**Flank incision**	**Flank laparoscopy**
Facilities needed	Squeeze chute with a kick panel and access to the perineum.	Squeeze chute, table fitted on a forklift.	Squeeze chute (may require access to both sides).	Squeeze chute (may require access to both sides), sling.
Equipment needed	Ecraseur, scalpel or bistury, blunt scissors, gauze sponges tied with umbilical tape.	Complete surgical pack, surgical drapes, gauze sponges.	Complete surgical pack, surgical drapes, gauze sponges.	Laparoscope, CO_2 for insufflation, surgical pack, surgical drapes, gauze sponges.
Equipment preparation	Ecraseur autoclaved or cold-sterilized in ortho-phthalaldehyde (OPA/28) >10 minutes then rinsed in sterile water, or use chlorhexidine for sterilization.	Autoclave or cold-sterilize instruments.	Autoclave or cold-sterilize instruments.	Prepare laparoscopic equipment – cleaned and cold-sterilized.
Sedation	IV injection to the jugular of xylazine + butorphanol + detomadine.	Horses are placed in dorsal recumbency following an induction dose of xylazine/ butophenol/diazepam and ketamine.	Local lidocaine infiltration of flank and IV butorphanol.	IV jugular catheter continuous sedation drip - 20 mg detomidine in 1 liter fluid for standing sedation.
Anesthetic /analgesic protocol	Horses remain standing under tranquilization: butorphanol + xylazine or detomidine . Add low dose ketamine as needed.	Anesthesia may be maintained by IV administration of triple drip (IV-ketamine, xylazine and guaifenesin 5%) or using inhalant anesthesia.	Horses remain standing under tranquilization: butorphanol + xylazine or detomidine. Injection of lidocaine in line or L-block (~100-200 ml) at incision site.	Horses remain standing under tranquilization: butorphanol + xylazine or detomidine. Epidural for analgesia of the reproductive tract, local anesthesia at portal sites.
Procedure	Administer antibiotic (Excede - ceftiofur) that lasts for 4	Administer antibiotic (Excede - ceftiofur) that lasts for 4	Administer antibiotic (Excede - ceftiofur) that lasts for 4	Administer antibiotic (Excede - ceftiofur) that lasts for 4

	Colpotomy	Ventral midline	Flank incision	Flank laparoscopy
	days. Wrap tail and tie up. Evacuate rectum/bowel, prep perineum. Make 1 cm incision in vaginal fornix. Expand incision via blunt dissection. Locate ovaries. Sterile 4x4 gauze soaked with 30 ml 2% lidocaine compressed over ovarian pedicle 3-5 minutes or lidocaine injected into pedicle. Remove ovaries via ecraseur. Repeat for other ovary through same incision.	days (or given 40 ml procaine penicillin and 10 ml flunixin meglumine post surgery). Surgical area is clipped and prepped with chlorhexidine scrub followed by chlorhexidine solution swabs. Incision into peritoneum made on ventral midline. Ovaries exteriorized through ventral midline incision. An emasculator is applied to the ovarian pedicle. #6 MSA used to ligate the ovarian stump proximal to the emasculator. The emasculator is removed and the ligated stump allowed to retract into the abdomen. Closure is accomplished in three layers, the outermost being a subcuticular layer using #6 MSA absorbable.	days. Surgical area is clipped and prepped with chlorhexidine scrub followed by chlorhexidine solution swabs. Line or L-block injections are administered. Wait until block has effect, then single incision in left flank through skin and fascia followed by blunt dissection into the peritoneum. 9-inch burdizzo (or emasculator) for removal of ovaries. Closure of the underlying layers of muscle and fascia such that only the skin requires suturing. Very bottom of the skin suture line is left open to prevent seroma formation.	days. Approach both flanks: surgical area is clipped and prepped with chlorhexidine scrub followed by chlorhexidine solution swabs. Make incision at flank. Insert cannula for instruments. Insufflate with CO_2. Lidocaine injected to ovary and pedicle. Ovary is removed through incision; incision may have to be enlarged to remove ovary. Suture incision. Repeat on other side.
Incision	5 cm in anterior vagina.	9-15 cm incision made just cranial to the udder.	10-15 cm in flank (on one or both sides).	One 1 cm incision and one 6-10 cm incision in the left flank, and three 1 cm incisions on the right side.
Standing or recumbent surgery?	Standing	Recumbent	Standing	Standing
Surgery time per	15-20 minutes	20-30 minutes	45 minutes	40-60 minutes

	Colpotomy	Ventral midline	Flank incision	Flank laparoscopy
horse				
Complications	1-2% seen within 2 days.	Infection of open wound; potential for evisceration; potential for injury upon recovering from anesthesia.	5% incisional complications under sterile conditions (more likely 10-20% under field conditions).	1-2% incisional complications (in sterile environment), 10% subcutaneous edema; puncturing a bowel; dropping ovaries in abdomen.
Recovery time (before release)	3 days	2-3 weeks	2-4 weeks	1-2 weeks
Contra-indications	Uterine infection/pyometra. Enlarged (>6 cm) ovary; pelvic or ovarian abnormalities. Heavy late gestation may prevent access to ovaries. Surgery may not be possible if the mare cannot be sufficiently sedated.	Very dirty animal; old/multiparous; any animal that is contraindicated for general anesthesia. Contraindicated in later term gestation due to risk of initiating labor and abdominal wall rupture during parturition. Pregnant mares in surgery have a 3x greater risk of pregnancy loss with general anesthesia.	Very dirty animal; any animal that is contraindicated for general anesthesia. Pregnant mares in surgery have a 3x greater risk of pregnancy loss with general anesthesia.	Dependent on technology; abnormal ovary.
Effect on pregnant/lactating mares	Late gestation may challenge access to ovaries. Pregnancy no issue following first ±70 days. No foal abandonment issues. No issues with lactation.	Late gestation may be challenging. May affect nursing (pain when the foal tries to nurse).	Unknown. May affect nursing (pain when the foal tries to nurse).	Unknown, but likely easily done.
Effect of breeding post-surgery	Vaginal incision usually healed by 7-10 days post-	Breeding may cause injury prior to recovery.	Breeding may cause injury to the incision line.	Breeding may cause injury to the incision line.

	Colpotomy	Ventral midline	Flank incision	Flank laparoscopy
	surgery. If open or <100 days pregnant administer long acting progesterone which should suppress receptivity/save pregnancy.			
Operator safety	Strapping the back of the mare helps prevent kicking, kickboard and tail tied dorsally aids operator safety. Short time with scalpel.	Animal fully anesthetized.	Operator protected due to small window of access on side of animal. Longer time with scalpel.	Operator protected when coming from the flank, but injuries can occur due to equipment and two people in a restricted space.
Cost per horse	$250-$300[1] Long-acting progesterone ($7/mare)	$350[2] includes all drugs and supplies. Plus long-acting progesterone ($7/mare).	$350[2] Includes all drugs and supplies. Plus long-acting progesterone ($7/mare).	$450-$500[3] Long-acting progesterone ($7/mare).
Pro of method	Fast healing and recovery, quick surgery, can be done on pregnant mares.	Low risk to operator, common surgery for companion animals.	Low risk to operator, common surgery.	Direct visualization, low morbidity, good public opinion.
Con of method[4]	Higher risk to operator, need for trained surgeons.	Risk of evisceration, risk of incision infection.	Risk of incision infection and pain.	Most expensive and time-consuming approach.

Notes:

[1] Colpotomy cost per mare: $100-1500 initial equipment cost (chain ecraseur Jorgensen J-37E $450 buy 2 or 3, replacement chain MidWest 350.01254.2 J37ED1 $95). Then $80-100/mare: OPA/28 4 gal $99.56 (estimate 50-80 mares: $2/mare), suture 2-0 monocryl 36/box $205 ($6/mare), #10 or #21 scalpel blade 100/$25.52 ($3/mare), Lidocaine 100ml $8 ($4/mare), Xylazine $1/100 mg ($4/mare), Butorphanol 10mg/ml 50 ml $250 ($5/mare), Detomadine $16/mare, Ceftiofur - excede 15 ml $30/mare. Once the ecraseur and scalpels have been purchased, the expense via colpotomy for each mare is the drugs, the lidocaine and gauze for the pedicles, and the sterilizing of the ecraseur.

[2] Ventral midline and flank incision – there will be a cost of drapes, gowns, sutures, etc., as for all abdominal surgeries, and a cost for the surgical equipment. Drug and other equipment cost will be as in [1].

[3] Laparoscopy equipment costs: $25,000 for camera, light core, light source, monitor, Thorston telepack, $5,000 – insufflator, $3,000 – microscope, $750 x 6 for hand instruments (need two sets): $40,000 total new, or could buy used. Likely need to replace 5 hand instruments per 100 mares. Drug and other equipment cost will be as in [1].

[4] It should be noted that all surgeries are associated with a risk of death. There are no published data available to assess the mortality risk of any spay surgery in wild horses, although preliminary data on domestic and wild equids were discussed by the panel.

Assessment of spay techniques for mares in field conditions

Panel meeting held at USGS Fort Collins Science Center
September 24, 2015

Transcript of Comments

Questions or topics are in **bold**.
The speaker is in *italics*. If the person introducing the topic made the comment it is indented with a bullet. If the speaker is not known the comment is indented with a hyphen. All attendees and invitees have been given the opportunity to edit this document. As these are notes taken from a discussion, what is written here may not capture the exact intended meaning of a given statement.

Attendees

In person: Zach Bowen (USGS), Jason Bruemmer (CSU), Doug Eckery (USDA/APHIS), Paul Griffin (BLM), Al Kane (USDA/APHIS), Sarah King (CSU), Joanna Ruffino (USGS), Kate Schoenecker (USGS).
By WebEx/Phone: Cheryl Asa (St. Louis Zoo), Gail Collins (NPS), Robert Cope, Jay D'Ewart (BLM), Bryan Fuell (BLM), Dean Hendrickson (CSU), Katrin Hinrichs (Texas A&M), Sue McDonnell (U. Penn.), Leon Pielstick (DVM), Patricia Sertich (U. Penn.), Mark Stetter (CSU), Regina Turner (U. Penn.), Julie Weikel (DVM).
Information provided after the panel: Paul Zancanella (DVM)

Introduction

Paul Griffin

- Purpose of the meeting is to discuss different procedures for spaying wild horse mares.
- We will discuss pros and cons of the various methods that could potentially be used for spaying wild horse mares.
- This is not a definitive decision making meeting for BLM, but to get the opinions of experts.

Background of study to be conducted by USGS/CSU

Sarah King

- Aim of the study is to look at the short-term impact of spaying on health and behavior of individual mares, specifically any effects on band fidelity, spatial ecology and population demography.
- Location: White Mountain HMA, Wyoming.
- We have proposed to spay 60% of adult mares (adult mares are 3 years old and older), which will probably be 36-48 mares, depending on the age structure, leaving 24-32 untreated controls plus juveniles and foals.

- We will collect 1 year of pre-treatment data, and then 3 years of post-treatment data.

Facilities at Rock Springs BLM Adoption Facility

Al Kane:

- It's a typical BLM facility.
- Two hydraulic squeeze chutes. Most facilities do not have a split tailgate. At Rock Springs, the door would need to be re-fitted to have a split tailgate that would allow access to perform a colpotomy. It's an open question about whether BLM would have the funding to get a new tailgate (that is split).
- Squeeze chutes give access to the left side and hindquarters of the animal. They are - padded and compress the animal front to back and side to side.
- For recumbent surgery the mare can be rolled out of the chute onto the ground; no access to a table.
- No hospital and no indoor facility.
- Transportation to and from the HMA: gooseneck, stock type trailer, or semi-trailers.

Zack Bowen:

- We will compile notes and comments and put them in a briefing paper. This will not be published. It will be a statement to BLM compiling information, not making a recommendation.
- Information will be compiled based on considerations that will be asked of each technique.

Kane: Today we will discuss some considerations for what technique may be most appropriate for this study, but another technique may be more appropriate in the future for spaying on a wider scale across HMAs. But it may be the same.

King: The method chosen by the BLM for this study should be the same as what is used in the future elsewhere, as this will have been the method we gathered data on.

Discussion of Colpotomy - *Leon Pielstick*

History:

- Leon is a veterinarian who has worked with the BLM since 1975, and has also been involved with the management of horses at Sheldon-Hart. At Sheldon he spayed horses which had been placed on a private pasture for the trial.
- Spayed mares in the field successfully: Out of 34 mares spayed, 31 were open, 3 were pregnant and successfully foaled. At Sheldon they used spaying as a management tool – they vasectomized males and spayed females that were considered unadoptable, then turned them back out to the range. The majority of such spayed mares were pregnant.
- He has spayed 188 mares by colpotomy, including 16 spayed at a wild horse sanctuary in California, 16 spayed as part of a PZP safety study in Oregon.

- Out of the 188 mares there were 2 fatalities: one bled to death internally due to a clotting abnormality, and one got sick, aborted her foal and died (anecdotal evidence indicated that she had a peritoneal infection). We can expect a 1-2% complication rate with colpotomy. For any given choice of spay method, the BLM must be prepared to accept some level of loss.
- 70-75% of mares are likely to be pregnant in late summer. Pregnant mares can still be sterilized by colpotomy because of the way the foal drops in the uterus: the ovaries are still at the top of the uterus so can be reached. At 7-8 months pregnant it gets harder to move the intestines to reach the ovaries, so it is more difficult to keep the intestine out of the ecraseur tool. The only mare that had an abortion was the mare that died.
- To do the surgery, give heavy sedation/analgesia (butorphenol + xylazine or detomidine + Dormosedan) and heavy analgesics (banamine + butorphenol); the surgery is performed with the mares standing. Banamine was added to eliminate mild post surgical colic which had occurred in a few of the mares the first year in which the procedures were done.
- Mares held off feed for 24 hours before surgery seemed to have good recovery after. Holding off feed means that fecal balls are reduced, which can resemble ovaries on palpation and thus take time to sort out, and reduces abdominal fill. Depending on the horse there was a little colic within the first few hours post surgery until Banamine was added to the procedure. Mares generally walked out of the chute and started to eat, some would raise their tail and act as if they were defecating, however in most you could not notice signs of discomfort.
- There was no squeeze chute at Sheldon, but the mares could be held at the back of the chute where there was an access window. Some mares needed additional sedation as they could not be squeezed. It should be possible to make any facility functional for this surgery.

Facilities:

- Needs a kick panel and an access window, so that there is access to the perineal area. Use a strap above the rump, to help prevent jumping and kicking. Most facilities can be made functional. At the BLM Burns facility, for example, there is enough room to slide in a 3 foot tall plywood kick panel.

Behavior:

- In the first group of 33 spayed mares with 10 intact mares and 2 stallions (on private land) – the group all stayed together as a herd.
- In another situation two groups of 8 spayed mares formed their own bachelorette band. They were new to the facility.
- In Sheldon it is assumed that they returned to their band.

Recovery time:

- The only complications were seen within two days of surgery. After 2 days there were no visual problems so they could be released to the range.

- It is typically recommended in domestic mares that they not be ridden for a month, but they seem okay after 2 days.
- The incision in the anterior vagina is not sutured; this heals rapidly (within days, cannot be identified easily on speculum examination). Evisceration through the vaginal incision is often brought up as a possible complication of colpotomy, but none of the panel participants had had this occur nor had heard of it actually occurring. In addition, being held off feed would reduce the chance of evisceration.
- Being held off of feed before-hand is important according to Pielstick, although others said it was less necessary.

Gail Collins - At Sheldon they had the opportunity to recapture spayed mares that were released (3yr, 5yr, 6yr later) to monitor progress.

- They were given a dose of antibiotic (Excede -- ceftiofur) that lasts for 4 days within the mare (single injection).

Procedure:

- Cold sterilize ecraseur. Give antibiotic.
- Wrap tail and tie up for procedure.
- Evacuate bowel, surgically scrub peritoneum and flush out vagina. Clean vagina with iodine. Others perform procedure without vaginal flush (vagina should be essentially sterile).
- Put on sterile sleeves, introduced hand into vagina, and make the incision in the anterior vagina.
- Mares have no nerve receptors in that area, so they only feel pressure/stretching. They show no outward sign of discomfort. Mares feel the pull on and compression of the ovarian pedicle, but lidocaine administered to the pedicle to minimizes this. This analgesic lasts a couple / few hours. The pain afterwards is similar to that of castration.
- Timeline: 15 minutes for the whole procedure. Speed is often necessary due to volume of horses. Can do 30-35 horses a day as it is not physically taxing.
- Controlling the level of dust is very important, but otherwise it is possible to keep the area 'field sterile'.
- Can make a portable chute for this procedure out of a hydraulic cattle chute; this would have an adequate tail gate.
- Mares walk out of the surgery.

Comments:

Julie Weikel served as an observer for Leon's procedure at Sheldon; wrote a review:

- Some mares would walk out from surgery and immediately want to eat (hunger pain worse than spay pain due to keeping them off food).
- Some (a few individuals) showed minor signs of colic for about a half hour, such as getting up and down repeatedly. These were collected so that they could be watched more closely for up to an hour, then turned out to join a bigger group with feed and

water. For these horses they did not use lidocaine, which might alleviate the colic symptoms. Leon now uses liodocaine in every spay.

Katrin Hinrichs: **Did you give any antibiotics before the procedure?**

- Antibiotics are given after sedation, so not as long before surgery as ideal. There isn't a large opportunity to give it long before surgery due to the circumstances of these being wild horses.

How is the Lidocaine injected in to the pedicle?

- Via needle, an assistant pumps the drug, but the surgeon guides the needle.
- Dr. Pielstick is trying to modify the ecraseur to also hold a needle, thereby reducing the overall risk of contamination (and needle stick); having the needle on the ecraseur would mean one less trip in and out of the abdomen.

Are the mares in pain after the drugs wear off? Are they observed at night?

Hinrichs: has seen mares in pain at night after the surgery; she now gives butorphanol for 24 h after surgery, or morphine + detomidine epidural at the time of surgery.

Pielstick: They are observed for several hours after. They seem fairly comfortable. Pain is at an acceptable level; the banamine helps.

- Domestic mares are given banamine and sent home.
- They seem more comfortable than castrated stallions.

Weikel: I walked pens every morning and evening at the gather observing behavior. Mares were mostly involved in social status behaviors.

Pielstick: Finished surgery at 4pm on a good day, so not a lot of light for observations after surgeries.

Regina Turner: **What is the effect of operator experience?**

- This is a practical technique. Leon taught 9 vets in Arizona when spaying 5 donkeys. Any vet who generally works with equine reproduction can pick it up, however there is a learning curve. On one donkey they had trouble getting the left ovary out, after they finally succeeded the donkey bled to death. They did a flank incision on two donkeys and this seemed better on that species.

Are there enough trained people?

- Plenty of vets would be interested in learning the technique. If there is a complication it can be used as a learning experience to avoid future complications. There were complications with the donkeys as multiple people were learning how to do it. The chance of complications increases with the number of times you go in and out of the animal.

Cheryl Asa: **Could we have more follow up on the AZ procedures?**

- Concerns about the Arizona project include the fact that one died, one lost a 50-70 day old fetus, and others had post-operative infections.

Pielstick: The burro that died had a left ovary with a membrane around it; there was no post mortem exam to discern exact cause of death. If a female has an unusual ovary it would be best to abort surgery and not proceed.

It seems as though the training contributed significantly to the mortality there?

- If there are unusual situations then generally complications arise, and this is used as a learning curve to create a better procedure.
- It is also worth noting that the number of learning surgeons entering the body cavity of those burros could have contributed to the one in five mortality rate for those burros. Dr. Asa noted that one of the major complications could have been the training itself.

Kane: **What are the contra-indications of this technique? Does the condition of the horse affect the procedure?**

- Generally body condition will affect the choice made to do the procedure. Best to pass on very old mares or mares in poor condition.
- Late gestation will increase the complication rate, but pregnancy will not be an issue in the first or second trimester.
- Surgeons should also pass on mares with pelvic abnormalities.
- 1-2% of mares could not be sedated heavily enough to do the procedure. 1cc of ketamine would help. If a mare fails to sedate the issue is that she moves, so the procedure is not done.

Of the big group of mares that you spayed, what percentage were pregnant? Which were checked before-hand?

- Of the first 33 mares that had the procedure, 3 were pregnant (about 60-70 days). All 3 foaled.
- The 16 spayed in CA were all open.
- At Sheldon 70-80% were pregnant - up to 7 or 8½ months gestation. Ovaries were easy to reach, but it was generally a little harder with the foal in the uterus.

Did it affect pregnant or lactating mares to keep them off feed?

Pielstick: They were only held off feed for 24 hours, and were given water. Mares were released with their foals. There were no abandonment issues.

Hinrichs: does not hold off of food and has not had an issue.

Pielstick: feels holding off food is important; the less abdominal fill, the better.

Hinrichs: **How long do you take to close the ecraseur?**

Pielstick: A few seconds.

Griffin: **Could we release into the wild and worry about potential breeding?**

Hinrichs: The result of ecraseur is a very clean pedicle, as she has had the opportunity to observe via flank incision during removal of ovaries with the ecraseur. Additionally, after 2 or 3 days you can barely see the incision in the vagina, but if she is mated at that time it could open up the incision.

- There is a chance they could be in heat, but if the procedure is in fall many will be pregnant.

- There is a concern that, if they are bred shortly after the procedure, the vaginal incision could be opened by the stallion's penis. This could result in peritonitis and death of the mare.

Patricia Sertich: Not necessary to hold off feed. Instead rely on careful palpation of the uterus and ovary. 32 years experience doing ovariectomy by colpotomy. The initial incision (<1cm) is made in the vaginal fornix with a no.10 scalpel and the incision is enlarged by blunt dissection. This method separates rather than transects the muscle fibers so the incision decreases in length when the vaginal muscles contract after the tranquilization wanes post-surgery. Three days post-op the incision edges are adhered, and healed after 7-10 days. If the mare is not pregnant or less than 60-80 days pregnant she will likely tolerate copulation by a stallion in 3-7 days due to the decrease in ovarian progesterone.

Hinrichs: They could be given an injection of a long-lasting progesterone (e.g. Altrenogest) to stop the mare tolerating stallion advances.

Asa: Estrus behavior is seen in pregnant mares, but in our study they did not not allow copulation.

Pielstick: could do a study to monitor how often mares show heat after spaying.

Sue McDonnell: Spayed mares can be receptive all year round. Typically can not even put them with geldings as they would be mounted. If given the opportunity a spayed mare would tolerate the sexual advance of an amorous gelding. If over 100 days pregnant at the time of ovariectomy, and don't immediately lose the pregnancy, the feto-placental unit has taken over from the ovary for progesterone to support the pregnancy, and without the ovary will likely suppress attractivity and receptivity.

Kane: **What are the long-term complications of spaying? What is the incidence of pyometras in spayed mares?**

Gail Collins: At Sheldon 85% of mares that had been spayed and released (plus 30 mares not spayed) were recaptured. The survival rate of spayed and non-spayed mares was not different.

Hinrichs: Breeding is not a problem if the incision is elsewhere than the vagina.

- There may be a long-term risk of vaginitis and pyometra in spayed mares if bred repeatedly after spaying. There is risk of penetration into the abdomen and peritonitis if mares are allowed to breed before the vaginal incision has healed.

Hinrichs: This is unlikely as the cervix will be open due to lack of progesterone (no ovaries).

Griffin: **How long should we keep mares in captivity?**

Collins: At Sheldon they kept them in for 7-8 days before release.

Hinrichs: Has just visited a lab that does a lumbosacral epidural on mares for oocyte recovery. This would eliminate the need for lidocaine and make sure the mare does not move during the procedure. May not be feasible in wild mares. The lumbosacral was given while the perineum was being prepared; done at the level of the tuber coxae. Lab she visited has used this on client mares over 300 times successfully.

Pielstick: It may be faster to just block the ovarian pedicle.

Discussion of ventral midline and flank incision approach - *Julie Weikel*

History

- Julie has spayed about 100,000 cows by either flank or vaginal approach. The vast majority of these were spayed by the vaginal approach, however several hundred were spayed through the flank at all stages of pregnancy. These experiences are the ones that inform our discussion about spaying mares as there are many similarities in the issues faced and potential consequences. The adult cattle spays were all performed as part of federal Brucellosis eradication efforts. Under the Brucellosis control program neither sexually intact nor pregnant animals could leave a quarantined premises, hence the need for both spays and C-sections, frequently in the same individual animals. Rarely were the conditions under which these surgeries occurred ideal in any way; cleanliness, weather, adequate manpower, etc. In other words, true field conditions. While Julie always tried to obtain follow-up information about complications and survival, she did not always get that feedback. Some of the problems in cattle might inform the discussion.
- In cows you cannot reach the ovary through the vagina when they are pregnant, so had to go through the flank.
- All the mares (domestic) Julie spayed were via a single flank approach, probably less than a dozen. They were all decades ago and were for either granulosa cell tumor removal or attempts to control "nymphomania." All were done in horses used to being handled and in a clinical environment.
- Entry for flank spays utilized a skin and fascia incision followed by blunt dissection that results in a "closure" of the underlying layers of muscle and fascia such that only the skin requires suturing. Only the very bottom of that skin suture line is left open. Julie came to this procedure as a result of dealing with seromas as a not uncommon sequence in fully closed suture lines. Seromas are not a serious post surgical complication, but in field situations where any secondary handling poses additional risk to the animal, they should be avoided if possible.
- In mares always used a 9 inch burdizzo rather than an ecraseur when doing flank incisions. Has never had a hemorrhage issue with castration using the burdizzo.
- P. 869 in Loesch and Rodgerson (2003) article is very thorough in regards to complications from any non-colpotomy approach in a horse.
- Always better to do surgery with a horse standing if possible – want to avoid lying horses down. Surgical vasectomies are done recumbent.
- Surgical recovery in a wild horse is already an extraordinary event (i.e., presenting unusual circumstances).

Conditions:

- Dust control is crucial (not completely sterile by any means).
- Heifer corrals mirror field conditions.
- Any surgery poses an issue for infection at the incision site.

Training:

- Julie has taught numerous vets to spay heifers. Only a small percentage are still spaying. All found it to be quite difficult. Adopting colpotomy for wild mares means that we need to find people who have gone through the steep learning curve. Some people can develop a good feel to correctly assess what tissue you have hold of, but it takes experience to learn.
- In the west, many dude ranches do not like cycling mares. Julie works with a veterinarian who spays these mares. Other vets with this experience are a potential for pool of people to choose from for the colpotomy method.
- While Julie has usually required 100 heifers to "train" a veterinarian to spay heifers, 100 mares may not be necessary to train an already accomplished equine surgeon to perform colpotomies. Training will vary with the individuals involved and hopefully could be accomplished with many fewer animals, maybe 5-10, with time to rest between surgeries for reflection during training.

Comments:

Hinrichs: Feels there is not such a problem with training veterinarians for equine ovariectomies via colpotomy. Did ovariectomies by colpotomy for PhD work – everyone wanted to learn the technique. Reproduction vets are best because they can tell if the ovary is covered by omentum, and recognize the anatomy better via palpation than surgeons. They are more familiar with the feel of it. Katrin has trained many veterinarians on ovariectomy via colpotomy, and they have been successful. They have conducted colpotomy on 22-30 year old mares with no complications. Reproductive specialists are used to palpating mares, so the colpotomy method is not difficult to teach.

Weikel: The biggest mistake is that trainees are too eager to jump right in and because the initial entrance into the abdomen is extremely important this causes an issue. During autopsies of spayed heifers the vaginal wall penetration site was difficult to find. This was observed in heifers only because she has never lost a mare.

Griffin: **Is there a minimum number of mares to use to teach people how to do the procedure?**

Hinrichs: 3-5 colpotomies should be done under supervision.

Weikel: People who were good had it after 1-2 animals. There are others who still wouldn't get it after 100. You need people with experience or competence.

- Dust control is critical – spray pens down every day, and re-spray during the day. In her opinion, field conditions make colpotomy an attractive option.

Kane: **Is there one incision or both flanks?**

- Single incision (left flank) and does the left ovary first. Dr. Weickel uses the burdizzo to access the second ovary, carrying the tool though the abdomen.

Chemical immobilization?

- Lidocaine and adequate sedation (butorphenol). Some mares respond differently – can't settle even when sedated. These are generally released without surgery.
- Sedation is an important variable.

Issues with flank incisions?

- It's an open wound. An external wound can become an abscess.

Recovery time? Or effects on pregnant mare?

- Cannot speak first hand on the flank approach in pregnant mares.
- In heifers Weikel would attempt vaginally or go in via flank. She would C-section (after 120 days pregnancy) therefore could see the ovary and proceed.

Turner: It will be harder to find people who are good at colpotomies. Some people prefer flank incisions, and more people are trained to do it. However flank incisions can take longer to heal and be more painful.

Weickel concurred that colpotomy appears less painful.

Kane: **Can anyone comment on the access to the ovaries on pregnant mares via flank incisions?**

[No response from the panel]

Kane: **Recovery time on flank incision?**

Weikel: There is a prolonged recovery time (2-3 weeks) due to the risk of abscesses developing after surgery.

Asa: Following up with data from the burro project: 2 were spayed via flank incision. These got infected and opened up within a week. They took a month to heal. These animals were closely monitored.

Turner: You will likely be able to find more veterinarians who are trained in and comfortable with flank incisions for ovariectomy. However, there are many complications in flank incisions even under sterile conditions. Most of these are related to healing of the incision line and discomfort. On the other hand, it will be hard to find people who are good at colpotomy. When untrained people perform colpotomies there is in an increased risk that things will go wrong and sometimes things can go very wrong.

Weikel: We need to be conscious of trained personnel who are good at colpotomy to teach others to do this procedure so they can take over if there are complications.

Hinrichs: has not had issues training people, however, she is very detailed in her training methods. She goes through every complication she has experienced with each new trainee. Reiterated that she prefers reproductive vets over surgeons.

Kane: **Safety of the people involved during the surgery?**

- Colpotomy – strapping the back of the mare helped stopped the mare from kicking, plus there is a kickboard and the tail is tied up.
- Flank incision – operator can be more protected. Small window of access. Weikel does not believe that this outweighs the consideration of the colpotomy.
- Hinrichs noted that the time with the scalpel in colpotomy is only a couple of seconds. This time is longer for flank incision, which means more opportunity for fractiousness and infection.

Ventral midline incision approach: *Jay D'Ewart*

Procedure

- 8 mares were spayed using the ventral midline approach at Rock Springs, WY. All survived. It was not a blind procedure. Done under anesthetic with the surgery similar to a dog or cat. Given three-layer sutures. After surgery the mare got up and wandered to the recovery pen. The mares were watched for 2-3 weeks at Rock Springs and then sent to long-term holding facilities.

Comments:

Kane: **Were the mares pregnant? How long after were they turned out?**

- Unsure if any mares were pregnant, but it is unlikely as they had been in holding for some time prior to surgery.
- These mares were never released to the range (they are in long-term holding somewhere).

Griffin: **How would this affect pregnant mares?**

Hinrichs: It would be difficult to access ovaries via ventral midline in a pregnant mare.

Kane: **How would this affect lactating mares?**

D'Ewart: Might be able to select mares that are close to weaning.

Weikel: It is likely to be more of a problem if the mare is still sore. In heifers there is soreness due to the calf poking around to nurse. The relationship between dam and foal could be compromised. Edema could affect lactation. Flank incision might also make some mares resistant to nursing.

Griffin: **With the ventral midline procedure is there a potential for evisceration?**

Weickel: Yes, definitely. This is a primary possible complication of this procedure, and it is an awful outcome.

Hinrichs: what is the incision size?

D'Ewart: 5 inches

[Referred to Loesch and Rodgerson (2003): 25-35 cm]

Weikel: depends on the size of the ovary and size of the operator's hand.

Doug Eckery: close to the mammary gland, so may affect nursing.

- Evisceration is a horrific consequence so we want to be careful of a method in which evisceration is a complication, as in ventral midline.

Hinrichs: Although evisceration is said to be a risk of colpotomy, she has not known of any evisceration post-colpotomy.

[Neither had people at U. Penn.]

Sertich: Domestic horses that have had a ventral midline incision are usually restricted to stall rest for one month, and then only hand walked for the second month. These horses are kept with very limited activity. No data on mares that had free access to exercise.

D'Ewart: Had a successful experience in Wyoming. After anesthesia the horses are turned out in a holding pen where they don't get a lot of exercise. No eviscerations. The vet thinks it is a very teachable procedure.

King: We will be sending the briefing statement to Paul Zancanella who conducts and advocates for the ventral midline approach. He can add comments.

Ventral midline procedure - Paul Zancanella (written comment submitted after the panel)

- Mares are restrained in a padded chute and administered an induction dose of xylazine and ketamine. Upon induction an indwelling catheter was installed in the jugular vein. Anesthesia was maintained using intravenous administration of triple drip (IV-ketamine, zylazine and Guiafensin 5%) to effect. Horses are placed in dorsal recumbency. Surgical area is clipped, pre-surgical preparation was done with chlorhex scrub followed by chorhex solution swabs. Seven-inch incisions were made just cranial to the udder. Ovaries are exteriorized through the incision. A serra emasculator is applied to the ovarian stump. Number 6 MSA is used to ligate the ovarian stump proximal to the emasculator. The emasculator is removed and the ligated stump allowed to retract into the abdomen.
- Closure is accomplished in three layers. The outermost being a subcuticular layer using #6 MSA absorbable. The surgical time, induction to completion, is twenty to thirty minutes.
- Mares all received 40 ml procaine penicillin and 10 ml flunixin meglumine post surgery.
- Mares are standing within thirty minutes of surgery and eating within two hours of surgery.
- In conclusion, a ventral midline ovariectomy is a viable field surgical procedure for fertility control in mares. The surgery is accomplished relatively easily with less risk and expense than other fertility control methods.
- The ventral midline can be performed with little or no modifications to the existing facilities.
- Postoperative pain is much less than colpotomy and is easily managed with intraoperative IV flunixin meglumine.
- Operator and assistants safety is much better than standing procedures.
- Some concerns expressed for mares being anesthetized is exaggerated or naïve. Equine practitioners anesthetize thousands of horses with minimal problems.
- Ventral midline surgeries are accomplished routinely on horses without complications.
- Having performed both the colpotomy and ventral midline I much prefer the ventral midline approach.

Kane: We need sedation and anesthetic protocols for the three incision approaches.
Griffin: **Is there something additional about lying horses down that is an issue?**
Weikel: There is a lot of weight on the tissues if on its back, pressure on the aorta (cardiovascular effects), and recovery issues; these are mentioned in the review article [Loesch and Rodgerson (2003)]. When a domestic horse that trusts you is recovering you can keep them calm and help them. With wild horses this is not an option. The goal is to get away from them (and get them

away from people) as soon as possible. They can sometimes run and buck/get acrobatic; level ground is a must for recovery.
[*Hinrichs* and *Turner* agree with not laying horses down for surgery if at all possible.]
Hinrichs: We always do ovariectomy surgery standing. Injectable sedation for ventral midline seems risky, as compared to gas anesthesia. People are moving away from abdominal incisions in humans; for example in women they are doing hysterectomy etc. via colpotomy and this is associated with less pain and much faster recovery.
Weikel: There is potential for adhesions to the ovary, for example due to a history of inflammation. Those are problematic. Leon will not take such ovaries out of mares. Individuals with these inflammations are high risk - heifers walked around with the hump in their back even if they lived. Would be skeptical if there are adhesions.
King: **Is this similar to cryptorchid stallions?**

- If a mare has an ovary left she will still cycle, even if one ovary is infertile. Generally in cryptorchids they do not have adhesions, so not really comparable.

Kane: **What are contra-indications for flank or midline methods?**
Weickel: No external surgical incisions should be done on any mare that is very dirty. Any mare that is especially dirty we need to ask why is she laying down and rolling a lot. Will she be doing this post-surgical procedure? How dirty are the field conditions? At Sheldon, the corral where the surgeries took place was clean, and not used at all for many months per year.
Sertich: Would avoid ventral midline incisions in late gestation in mares (in last month before parturition) as they may go into labor. Some mares have delivered foals after midline surgery for colic, but they were watched carefully. The concern is that there may be a breakdown of the abdominal wall during delivery.
Kane: **Is there an age effect for these approaches?**
Sertich: No specific numbers, but as mares get older they are more at risk for abdominal wall rupture.
Turner: Older/multiparous mares have an increased risk under general anesthesia because they may be at increased risk for catastrophic fracture during the recovery process. Also, many physical conditions would preclude the use of general anesthesia and subsequent recovery (e.g. neurologic horses, horses with heart conditions, etc.) Any of these conditions would be contraindications for general anesthesia and therefore the ventral midline approach. Mares would need to have a full physical examination prior to anesthesia, which is unlikely in the field.
Sertich: Mares that lactate year round every year probably have lower bone density, so are more at risk of catastrophic fractures during recovery from general anesthesia. These risks are inherent to the recovery process following general anesthesia (not the method of surgery chosen). But since the ventral midline approach can only be done under general anesthesia, these potential complications would be unavoidable with ventral midline (vs. other procedures that could be done standing).

Weikel: The Sheldon mares (colpotomy) spayed were skewed towards older mares. The recovery was the same as the mares that did not have the procedure, but all of these mares were done standing and did not have general anesthesia.
Hinrichs: 8 mares 21-30 years old were given ovariectomies by (standing) colpotomy without complication.
Kate Schoenecker: **How does an ovariectomy affect the fetus?**
Hinrichs: [will be sending the research paper cited to Joanna] Mares ovariectomized earlier than 50 days – all lost their fetus. At 50-70 days about half lost their pregnancy. >140 days none lost their foal. In her own research, took ovariectomized mares off progesterone after 100 days of gestation and then had no problem.
Sertich: When mares get abdominal surgery of any kind they are also administered progesterone/progestin before and for a few weeks after surgery to prevent pregnancy loss. There is less risk of losing a foal with a colpotomy. General anesthesia for a ventral midline incision requires that the horse is off its feed, which means more muscle trauma and more stress.
Turner: Pregnant mares in surgery have a 3x greater risk of pregnancy loss with general anesthesia.
Griffin: **Due to colpotomy sometimes early stage pregnancy can be lost. Can we give them long term progesterone injection to save the pregnancy?**
Hinrichs: It could be possible to keep ovariectomized mares pregnant by injecting progesterone. At <50 days of gestation won't find evidence of fetal loss. Pregnancies of 50-70 days might be helped by a long-lasting progesterone shot. Pregnancies over 70 days are likely to be maintained even without exogenous progesterone.
Kane: **What product would you recommend?**
Hinrichs: Not sure on commercial availability of certain products. BET had a long-lasting progesterone and long-acting altrenogest that was available. (Checked subsequent to the panel: 30-day altrenogest injection still available).
Kane: **Potential for a feed through on mares that die due to this procedure in the wild?**
Hinrichs: Main issue is if an animal were to eat the area of the mare where the progesterone is injected, but progesterone is a natural hormone and the consumer would just receive an oral dose. (Note added later: Altrenogest does have the potential for feed-through).
Kane: If gestational stage is <50 days, we should consider accepting fetal loss. If stage is 50-70 days, one could consider injecting a drug to maintain the pregnancy.
Hinrichs: **Does anyone know what time in gestation an abortion is visible?**
Kane: It is very difficult to find aborted fetuses, even in a stall. Most that are found are close to term (size of a beagle). There are behavioral signs preceding abortion. We want to avoid abortions to the extent possible.

- Beagle size is 150 or more days (5-6 months) gestation.

Collins: We rarely found aborted fetuses in corrals. Only a few times did we find beagle size fetuses.

How often is the fetus reabsorbed?

Sertich: It is thought to be expelled/passed out through the cervix and not found, rather than resorbed.
Weikel: When it's hormonal abortion we are less likely to see discharge from the mare. When it is infection one sees a messier discharge.
Kane: Horses are corralled for testing purposes and held for 30-90 days. We vary rarely see secondary complications in mares that abort a fetus. The complications with abortion of foals are minimal. The mares do not show signs of illness, pyometra, or founder. The mares are generally farther along when we see complications, if any.
Hinrichs: In Argentina the polo industry is aborting hundreds of male foals, then within a few weeks transferring another embryo to the same mare with no ill effects.

Discussion of Laparoscopy: *Dean Hendrickson*

Procedure:

- Done a lot in horses at CSU. Performed in domestic horses with standing sedation. Typically approach both flanks. Can do it through one flank but this takes more time. Typically it takes 40 mins to remove both ovaries (doesn't include time for preparing/sterilizing horses for surgery, which brings the total to 50-60 minutes per animal).
- Equipment needed is a laparoscopic tower, light source and monitor, camera box, CO_2 canister, insufflator, and one standard surgery pack per animal (scalpel, blades, clamps, etc.).

Recovery time:

- Some are sent home the same day or some stay over night. There is minimal postoperative care.
- Has done the surgery in a field setting (laparoscopic vasectomy of elephants in Africa). Can keep the animals/instruments clean and sterile enough with cold sterilization.
- Standing sedation may not be possible in a wild horse for this surgery.
- The effect on the fetus if the hormones are not maintained (at less than 50 days gestation) is that the mares will generally abort. It is most likely that there is the same level of risk as for other procedures.

Comments:

Hinrichs: **What is the sterilization time for the instruments?**

- About 20 minutes to sterilize between animals (would work better with 2 sets of instruments to avoid wasting time).

Jason Bruemmer: **If you were to do it from only one side does it affect your time of completion?**

- Yes, it can take up to an hour standing sedated in a squeeze chute if surgery is from one side.

Sertich: When we do ovariectomies by laparoscopy they allow 1 to 1.5 hours, which includes scrubbing the flanks. They make incisions from both sides to get both ovaries.

Collins: **How is sedation maintained?**

- IV jugular catheter, continuous sedation drip. More consistent than with a bolus.
- Have done epidural (caudal) which also works well, but not sure this will work for wild mares.
- With this procedure in elephants they are under general anesthetic and hung in a sling from a crane so there is access to the flanks. Not sure if this is possible for horses.

What is the size of the incision?

- Ovaries are removed through the flank. Incision size is dependent on the size of the ovary (just enough to pop the ovary through), normally 4-10 cm.
- In 2% of cases there is incisional drainage, and in 10% there is subcutaneous emphysema from the CO_2 insufflation.

How are they managed post operatively?

- 1 dose of antibiotics pre-op and 3-5 days of NSAID post-op.
- Kept in a pen for 1 week, and then normal work after 2 weeks. But would not have a problem waking the mares up and sending them off.

Is it possible to use the laparoscope vaginally?

- Yes, there have been two reports using it to aid in colpotomy.

King: **Have you ever carried out this procedure on a pregnant mare?**

- No.

Hinrichs: **What type of epidural do you use?**

- Caudal epidural as it reduces movement. But there is no flexibility in that method of sedation as if you put in too much it's hard to keep them standing, and too little means they aren't sufficiently sedated.
- 40 µg/kg detomidine is used. Lidocaine would require too much volume to be used as an epidural.

Do you use lidocaine on the ovary?

- Yes – injected into the ovarian pedicle (15 ml local anesthetic injected to the pedicle).Give an epidural for analgesia of the reproductive tract, rather than the flank.

Kane: **Can you comment on the ventral approach?**

- It makes laparoscopy more difficult. When dorsal it is harder to get to the ovaries and find them. Ovaries present better when standing from the flank.
- In the small animal world they put the animal head down tail up and tip them from side to side. It might be possible to roll horses one side to the other to get to the ovaries.

Kane: **What are the general equipment costs?**

- $25,000 for camera, light core, light source, monitor, Thorston telepacs (sp.?)
- $5,000 - insufflator
- $3,000 - microscope

- $750 x 6 for hand instruments
- $40,000 total new, or could buy used.
- You would need two sets of instruments to be efficient, so that one set could be cold sterilizing while the other was in use.

Kane: **Contra-indications for this method?**

- You are very dependent upon technology – if something breaks and you don't have a duplicate then you need to stop. It takes time to get used to the approach and do it.
- Worst-case complications would be puncturing a bowel. Withholding feed tends to reduce this risk.
- It is possible to drop ovaries in the abdomen. There is no proof that this is an issue but could be.

Durability of equipment?

- Not a huge worry – machinery is not that delicate. The monitor is most delicate - if kicked it could break the screen.

What do you recommend for pre-op?

- 12 hours off feed but not critical. The more comfortable you are with the tech the less you need to worry about feed – 3 hours would be fine. Time off food possibly upsets them. Elephants were never held off feed and they have similar digestive tracts.

Hinrichs: **How long does it take to learn the procedure?**

- With 3 days and 5 or 6 mares you can get someone up to speed. Doing it regularly helps. Has learned how to teach it well. Ovaries in a standing mare are easy to access. It is not difficult to do an ovariectomy.

Kane: **Can you guess what would happen in a pregnant mare?**

- Pregnant mares present ovaries well if standing. It could be easily done.

Do you have any ideas about slinging them under anesthesia in a chute?

- Can use the same concept as hoisting horses up and out of ditches. You can use this to keep them into position.
- You could potentially have two teams working on either side of the abdomen and cut your time shorter.

Hinrichs: **What is the infection rate?**

- It's about 1/10 less when the instruments go in and out of the body cavity through a cannula, rather than flank laparotomy where they go repeatedly in and out through an incision. Instead of 5 in 100 you'll get 1 in 100.
- We don't see them commonly in open flank procedure.

Kane: **How safe is this procedure for the operator?**

- Coming from the flank you can avoid the feet, but he has still ended up with bruises and a broken toe.
- The difficulty is the amount of equipment and two people puts you a little more at risk than standing flank ecraseur, but slightly safer than a colpotomy.

How do you feel about training on a large scale?

- He feels good about training people fully. Colpotomy is rough because you cannot see what you are doing. One could use the laparoscope to train colpotomy.
- Won't walk away from someone until comfortable that they could do it.

Hinrichs: When training students on colpotomy, the student will find the ovary and place it in the loop of the ecraseur, then she will put her hand in and make sure it's an ovary before anything is cut.

Griffin: **Can you comment on the approximate cost the procedure will be per animal?**

- CSU charge is $1400 to do mare ovariectomy at the hospital (including technician time).
- Could do it in the field for $250/ horse for procedure alone (not including salaries).
- Did ovariectomies on dude ranch mares in Durango – 11 horses in 3 days. The real cost was $450-$500 per horse.

What is the cost per colpotomy and ventral midline?

Collins: Colpotomy was $250-$300 per horse at Sheldon NWR.

- Ventral midline is approximately $350/horse at Rock Springs, WY.

Fragility of the equipment? Is the jostling of the wild mares going to be an issue for the equipment?

- Wise choice is per 100 mares look to replace 5 instruments (this is the rate with people who are not fiscally responsible for the equipment).
- More likely to break hand instruments than the scope.
- Laparoscopy is the most expensive approach, but there is direct visualization, low morbidity, and better public opinion.

Schoenecker: **Do you cold sterilize?**

- Yes. Clean with soap and then water, rinse with sterile water.

What happens with dust?

- Blowing dust is less of a problem as the instruments are solid. Has been able to brush dust off during elephant procedures.

Sertich: **Infection issues or seromas?**

- One does get some seromas but no big problems with them. 1-2% incisional complications, but those rates are for mares in a clean and controlled environment. Opening and draining generally fixes this.
- No long-term negative or infection rates. In a field setting there may be a 2-3 x higher risk, but this is still small and corrects on its own.

D' Ewart: **Is there a difference in laparoscopy vs just through the flank?**

- You can see what you are doing with lap.
- You can ligate the pedicle with two ($10) ligatures to cut sharply, rather than putting the ecraseur through with a flank incision.
- Two ligating loops is faster than the radiofrequency "Ligasure".

- There is a radio frequency device for cauterizing the ligated tissue, but it is expensive and finicky, and is just one more piece of equipment to worry about that may halt surgeries.

Bruemmer: **Can you use umbilical clamps?**

- Seems like you could. Thicker pedicle in the horse than cattle.

D'Ewart: **What is the infection rate difference in flank than scope?**

- 5% incisional infection for flank.
- 2% with laparoscopy.

Hinrichs: **Is the opening for the ovary a straight incision? How is it closed?**

- Incision of 5-10 cm modified grid. Close fascia and skin in continuous pattern.

D'Ewart: **How do you manage pain?**

- A lot of the pain comes from the flank incision. It is helpful to anesthetize the pedicle (with carbocaine).
- Since laparoscopy incision is smaller than with the flank laparotomy method, it is mildly less painful but not a huge difference.

Behavioral estrus post spaying: *Cheryl Asa*

History

- 10 ovariectomized mares and 10 anovulatory mares were put with stallions and monitored for 15 days in January. The study was published in 1980.
- Of the 20 mares all showed at least one day of weak estrus. One mare showed full estrus all 15 days. Most had at least some mounting and were accepting of a stallion to full copulation.
- Most notable is that there was nothing cyclic (it was on and off day to day).
- Considering our various studies, only when a mare had elevated progesterone (e.g., diestrus, pregnancy) was there an absence of estrus and copulation. The adrenal cortex produces sex steroids. Estrus was not shown in mares with adrenal cortical hormone production was blocked with dexamethasone, suggesting that it is adrenal sex steroids that support estrus in mares that are ovariectomized and during the non-breeding season.
- It would be interesting to know whether this represents what mares would do in a naturalistic circumstance.

Comments:

Weikel: **What was the relationship between stallion and exposure? And relationship to spay?**

- Mares and stallions were kept separate and introduced (several mares and a stallion in a paddock) and allowed to interact freely.

Weikel: The increase in breeding activity may have been because the mares were new to the stallion. In Asa's study, the stallions were not with the mares except during the study.

Collins: Didn't monitor for sexual behavior at Sheldon but did analyze band association. Spayed mares maintained their band associations, and were in mixed groups. They were not found in bachelorette bands, or as solitary animals.

Is there a more natural setting?

Asa: Horses stay together year round (unlike most animals), so behavioral estrus may be a mechanism for the band to stay together outside of the breeding season.

Collins: Mares that were not coming into estrus were not driven off or kicked out the band.

Hinrichs: **In an intact band full of intact mares what portion of those mares would not be pregnant?**

Collins: 45-50% recruitment in Sheldon.

Asa: Reviewed horse literature for the NAS report. It looked like there was a trend where mares on BLM range were reproducing about every other year.

Kane: varies from HMA to HMA. 60-75% of mares are coming up pregnant based on fecal testing or foaling. There is 75-80% pregnancy rate based on fecal testing or foaling, observed at Teddy Roosevelt NP.

Collins: Most foal deaths were within the first 2 months of life.

Griffin: If a foal survives to fall, then typically BLM would consider it part of the population. "Recruitment" is survival to nearly a year old.

King: **Any other observations of behavioral differences in mares post ovariectomy?**

Sertich: Teaser mares (stimulus mares for semen collection) are ovarectomized. They are sexually receptive to stallion advances every day for the rest of their lives. Sometimes ovariectomized mares are administered estrogen to encourage proceptivity, but most of the time they are not. Ovariectomy to prepare a stimulus mare is common in the horse breeding industry for semen collection to be used for artificial insemination.

Griffin: The main reason we are asking USGS to conduct this study is because this behavior is not well known, so part of the purpose of this study is to discover that.

Griffin: **An injection of progesterone would create more time for the mare to heal by making her less receptive to being bred?**

Hinrichs: Yes. A long-acting progesterone given at a concentration of 1-2 ng/ml could suffice. They should reject the stallion for about 7-10 days.

Kane: **Any concern about exogenous progesterone affecting post-operative uterine infection rates?**

Hinrichs- There could be concern, but because the cervix may open shortly thereafter, when the progesterone level falls, this should be self-limiting. Some contamination could occur during the procedure, but because of antibiotics the likelihood of infection would be low.

Collins: **If you are doing the surgery when is the injection being administered?**

Collins: At Sheldon we held animals for a week, and restricted the number of times they handled the animals.

Hinrichs: If you give injection at the time of ovariectomy it should last 7-10 days, which would be OK if the mares are released in 2-3 days. Otherwise would need to be given later for a later release, and it would be better not to handle the horses again.

Were the stallions held?

Collins: Depended on the studs (they were doing vasectomies). Some were turned out sooner than the mares, but not always.

Did the mares get back to the same harem group?

Collins: They were not released all at once and this was not monitored as some were adopted.

Griffin: **Is a week enough to heal?**

Weikel: Yes, the healing rate is very quick with colpotomy.

Hinrichs: To be safe maybe one should allow 2 weeks before the mare is repeatedly bred in the wild in order to avoid catastrophe. A stallion can rupture even an intact vagina. It takes a surgical incision 7-10 days to fully heal.

Risk is running through chute once or keeping them in captivity?

Weickel: Checking the BET website, there is a 30 day progestin injection, Altrenogest, that could also be given. Could it promote uterine infection?

Hinrichs: if the uterus was contaminated at surgery, but as noted before, this is unlikely.

King: **Could progesterone injections also be given to control animals** (to reduce any confounding effects of progesterone injection on comparisons of observed animal behavior)?

Hinrichs: This drug has been thoroughly tested in pregnant mares and shown not to be detrimental to the mare or foal.

Mare menopause approach: *Doug Eckery*

- The aim is to develop a vaccine. GonaCon and PZP are currently available and both have been shown to be effective, but for a limited time. PZP prevents fertilization; GonaCon prevents ovulation. A vaccine is only good as long as antibodies can be maintained. PZP, in particular, requires many booster shots. Follicles are constantly replenishing, so mares need a booster throughout their lifetime.
- The idea is to target something earlier in ovarian function. Successful reproduction in female mammals depends on an adequate supply of eggs that are found in primordial follicles. However, there is only a finite supply of eggs in the ovaries, that if destroyed would cause permanent sterilization. Every day a certain number of primordial follicles begins to grow, and only a very few ever reach the final stages of maturation and go to ovulation. The initiation of growth is a committed step and controlled largely by local growth factors. Oocyte-specific growth factors are involved in the early stages of follicular growth. A research team in New Zealand found that a certain group of infertile sheep had a mutation causing infertility. The research goal is to come up with a vaccine to mimic this mutation but for horses. If the primordial follicles do not mature then the animal is sterile. For sterility you need to deplete the ovaries of eggs by directly killing the eggs/primordial follicles or by

causing a mass activation of the primordial follicles (once growth is started it cannot be stopped).

- We are going to test vaccines against oocyte specific growth factors in horses. This will prevent follicular growth and ovulation in the short term, but hopefully will eventually deplete eggs leading to mare menopause, but this is a hypothesis.

Timeline:

- Start research in November by vaccinating mares against the growth factors. After 1 year will conduct a unilateral ovariectomy and look at the ovaries under the microscope. Throughout the year will conduct hormone testing. AMH levels will be measured, as this can be one index of follicular reserve. The project will run for 2 years. Also will track ovarian function through ultrasound and behavior through teasing.

Comments:

Kane: **When would you expect them to be sterile? Are you planning a fertility challenge?**

- We do not expect animals to continue to cycle. The follicles will not produce any steroids so behavior will be changed. Eventually we will conduct fertility testing. It is unknown how long it will take to deplete the ovaries. This is a test to see if the mechanism will work.
- Vaccine and booster will be 6 weeks apart. Next step is to develop a vaccine.

Kane: single shot vaccination is a lofty goal.

Schoenecker: **What happens if given to a pregnant mare and depletes her eggs?**

- The vaccine should have no effect on an existing pregnancy as it is not affecting the corpus luteum.
- The time frame of egg depletion would take longer than the term of a pregnancy, so therefore no effect on the pregnancy.

Kane: **What about the effect on the fetus?**

- Antibodies would probably not pass through the placenta to affect the foal, and probably not in the colostrum long enough to affect the foal's antibodies. But it is unknown whether there would be a long-term effect on the offspring.