

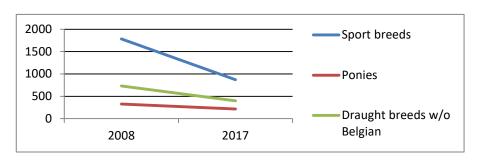
Conserving Equine Diversity: Time for Action

Targeting issues and finding solutions

For over 40 years The Livestock Conservancy has worked to protect endangered livestock and poultry breeds from extinction. Although all rare breeds face challenges, it has become apparent that the recent market downturn has particularly impacted our equine breeds. Many horse and donkey breeds face the threat of extinction more so today than at any other time in history. Thanks to the grant from the USA Equestrian Trust, the effort to stabilize equine breed decline and secure the remaining diversity began in 2017. It started with collaboration between Texas A&M University, Virginia Tech, and The Livestock Conservancy to bring together representatives from approximately 50 endangered horse breed associations and registries to participate in the first National Endangered Equine Summit. This group represented thousands of horse owners. Together with leaders of the scientific, rare breed, and horse communities, the group was tasked to identify what they believe are the leading causes of breed population declines, decide what actions may be taken to stabilize the loss of breeds, and develop a list of achievable and fundable action points. It was envisioned that attendees could work towards developing long lasting partnerships that would serve to strengthen and revitalize efforts long diluted by many small groups working independently, and the summit was structured to encourage networking.

Counting our equines

To begin to understand the full scope of the breeds' population status The Livestock Conservancy, Heritage Breeds Canada, and the UK's Rare Breeds Survival Trust collected the most up to date census numbers for each of the breeds we share in common. Participation by registries was nearly universal. As everyone had feared,



many of the breeds have seen sharp declines. A more detailed listing of the numbers can be viewed in the **Appendix I** of this document.

Survey and pre-summit homework

Prior to the summit, a survey was distributed to the association representatives and used as a tool by the organizers for targeting the most pressing areas of concern among the breed associations. These ideas set the agenda for the summit. Eight issues were in the forefront of concern and these fell into three main categories: biological challenges, breeder education & technical assistance, and marketing assistance.

- 1. Biological- Addressing genetic diversity challenges within breeds
- 2. Biological Improving availability of scientific advice for breeders and owners
- 3. Biological Creating a national semen repository/collection program
- 4. Technical Improving member engagement within breed associations
- 5. Technical Improving communication between and within breed associations
- 6. Marketing Building interest/demand for breeds
- 7. Marketing Developing marketing skills for owners
- 8. Marketing Exploring collaborative marketing between breeds and associations

Preparation for the summit continued with "homework" assignments for attendees so that upon arrival to the meeting, they would all have background and educational information on some of the more complicated topics to be discussed. A gathering the night before the summit broke the ice as people got acquainted and began to discuss the issues facing their breeds. The next morning, everyone was ready to get to work.

The experts

Three expert panels were assembled, including some of the best minds within their professions. The panelists were asked to give an overview of the work they do and how it pertains to helping solve the key problems within the panel's theme.

<u>Biological</u> - D. Phillip Sponenberg, DVM, PhD, Professor of Pathology & Genetics, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Gus Cothran, PhD, Clinical Professor, Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Science, Texas A&M, Leif Andersson, PhD, Professor, Functional Genomics, Uppsala University, Professor, Animal Genomics, Texas A&M, Guest Professor, Molecular Animal Genetics, Swedish Univ. of Ag Sciences, Scott Dindot, PhD, Associate Professor, College of Veterinary Medicine, Texas A&M, Katrin Hinrichs, Professor and Patsy Link Chair in Mare Reproductive Studies, College of Veterinary Medicine, Texas A&M and Tullis Matson, Trustee, Rare Breeds Survival Trust, Managing Director, Stallion Al Services/Cryogenetics Chapel Field Stud.

The biological panel discussion was a highlight at the meeting simply because recent technology advances in equines were previously not known by a majority of the audience. Assistive reproductive technologies and an explanation of how DNA testing can be more productively utilized for conservation purposes were among the most exciting topics. The idea of establishing a national rare breed semen repository also generated useful discussion as we learned how such a collection is being utilized by our breed conservation

Marsh Tacky mare & foal

counterparts in the UK. A detailed reporting of the assistive reproductive technologies and of DNA technologies can be found in **Appendix II** and in **Appendix III**.

<u>Breeder education & technical assistance</u> - Alison Martin, PhD, Executive Director, The Livestock Conservancy, Cliff Williamson, Director of Health and Regulatory Affairs, American Horse Council, Debbie Fuentes, Registrar and Sr. Director of Registry and Member Services, Arabian Horse Association, Brian Larson, PhD, Past President, Natural Lincoln Sheep Breeders Association, Board Chair, The Livestock Conservancy, Patti Strand, President, National Animal Interest Alliance

The second panel was another great discussion because many in the audience realized they share a good number of problems in common. Debbie Fuentes admitted that even registrations for the popular Arab horse are down. She summed it up best by quoting the registrar for the Paint Horse Association who said "Flat is the new up." This meant that maintaining annual registration levels is something to be happy about in the modern horse market. Good identification of horses (such as tattoos and microchips) was discussed as crucial to "recovering" genetically important horses back into a registry should their records be lost through multiple owners or sold without records. The American Horse Council's *Unwanted Horse Coalition* program helps groups organize microchipping and gelding clinics that the audience thought could be of great use to the breed associations. Dr. Larson discussed breeder

education, while Patti Strand shared marketing tips and the audience discussed various ways to engage youth with horses.

Marketing assistance - Jeannette Beranger, Senior Program Manager, The Livestock Conservancy,
Ashlee Watts, DVM, PhD, President, U.S. Dressage Federation's Brazos Assoc. for Classical
Horsemanship, Assistant Professor, Large Animal Surgery, Texas A&M University, Keith Yutzy, Southwest
Regional Director, American Driving Society, Owner, Lone Star Dartmoors, Tim Jobe Co-Founder, Natural
Lifemanship Equine Assisted Therapy, CEO & Founding Board Member, Equine Assisted Growth &
Learning Association, Jas Shearer-McMahon, American Endurance Ride Conference

The third panel took on the subject of finding new markets that perhaps many of the rare breeds had not considered. Using smaller breeds for competitive driving offers great opportunities for potential new owners wanting smaller, easier keeping horses. For draft horses dressage and competitive jousting could be an option. Many breeds could be finding new jobs within hippotherapy and rare breeds are performing well in endurance rides for pleasure or competition. The points made were that there is a wide host of activities for which rare breeds are well suited and that may bring new attention to them. The other important issue is that owners need to know how to "work the crowd" so as to create interest and excitement for their breeds among both horse owners *and* non-horse owners.

Breakout work

The panel discussions were then followed by a 30 minute Q&A session with the audience. Using what was learned from panels and from the "homework", the final exercise of day one was facilitated break out groups assigned several of the key issues for discussion. Each group outlined 2-3 possible solutions for each topic. The diversity of the attendees yielded a broad array of ideas and these were compiled that evening in preparation for day two. The following day participants once again broke out into facilitated groups, taking care to change the composition of the groups. This time each group was assigned only one of the eight key issues. Armed with the ideas from the previous day, the groups identified the top 2-3 solutions that had the best chances for success, and what would be needed to implement them.



The intense brainstorming of the summit accomplished what we all had hoped it would -1) identify the major problems endangered equine breeds and their owners face today, 2) identify which of those problems we can actually do something about, and 3) identify the problems for which we could we find resources to attain solutions. These are the ideas generated from the final discussions of the summit:

Ideas and solutions - Biological

Addressing genetic diversity challenges within breeds

- 1. Educate breeders and communicate the value of DNA testing. Many breeders and breed associations are unclear on both the benefits and drawbacks of DNA testing for rare breeds. It can be a powerful tool, but as with all power tools, in the wrong hands it can be deadly (in this case to a breed!) Therefore breeder education on diversity management, genetic disease, etc. is a priority. Educational materials should be developed, and an action plan for the associations to promote the information.
- 2. Use DNA analysis to identify diversity and individual sources that could be important for a breed. This would be accomplished by sequencing a diverse cross section of mares and stallions from each breed. The effort would help target rare bloodlines or individuals within the breeds that are a priority for conservation.
- 3. Recruit new breeders and help them develop a breeding strategy for using and securing the remaining diversity in the breed herd. The 2017 equine survey showed that three quarters of horse owners are between the ages of 40-60 and many of the long time breeders are aging out. The associations need to actively engage all breeders along with the registry to develop a breeding plan that includes stallion swapping, artificial insemination, semen & germplasm banking, and possible outcrossing as a last resort for those breed in extreme danger of extinction.

Improving availability of technical advice for breeders and owners

- Participants recommended that the Livestock Conservancy serve as a primary conduit for
 information by developing an equine resource webpage containing info on breeding, training,
 events, and more. The Conservancy will also facilitate communication between breeders,
 outreach via multimedia, social media and/or hard copy. The list would be used to solicit input
 from breeders and the scientific community to share among all the breed groups.
- There will be continuing need to identify and support the most pressing issues for the rare breeds through regional conferences and by more broadly promoting through organizations such as American Horse Council or the Equine Education Association. A free app similar to Texas Horse Help https://spark.adobe.com/page/vEWBx0FVK9Nsz/ but centered on rare breeds was also suggested.

Creating a national semen repository/collection program

- 1. A national repository of equine germplasm in the U.S. would conserve genetic diversity for the long term. This initiative would begin with a search for a national storage facility, identifying sponsor(s) for the program, and having the Livestock Conservancy provide logistical support. Zoos and Rare Breeds Survival Trust might offer useful examples of such collections. Optimally, a reserve would have three tiers one available for immediate commercial use by the studs' owners, one to be used when needed for conservation, and one reserved for breeds in serious trouble and facing a "doomsday" scenario. The model needs to be sustainable, transparent, and have a solid business plan in place.
- 2. An interactive database will be a crucial element for the repository so it can be effectively used by breeders. It should include a "universal database" with free access to all parties involved including universities, breed associations, and breeders. The purpose of the database should be clearly defined by determining how it will be used and by whom. Information in the database would include microchip id, genetic testing results, and pedigree. It would be maintained by a single organization that make sure it is user friendly and accessible for all.

<u>Ideas and solutions - Breeder education & technical assistance</u> *Improving member engagement within breed associations*

- 1. Communication can be improved by centralizing member information on a website and choosing appropriate social media means to convey current events and interests. Many breed groups have already taken steps in this direction.
- 2. Member support and recognition for volunteering is an important way to encourage members to be more engaged. Association leadership should develop and promote opportunities for members to participate in a broad array of activities and events. They also need to publically recognize and reward those that do. Reward systems could encourage "outside the box" activities such as mentorship, participating in local historical field days, or hand-on youth activities.
- 3. Associations should consider opportunities to partner and the advantages of sharing resources with other breed groups. This "cross pollination" between associations offer a wider field of opportunities for each other's members. The bottom line is to continually encourage participation, share experiences, and cultivate a culture of mentorship.

Improving communication between and within breed associations

1. Establish regional ambassadors within breed organizations and identifying two ambassadors within each association for regular communications between breed clubs and groups in the same region. This network of ambassadors will establish the best means of communication (telephone, email, print, etc.) and regular regional teleconferences. The ambassadors can then begin joint outreach to broader markets such as Pony Club, 4-H, FFA, heritage lifestyle enthusiasts, and historical societies.

Building interest/demand for breeds

- 1. Building demand means getting horses into public venues that showcase the breed's best qualities. Each association should create a display and fund the cost of a booth at events. They should create incentives for owners who actively reach out to promote the breed and promote mentorship opportunities for those owners that are new to the breed.
- 2. Creating experiences for the "non-horse" public to enjoy and appreciate the breeds is an important part of building demand. Owners should be encouraged to attend local/regional events for the general public that allow hands on experience (ex: "hook" for parents "Are you tired of your kids being glued to their devices?") Other opportunities can be found in leasing or donating to equine assisted therapy programs
- Association members must learn how to "work the crowd". Associations should develop
 promotional handouts and books, and provide marketing messages along with stories. Ideally
 there would be two sets of messages, one for horse owners (Why this breed? Cost comparisons,
 etc.) and another for the non-horse owners (passion/pride for the breed, history, safety, cost of
 ownership.)

<u>Ideas and solutions – Marketing assistance</u>

Developing marketing skills for owners

The development of a database of skills and best practices will be useful for helping owners
attain the skills they need to market and sell horses. This could include a series of tutorials for
"DIY" social media such as the basics of how to find and use social media in a step by step
format and how to use algorithms to target markets. Associations can also develop a media kit
for owners to use at events including brochures, banners, flyers, and a media stick with quality
photos.

2. Request and develop recommendations of professionals of marketing that may be found among the members within the breed associations. The associations should seek out "Spin doctors" or writers to help produce content to reach their targeted audiences. These professionals can also help identify social media outlets (Facebook, etc.), and ways to market the horses to the news to tell your breed's story.

Exploring collaborative marketing between breeds and associations

- 1. Bringing a variety of breeds under a common banner for public venues (ex. Homesteader fairs, state fairs, etc.) could be a powerful promotional tool for all the associations. A master list of events could be created from individual input so as to highlight opportunities across the country. Reciprocal sharing of e-newsletters would further support an exchange of ideas between associations.
- 2. Regional or webinar clinics could be developed to help build marketing skills for all breeds. Until those can be developed, a rare breed marketing campaign on social media and an online marketing hub for all breeds will help connect prospective new owners with the breeders.
- 3. Building awareness of non-traditional sport disciplines and associations will help expand the scope of opportunities for rare breeds. Associations should identify members within that may already be active in specific disciplines and provide ways for these members to share their experience.
- 4. In order to brand the rare breed cause, an endangered equine logo could be developed and used to create promotional materials (for example saddle pad patches, fly hoods, etc.) that identify the horses as rare breeds when they are out in public.

Summit participants also created a list of potential funding sources to assist groups in developing these ideas. There was a wide range of ideas including: donation of services, grants, Regional Association of Grantmakers (RAG locator site https://www.unitedphilforum.org/find-your-regional-association) agricultural based community foundations, corporate sponsorship, universities (facilities and/or students to help with marketing, production, and media merge), private donations, online crowd sourcing, raffles, and fundraising campaigns.

Where do we go from here?



At the conclusion of the summit, it was clear that participants wanted to turn that one meeting into a longer lasting program to act on the ideas generated by the group. The Livestock Conservancy has committed to continue its leadership role and serve as the central hub for information and coordinate the programmatic ideas generated from the summit. The initiative, now known as the **Endangered Equine Alliance**, has become a collaborative effort including the groups that participated

in the summit along with new partners that continue to come forward and want to be involved. The website www.endangeredequinealliance.org is nearly complete and is now ready to be populated with information and educational materials. Social media content is being generated and will be launched once the website is complete in spring of 2018. A listing of participant organizations can be found in Appendix IV and will be posted online as well.

One surprising outcome of the summit came from our British counterparts. The leadership of Rare Breeds Survival Trust was taken by the energy and collaborative spirit of the summit and has now

planned to mirror the effort in their own country in October 2018. They will bring together the leadership of their rare breed equine associations for a meeting that is set to accomplish similar objectives that were reached in Texas. A representative from The Livestock Conservancy will attend the meeting to help facilitate relationships between the associations that work with breeds that the U.S. and the U.K. share in common.

More exciting developments continue to arise from post-summit activities. Because the information on assistive reproductive technologies was so well received, a more detailed manual is now being developed through scientists at Texas A&M University and The Livestock Conservancy. The manual will be geared toward owners and veterinarians so that they can have an understanding of what technologies are available and how sometimes simple sample collections can be taken by local vets. Those samples can then be sent to specialized reproductive labs that are able to "work their magic" to secure the critical genetics within. The manual is set to be launched in time for the December 2018 American Association of Equine Practitioners annual conference and will be available at no cost on line.

One of the greatest accomplishments of the summit was in the gathering of a wide diversity of groups in one place and enabling them to see the great possibilities in unifying to conserve their breeds. Together the group generated a powerful list of ideas and action points and now gives us all a clear direction on next steps. Efforts are underway by the Endangered Equine Alliance to take on each of the suggested action points and find the resources to make them a reality. The objective for 2018/2019 will be to secure funding for the hiring of a program coordinator who can work to develop rare breed events, provide technical assistance for breeders and owners, and act on the ideas generated at the conclusion of the summit.



We sincerely admire and respect the remarkable group of people who came together at the summit. We thank them for their time and effort and hope that together, we will turn the tide for rare horse breeds in America.

The Livestock Conservancy

Appendix I

Counting Our Equines

Planning for the 2018 National Endangered Equine Summit began with The Livestock Conservancy collecting current census numbers for each of the breeds listed on their Conservation Priority List. This was a challenging task because there is no uniformity in the way registries report on the status of their breeds in the United States or even globally. This point has generated discussion among the registries and it is hoped that in the coming years that will change. In the meantime The Conservancy was able to collect the following data on the breeds and placed them in the appropriate categories within our 2018 Conservation Priority List.

<u>Donkey Breeds – Critical: Fewer than 200 annual registrations, estimated global population less than</u> 2000

American Mammoth Jackstock

- 2016 annual registrations ~300 per year for the past several years reported by American Donkey & Mule Society
- 2015 annual registrations 60-70 reported by American Mammoth Jackstock Association

Poitou Donkey

- 2017 No annual registrations reported, The Livestock Conservancy estimates <60 purebred left in the U.S.
- 2014 The United Nations Food & Agriculture Organization (FAO) reports 515 Poitous in France, no numbers from U.K. or Belgium

Pairau dankey

<u>Donkey Breeds – Recovering: Numbers are good but breed still needs monitoring</u> Miniature Donkey

• 2016 annual registrations 938 down from a high of 4000 registrations in 2008, estimated current total population 20,000 reported by the American Donkey & Mule Society

<u>Horse Breeds – Critical: Fewer than 200 annual registrations, estimated global population less than</u> 2000

American Cream Draft

- 2017 fully registered horses 203 mares and 207 males (includes geldings) with 8 foals reported in 2016 by the American Cream Draft Horse Association
- 2012 annual registrations 7 with estimated 210 mares and 6 active breeding stallions reported by ACDHA

Canadian

 2017 annual registrations 143 with 1090 stallions and 4263 mares between the ages of 3-30 reported by Canadian Horse Breeders Association

Caspian

 2016 Livestock Conservancy census estimates approximately 600 horses total and annual registrations <20 per year

Cleveland Bay

- 2017 annual registrations 3 with 43 stallions and 73 mares reported by the Cleveland Bay Horse Society of North America
- 2017 population 35 stallions and 67 mares including 7 births for 2017 reported by the International Cleveland Bay Registry

 2016 annual registrations 20 with 51 breeding mares reported by the Cleveland Bay Horse Society (UK)

Dales Pony

- 2018 an estimated 5-8 stallions and 10-20 mares actively breeding reported by U.S. breeders to TLC noting many mares aging out
- 2011 annual registrations 5-10 per year with approximately 300 horses in the U.S. including 22 stallions and 100-125 mares reported by the Dales Pony Society of America Inc.
- 2016 annual registrations 77 with 267 breeding mares reported by the Dales Pony Society (UK)

Dartmoor

- 2017 annual registrations 18 and in 2016 there were 20 with an estimated population ~524 reported by the Dartmoor Pony Registry of America
- 2016 annual registrations 72 with 456 breeding mares reported by the Dartmoor Pony Society (UK)

Exmoor

 2016 annual registrations globally 111 with 462 purebred females reported by Exmoor Pony Society (UK)

Fell Pony

- 2017 annual registrations 39 with 27 licensed stallions, 227 mares, and an estimated population of 450 reported Fell Pony Society & Conservancy of the Americas
- 2016 annual registrations of 95 mares and 107 stallions with estimated 640 total breeding mares reported by Fell Pony Society & RBST (UK)

Galiceño

 2017 annual registrations <10 with 9 stallions and 40 mares reported by the Galiceño Horse Breeders Association

Hackney Horse

- 2017 annual registrations 24 reported by American Hackney Horse Society
- 2016 annual registrations 14 reported by AHHS
- 2016 annual registrations 52 with 182 breeding mares reported by the Hackney Horse Society (UK)

Morgan – Traditional

- 2017 annual registrations 15-18 with ~1500 total reported by the Lippitt Club, Inc.
- 2017 annual registrations 60 with 80 stallions and 300 mares reported by the Cornerstone Morgan Horse, Inc.
- 2013 annual registrations 14 reported by the Lippitt Morgan Horse Registry

Newfoundland Pony

- 2017 annual registrations 27 with estimated breeding population of 64 stallions, 167 mares,
 2016 annual registrations 44 reported by the Newfoundland Pony Society
- 2015 90 breeding stallions reported by NPS
- 2013 400 horses total reported by NPS

Suffolk

- 2016 annual registrations 80 with 44 stallions, 163 mares, and 1586 horse total reported by the American Suffolk Horse Association
- 2015 annual registrations 57 with 24 stallions, 33 reported by ASHA
- 2016 annual registrations 27 with 73 breeding mares reported by the Suffolk Horse Society (UK)



<u>Horse Breeds – Threatened: Fewer than 1000 annual registrations, estimated global population less</u> than 5000

Akhal-Teke

• 2017 annual registrations have averaged <20 over the past decade, less than 500 in U.S. with global estimate of 3500-5000 reported by Akhal-Teke Association of America

Colonial Spanish – Multiple Strains

- 2016 annual registrations 62 reported by the Spanish Mustang Registry
- 2016 stallions 785, mares 1417 with total of 2202 reported by the Horse of the Americas

Colonial Spanish – Individual Strains

Baca-Chica

- 2017 annual registrations 4 with 2 stallions, 16 mares, and 33 total horses reported by the Baca Colonial Spanish Horse Alliance
- 2017 Baca family census (including BCSHA horses) include 2-3 stallions with 4 more in process of approval, 31 mares (3 in foal), and 3 full Baca foals reported by Annette Baca-Garcia

Banker

- 2017 population estimated 120 on Shackleford reported by Foundation for the Shackleford Horses
- 2017 population estimated <100 on Corolla reported by The Livestock Conservancy



Choctaw

- 2018 registered horses 193 mares and 56 stallions with 21 new registrations in 2017 reported by the Southwest Spanish Mustang Registry
- 2012 total population estimated 200-300 reported by The Livestock Conservancy

Florida Cracker

- 2017 annual registrations 33 reported by the Florida Cracker Horse Association
- 2016 registrations 30 reported by FCHA

Wilbur-Cruce

• 2017 total population 111 with 8 stallions and 43 mares reported by the Spanish Barb Horse Association

Marsh Tacky

- 2017 annual registrations 32, with 100 stallions (11 actively breeding) and 222 mares reported by the Marsh Tacky Pedigree Registry
- 2016 annual registrations 43, with 92 stallions (only 8 actively breeding) and 214 mares reported by MTPR

Santa Cruz

- 2017 annual registrations 11 with approximately 60 horses total, *note 28 horse herd dispersed and currently unaccounted for in FL reported by Santa Cruz Horse Pedigree Registry
- 2016 annual registrations 16 with < 50 horses total reported by SCHPR

Sulphur

• 2018 registered horses 25 stallions and 33 mares reported by the Horse of the Americas Registry **Gotland**

- 2014-2017 annual registrations total 15 with an estimated population of <100 reported by Swedish Gotland Breeders Society
- 2009-2017 annual registrations total 5 and an estimated population of less than 200 reported by Gotland Pony Preservation Society

• 2011 population estimate approximately 5000 with 135 stallions and 1000 mares reported by the Swedish Board of Agriculture

Lipizzan

- 2017 annual registrations 32 with 54 stallions, 126 mares, and 929 total populations with additional horses being considered for breeding in 2018 reported by the US Lipizzan Federation
- 2017 estimated North American population 1800, with approximately 50 annual new registrations, reported by the Lipizzan Association of North America.

Clydesdale

- 2017 annual registrations 354, 2016 annual registrations 351, 2015 annual registrations 330 reported by the Clydesdale Breeders of the USA
- 2016 annual registrations 221 with 803 breeding mares reported by the Clydesdale Horse Society (UK)



Mountain Pleasure

• 2017 estimated 2000 horses in the U.S. but only 3 foals produced in 2017 and 1 in 2016 reported by Mountain Pleasure Horse Association

Shire

- 2017 annual registrations 108 (50 stallions & 47 mares) and estimated 2500 total reported by the American Shire Horse Association
- 2016 annual registrations 101 (37 stallions and 54 mares) reported ASHA
- 2016 annual registrations 235 with 978 breeding mares reported by the Shire Horse Society (UK)

<u>Horse Breeds – Watch: Fewer than 2500 registrations, global population less than 10,000</u> Rocky Mountain

 2017 over a seven year period average of 350 horses registered each year with 1450 active breeding mares and 450 active breeding stallions during that period reported by Rocky Mountain Horse Association

Belgian

- 2016 annual registrations 2105 reported by the Belgian Draft Horse Corporation of America
- 2011 annual registrations 3000 reported by the BDHCA

Brabant

- 2015 total population includes 20 mares and 15 stallions, 4 additional stallions imported after
 2015 reported by the American Brabant Association
- 2013 estimated population in Belgium is 1774-2811 reported by the Belgian Department of Agriculture



Appendix II

Assisted Reproductive Technologies for Conservation Breeding of Equines

Charlene R. Couch and Katrin Hinrichs

Conservation of rare or endangered horse or donkey breeds depends on successful reproduction to propagate the animals and to conserve the genetic health of the populations. Despite the continued genetic health of some breeds, in general, rare breed foal registrations are down by 50%. The time to start conserving these genetic resources is now. Recent advances in assisted reproductive technologies (ART) are now available to support equine conservation efforts. Cryopreservation of semen, embryos and somatic cells can extend the reproductive lives of both mares and stallions.

Semen collection for cryopreservation—Semen can be collected from a stallion as he mounts a mare, or a phantom ("dummy") mare, and ejaculates into an artificial vagina. When a suitable mount is unavailable, e.g. for ponies, semen can often be collected by placing an artificial vagina on the penis while the stallion is standing. A teaser mare may be required. In the laboratory, the collected ejaculate is centrifuged to remove the seminal fluid then the sperm is diluted in a cryoprotective extender and frozen at about 100 million sperm per straw. A single breeding dose is typically 200-300 million progressively motile sperm. The number of straws needed depends on



the quality of the sperm after thawing; typically about 1 billion sperm total is used per dose. Once the sperm are frozen in liquid nitrogen, they can be stored indefinitely, with an estimated half-life of ~50,000 years. The average stallion produces 4-8 doses per collection. Semen is typically collected every other day. Very young stallions and older stallions may have fewer sperm or lower-quality sperm, but 80-90% of adult stallions produce freezable semen. Use of cryopreserved sperm is often more efficient than is shipping a stallion or a mare and makes possible the national or international shipment of semen. One series of collections yields multiple doses that can be used over many years, far extending the natural breeding life of the stallion. Frozen semen can be preserved long-term in a semen bank for an unforeseen future. The frozen semen (as well as other genetic material such as embryos) must be stored in a dedicated semen-storage facility to avoid the possibility of liquid nitrogen failure and thus loss of the samples. Dividing the sample among locations can further safeguard the collection. One estimate of the cost to house such samples is \$50/quarter for 250 straws, but some facilities may offer lower prices. Collection and freezing of semen can cost about \$500 -\$750 per ejaculate; typically with board and multiple collections to provide a reasonable supply of frozen semen for future use, total cost is \$2500-\$3500.

Chemical ejaculation—In situations where standard semen collection is not possible, chemical ejaculation can be used to collect sperm for cryopreservation. The stallion does not have to travel to be collected, but can instead be collected at home under the supervision of a trained veterinarian. A medication is administered to reduce the threshold for ejaculation, which sedates the horse. In many cases, sedation will cause ejaculation and the concentrated fresh sperm can be harvested and shipped directly to a cryopreservation facility. Cost is variable for the sedation procedure (your veterinarian); cryopreservation of shipped semen can cost about \$500-\$750 per ejaculate.

Epididymal sperm collection—In the event of injury or death, or at gelding (if the testes are mature enough), testes and their associated tissues (epididymides) can be harvested and shipped cool to a freezing facility where sperm can be recovered from the epididymides and frozen. It is possible to retrieve many (30-50) breeding doses of frozen sperm from epididymal tissue of a normally-fertile stallion. For best results, freezing of semen should be completed within 48 hours of tissue harvesting. Cost of harvesting the epididymal sperm is \$500-\$750. Cryopreservation of the recovered sperm can cost about \$750-\$1000.



Oocyte harvesting from live mares—Mares may not be able to carry a pregnancy to full term due to a number of causes. In such cases, oocytes (unfertilized eggs) can be collected from the mare's ovaries and shipped to a laboratory for fertilization. The resulting embryos can be carried to term in a recipient mare or frozen for later embryo transfer. The half-life for frozen embryos is estimated to be ~50,000 years. Cost is ~\$1000 for the oocyte harvest. Costs for the fertilization procedure (ICSI) are given below.

Post mortem oocyte recovery—The death of a mare does not mean that her reproductive potential must be lost. Instead, ovaries can be harvested post mortem and shipped at room temperature to a laboratory. This must be done quickly; best results are obtained if the laboratory receives the ovaries within 6 hours of death. Unfertilized oocytes are not stable for freezing. Oocytes can be recovered from the fresh ovary and introduced surgically into an inseminated recipient mare for immediate fertilization, or the oocytes may be fertilized in the laboratory and the embryos frozen or transferred. Therefore, at the time of ovary recovery, steps should be taken to make suitable semen available to the laboratory. Cost is variable for ovary removal (your veterinarian); ~\$500 for dissection of the ovary to recover oocytes. Costs for the surgical transfer to an inseminated mare are ~1500 per surgery; costs of the laboratory fertilization procedure (ICSI) are given below.

ICSI—intra-cytoplasmic sperm injection—Oocytes harvested from live mares or harvested post mortem can be fertilized by the injection of a single sperm into the cytoplasm of the oocyte. This technique is especially useful when numbers of oocytes or sperm are limited or sperm are of lower quality. The resulting embryo can be cryopreserved for later use or placed into a recipient mare to produce a pregnancy. The cost is about \$500-\$1000 for the oocyte maturation and ICSI procedures, then most laboratories charge an additional \$500 to \$1000 for each embryo produced as a result. The cost to transfer an embryo to a recipient mare and purchase the pregnant recipient is typically ~\$2000-\$5000.

Somatic cell preservation and cloning—Cloning can be used to produce a live foal having the same genetics as the donor animal. Cloning involves transferring the nucleus of a somatic cell (usually a skin cell) of a donor animal into a host oocyte whose own nuclear material has been removed. The oocyte is stimulated to divide and develop into an embryo, which can then be transferred to a recipient mare. A clone has the nuclear genetic material of the original skin cell donor. The purpose of cloning for conservation is not necessarily to produce a copy of a horse for performance, but instead to produce a clone that is a genetic twin of the somatic cell donor. This cloned animal can be used for breeding. This is especially valuable if the original animal was lost or was gelded before his or her genetic value was known. Although the cost of cloning an equine is high (currently about \$70,000 commercially), prices continue to decrease. In any event, tissue harvesting (needs only a pea-sized bit of skin tissue from the donor animal) and freezing of somatic cells from important animals is both highly feasible and

affordable. The decision to clone the animal can be made in the future. Cost for tissue collection is variable (your veterinarian); processing of the sample for cell culture and storage is ~\$1000-\$2000.

Breed conservation using Assisted Reproductive Technologies.

These techniques can be used by the individual horse owner as they



see fit. When these techniques are used by breed associations to preserve selected genetics for long term use or as a "backup," associations should actively participate in selecting animals for preservation. The optimal collection is a broad representation of the breed, including both major and minor bloodlines. At the very least, the sample should reflect the genetic ratios in place at the time it is created. More optimally, the sample would contain equal representation of all genotypes present in the breed, whether currently desirable or undesirable. In addition, a protocol should be standardized to collect information on the origin of germplasm in the collection, for example pedigree of the donor, and phenotypic characteristics.

For breeds with populations in more than one country, it is worthwhile to investigate health testing requirements in all of the possible countries to which germplasm or tissue samples might be exported (including the U.S.A.) Following required procedures (such as quarantine and testing before collection and storage), including use of approved facilities, may add to the additional cost but this will preserve options for future transfer of critical genetics between countries.

Online Equine Assisted Reproductive Technology Resources Colorado State University: College of Veterinary Medicine Select Breeder Services Equine Medical Services

Texas A&M University: College of Veterinary Medicine & Biomedical Sciences. Equine embryo laboratory http://vetmed.tamu.edu/equine-embryo-laboratory; Theriogenology laboratory http://vethospital.tamu.edu/large-animal-hospital/equine-theriogenology/stallion-services.

Appendix III

Common Questions About Genetic Techniques for Conservation of Equines

1) What is the price for whole genome sequencing of a horse? Pricing for DNA genome sequencing continues to fall. Currently the cost of whole genome sequencing for a single individual is about \$1000. Interestingly, the cost for whole genome sequencing of 30 pooled individuals is also about \$1000. Leif Andersson suggested that it would be easy and affordable to pool resources within a breed association and sample a breed as a whole. Whole genome sequencing of 30 pooled individuals is useful for population genetic description of the breed, but sampling must not be undertaken lightly—sampling must either be completely random to capture the true structure of the population, or targeted such that the 30 samples truly represent the populations' genetic structure. This may necessitate herd book analysis in advance of sampling and working closely with the breed association. Samples should be divided equally between mares and stallions. If the entire herd consists of fewer than 30 horses, sample

them all. Landrace breeds, like the Colonial Spanish horse or Newfoundland Pony may need additional sampling to fully capture the genetic profile of the breed. Blood samples are best for this type of analysis, and parentage tested animals are preferred for this work. Collect and ship blood in clean, purple-topped EDTA Vacutainer-type tubes. Second day air is adequate for shipping fresh blood but tubes should not be in direct contact with cold packs—avoid freezing of blood samples! Equivalent pooling of DNA from each individual is critical to the accuracy of the analysis.

- 2) Is it possible to compare an older generation against a younger generation to see what is being lost as stallions age out? This analysis can be done using the techniques described above for DNA genome sequencing. Two pools (\$2000) would reveal differences in alleles between the older and younger generations. This could also be used to compare populations of the same breed in the U.K. and U.S.A.
- 3) Is there a written sampling protocol easily available to breed associations? Yes. This will be made available on The Livestock Conservancy resources page. TLC through its Endangered Equine Alliance will act as central hub to connect everyone with information and resources on genetics and assistive reproductive technologies.
- 4) When setting up a semen bank, the choice of stallions to be banked is very important. It is important to maintain a diverse representation of stallions in the bank, not just a few popular sires, in order that the genetic structure of the population is represented as well as possible. Representation of uncommon or unpopular lines can be important, despite their commercial value at present. Phil Sponenberg weighed in that caution must be taken to collect a wide sampling within the breeds and not just the "best of the best" since the effort will fail to capture diversity within the breed. It is also important to collect as much phenotypic information as possible at the time of collection for future work, and to take somatic cell samples for possible cloning work. Tullis Matson commented that it time of collection can be dependent on the breed. Some stallions cannot be collected until they are 4 or 5 years old as is the case with the Suffolk breed. Collecting from stallions that are too old is not optimal but Katrin Hinrichs noted that even if the semen is not optimal, it can still be used by utilizing intracytoplasmic sperm injection (ICSI) techniques which is now commonly done. She suggests freezing what you can despite quality. Another option is somatic cell collection for the production of clones. Katrin said the current cost is about \$70K to produce a clone but the costs are constantly becoming less. This is half of what it cost 5 years ago. For cloning you need a pea sized tissue sample. You can also get useful material from harvested testes in a horse that has suddenly died or is to be put down. Pack the organs cooled, not frozen (not to touch ice pack in box) and ship next day to facility that can harvest the material. Collecting testicles from young geldings may not produce viable semen but the tissue could be used for cloning later. We can also look to the American Zoological Association's Species Survival Programs (SSP's) for models for conservation of material. The Rare Breeds Survival Trust semen bank starts with RBST allocating funds for collection. The money pays for the collection which is suggested to be saved

- in thirds one third for use by the owner, another to the semen bank where it can be used if needed, and a third for a doomsday scenario and used only in extreme circumstances.
- 5) Who owns the data? Ownership of the data depends on who owns the horse (s), where the work is done, and who provides the funding. Scott Dindot suggested that if the data are entered into a public database, the data are then open to the public for viewing. Most laboratories are affiliated with universities. Their "payment" for the scientific work is to be able to generate a thesis or manuscript from the work and once published, the manuscript is available to the public. Data can be shared across laboratories if the proper controls are in place.
- 6) Shipping oocytes from Canada requires only a Coggins test. Shipping semen across country lines requires an approved quarantine and collection center and stallion health testing that may cost as much as \$1500. It's easier to ship from U.S. to U.K. verses U.K. to U.S. These USDA import/export protocols were developed by countries based on disease history and are important for protecting the herd health of the country. Contact the American Horse Council to intercede if having problems with importing or exporting. It was mentioned that collaborative activities have increased greatly between the U.S., Canada, and the U.K. in recent years. The idea of using DNA analysis on all sides and having an agreed best practices agreement was mentioned.
- 7) Is there value for mitochondrial DNA (mtDNA) studies? Horses (and all mammals) contain DNA in their cells at two locations: the nucleus, where the chromosomal DNA resides, and the mitochondrion, the power house of the cell, where a short bit of circular DNA resides. Total DNA extraction contains both nuclear and mtDNA. In some cases, lost female lineages can be identified with mtDNA. Using mtDNA analysis can be valuable for identifying a suitable oocyte donor (to get a genetically similar cytoplasmic, and therefore, mtDNA portion) if the donor mare's ooctyes cannot be used.



Appendix IV

Endangered Equine Alliance Partners

Breed Associations & Registries

Akhal-Teke Association of America

American Cream Draft Horse Association

Dales Pony Society of America, Inc.

Dartmoor Pony Registry of America

American Donkey and Mule Society Exmoor Pony Society

American Hackney Horse Society Fell Pony Society of North America
American Heritage Horse Association Florida Cracker Horse Association
American Indian Horse Registry Foundation for Shackleford Horses

American Mammoth Jackstock Association Galiceno Horse Registry

American Mammoth Jackstock Registry Gotland Pony Preservation Society

American Shire Horse Association Horse of Americas

American Suffolk Horse Association International Cleveland Bay Registry LLC

Arabian Horse Association Irish Draught Horse Society of North American Australian Suffolk Punch Registry Lipizzan Association of North America

Baca Colonial Spanish Horse Alliance Lipizzan Rescue Foundation

Baca Horse ConservancyMountain Pleasure Horse AssociationCanadian Horse Breeders AssociationRocky Mountain Horse AssociationCarolina Marsh Tacky AssociationSouthwest Spanish Mustang Association

Caspian Horse Society of the Americas Spanish Barb Horse Association Caspian Registry Services Spanish Mustang Registry

Cleveland Bay Horse Society of North America Swedish Gotland Breeders Society/Gotland

Clydesdale Breeders of the U.S.A. Registry Services
Cornerstone Morgan Horse, Inc. The Lippitt Club

Corolla Wild Horse Fund United State Lipizzan Federation

Universities & Technical Service

Brooks Equine Genetics Laboratory Texas A&M University

Mississippi State University

ViaGen

Stallion AI Services

Virginia Tech

Organizations

American Horse Council National Animal Interest Alliance

Colonial Williamsburg Natural Lifemanship
Carolina Marsh Tacky Outfitters Rare Breeds Survival Trust

Heritage Livestock Canada TREC USA

....And the list continues to grow. A regularly updated list will be maintained on the Endangered Equine Alliance webpage.