Comparing the physiological and biochemical parameters of mules and hinnies to horses and donkeys

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Figure 1. Mules and hinnies comparing the commonalities and differences of these hybrids to their parents the donkey and horse. One of the most challenging things to do is distinguish a hinny from a mule based only on its appearance, which is the mule and which is the hinny in this figure?

Abstract: Mules and hinnies are hybrid offspring from donkeys (*Equus asinus*) and horses (*Equus caballus*). Little scientific information is known regarding mules and even less is known about hinnies the reciprocal cross. There have only been a few studies that have attempted to define commonalities and differences of these hybrids especially in terms of comparing and contrasting them to their sires and dams (donkeys and horses). This presentation will take a closer look at hinnies and mules from around the world and how they are used today as well as comparing both physiological and biochemical parameters of these unique creatures while addressing many of the fallacies and myths commonly associated with such equids with special attention being paid to hinnies. Recent studies conducted by the author and colleagues on comparing physiological, haematological, and biochemical parameters of both hinnies and mules from several countries will be shared in this review. In addition, interviews from both hinny and mule owners and breeders from Mexico, Colombia, US, Spain and Portugal will also be included in the presentation for a more comprehensive understanding of behaviour and training and how hinnies and mules are used today (Figure 1).

Introduction: Until recently very little scientific research or time has been dedicated to the working equid or beast of burden. Interest from many nongovernmental organizations, charities and some universities with faculty interested in persevering indigenous breeds of donkeys or donkeys being used for milk production have lead to an

increased amount of scientific data found on donkeys. An interest from veterinarians in industrial countries has also increased due to the number of people purchasing donkeys as companion animals and a few for performance or recreational purposes. Veterinarians and owners treating or caring for donkeys generally learn quickly they are not “horses with big ears” and there are differences in their behaviour, nutritional needs, hoof care, and medical treatment (e.g. sedation).

The increase in donkey owners in countries such as the US as well as the UK has created a demand for donkey knowledge (e.g. the start of the Donkey Welfare Symposium at the University of California at Davis, Ca). In addition, there has been a surge of studies and research published by Italian Universities and researchers where the donkey dairy industry has begun to boom due to use of donkeys’ milk for human consumption. Many of the studies being performed and published from Italy are very applicable to all donkey owners and such work is helping bridge gaps in knowledge such as improved feeding protocols and enhanced understandings of biochemical profiles of donkey blood chemistry. However, the increase in resources and scientific information about the donkey has still left owners and veterinarians in the dark about the hybrid offspring’s of the donkey and horse, the mule and hinny.

Generally, the first question a new mule owner will ask is which parent should the animal be compared to, the horse or the donkey? That’s a great question and the answer is not so simple as always compare a mule to the horse and or always compare a hinny to a donkey. In reality, we don’t know and current research has shown that some parameters a mule may resemble more closely its’ dam (the horse) and in other cases the mule and hinny may stand-alone and are not like the horse or donkey (McLean and Wang, 2013 and McLean et al., 2014). More importantly is how we can distinguish a mule from a hinny, since we have found some physiological and biochemical differences (McLean et al., 2014). This paper will review studies and owner/breeders’ perspectives of mules and hinnies to provide a more comprehensive understanding of these animals. The objective of this work is to attempt to compare and contrast both physical and physiological parameters of mules and hinnies to those of their parents, the horse and donkey, to help provide a better understanding of the similarities and differences.

Attitudes and Perceptions towards Mules and Hinnies: We know from speaking with owners in various countries why some prefer mules to hinnies or vice versa. In most countries mules are considered the best choice for work yet they are not always available. In Central and South American countries mules are still used for draught purposes such as farming and packing goods (e.g. coffee). In some areas in Mexico, dealers will sell mules imported from the US to local farmers and range in price from $1,500-3,000 depending on size. In southern Mexico, Cattlemen Associations have imported larger jacks from the US for mule breeding purposes and if you are a member of the association then you can breed for free to the jack. In other countries such as India, the government actually has a mule-breeding program and will supply mules to farmers for farming and hauling bricks. In South America mules are generally prized animals and used mainly for work on cattle ranches. One breeder in Colombia supplies large Catalonian type donkeys to ranchers for mule breeding purposes. Those that do not have access to mare horses often times raise hinnies. Then in some countries such as Mali, West Africa you will only find donkeys and a few horse but no mules. However, other countries such as Morocco or Ethiopia in Northern Africa you will find many mules and hinnies used for draught purposes.

Figure 2. A hinny from Miranda, Portugal an area where there are many hinnies used in the communities for ploughing potatoes and helping harvest grapes. Although, most of the hinnies are quite old averaging 24.3 + years and soon there will be less hinnies in this area and less people using hinnies.

In Baja, Mexico cattle ranchers prefer hinnies to mules due to the very rough and desert type terrain. The ranchers believe that hinnies perform and work better in this dry, desert climate and can withstand longer periods without water compared to a mule. This attribute more closely resembles characteristics of a donkey versus a horse (Corazon Vaquero, 2008). Other places like Portugal, Colombia, and Mexico, hinnies are found based on the availability of female donkeys and mares. In some communities it's easier to find mares to breed with jacks (male donkeys) and some times in more resource poor communities donkeys may only be available, therefore, a female donkey is then mated with a stallion to produce a hinny. In some cases the hinny has even been referred to as the "poor man's mule."

Figure 3. Hinny being used for cow working competition at the World Mule Show in Bishop, CA (World Champion Cow Mule (Hinny), LeMoan and Walter Nunn, Bryan, Texas)

Breeding for Mules and Hinnies: Hinny breeders in many countries have all shared challenges with raising hinnies. One such challenge is convincing a stallion to breed a female donkey but the same can be true when asking a jack to breed a mare horse. Some breeders have even begun using artificial insemination and to improve the size and quality of hinnies being produced. In Colombia like in the US, the perception has generally been that hinnies are smaller and somewhat inferior to mules. However, some breeders have started using artificial insemination and using semen for example from champion Paso Fino horses to produce outstanding hinnies to prove there's little to no difference in size or quality when producing hinnies. However, some breeders have also reported difficulties in getting jennies to settle or conceive when breed to a horse. Several theories have been proposed that the decreased conception rates are due to acrosomal differences on the acrosome of the spermatozoa of the stallion compared to the donkey and other theories have been based on ideas that the intrauterine environment is somewhat different in a jenny compared to a mare. Generally, once a stallion is found to breed jennies and produce live foals, that stallion will continue to be used for hinny production (refer to Figure 4).

Figure 4. A stallion in the state of Veracruz, Mexico used for breeding to jennies (female donkeys) for hinny production.

Reports of Fertility in Mules and Hinnies: There have been several reports of hinnies and mules having foals. A hinny stallion imported from Texas in the late 60's was reported to produce live and mature spermatozoa's (Trujillo et al. 1969). However, there was much controversy over the fertile animal but it's possible. Another case of a fertile mule in China during the late 80's later turned out to be a fertile hinny that produced a live foal named Dragon Foal (Rong et al., 1985, see Figure 5). Figure 6 refers to the schematics and genetics of hinnies and mules that produce live foals and if they are mated with a horse or jack and what the outcome would be. More and likely there are more fertile mules and hinnies than we know of but most people do not attempt to breed them. Depending on where they are born and the beliefs associated with mules and hinnies having foals there's probably more live foals born in developing countries than we know of. In some cases the live foal of a mule or hinny maybe seen or considered a blessing and in other cases a curse. Recent reports of live foals from mules have come

from Colorado and Morocco. Some horse breeders are now using mules as recipient mares/mothers to carry horse foals due to their outstanding mothering abilities.

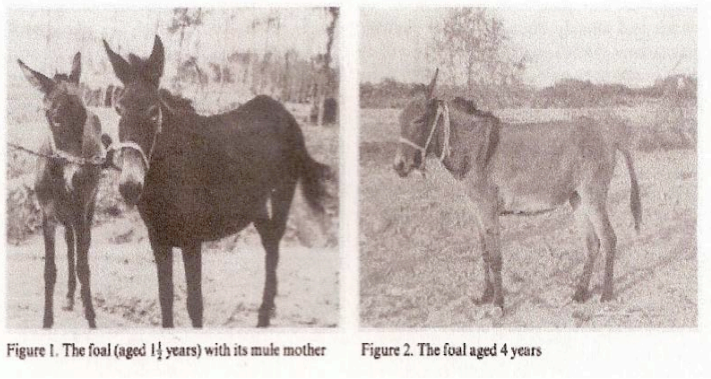

Figure 1. The foal (aged 1½ years) with its mule mother

Figure 2. The foal aged 4 years

Figure 5. Mule mother produced a live foal named Dragon Foal in China in 1981 (Rong et al., 1985).

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Table 1 Maternal chromosome-passing pattern in hybrids of horse and donkey[a]

	Mule (♀, 2n = 63)	Hinny (♀, 2n = 63)
Horse (♂, 2n = 64)	Horse (♀ or ♂, 2n = 64)	Hinny (♀ or ♂, 2n = 63)
Donkey (♂, 2n = 62)	Mule (♀ or ♂, 2n = 63)	Donkey (♀ or♂, 2n = 62)

[a]The first row and column are maternal and paternal parents, respectively, and the rest are the offspring from the corresponding parents in the first row and column.

Figure 6. Fertile hinny and mule schematics when bred with a horse or donkey.

Physiological Parameters of Mules and Hinnies: Even to a well-trained eye, it's difficult for one to distinguish a mule from hinny based solely on physical appearances (refer to Figure 1, which animal is the hinny and which is the mule?). A few areas that seem to be different compared to a mule but again they do not always significantly show a difference is in the face, loin and hip area.

Comparing and contrasting conformation: Many hinnies tend to have a longer space from the eye to the bridge of the nose. This area in the forehand is more pronounced or dish shaped when compared to a mule. The loin of a hinny similar to a donkey is often longer and the croup may be shorter and stepper again resembling that of the donkey. However, these slight differences are not always true and it's still more reliable to ask the owner if the animal is a mule or hinny. In countries such as Colombia or Mexico they may refer to the mules as "normal" and the hinnies as "Romo's." There are many fallacies associated with the conformation of the hinny such as their ears are shorter and their backs are longer and weaker. In fact, many hinnies have the same underline to

topline ratios and in some cases is what we call balanced better than some mules. Balance refers to how the equine's body if divided into thirds fits together, ideally with each third being equal in length. We have found in our studies that the length of the neck and back will generally be the same but the hips in all three equids (mule, hinny and donkey) is much shorter that what we would find in a horse. When we look at balance by measuring the top to bottom ratio, the underline (the area behind the elbow to the flank) compared to the topline (the top of the withers to top of the hip) we find in horses that a balanced horse will have a 2:1 ratio (underline is twice as long at the back or topline) but in a well balanced mule or hinny we have found this ratio to be 1:1 (refer to figure 7).

Figure 7. Measuring the length of the back of a hinny in Toro, Spain. The idea is that the equine can be divided into 3 equal parts and they are then considered to be balanced because the length of the poll to wither equals the length of the back and the length of the hip. We have found in mules, hinnies and donkeys that the length of the neck and back are often close but the hip falls short.

Behavioural Aspects: The ability to identify a "known" population of hinnies or distinguish a hinny from a mule can be challenging, considering their similar phenotypical characteristics. Generally this can only be accomplished if the owner who bred the animal knows the parentage of the animal. There are many fallacies associated with both hinnies and mules in terms of training. Some may claim a hinny is harder to train than a mule or vice versa but in all reality each animal is an individual and must be trained in that format. One of the biggest behavioural differences that maybe noticed is which animal a mule and hinny will associate with when roaming free in a pasture setting. Most people claim a hinny will associate the most with donkeys and mules will group or bond strongly with horses. Considering, each equid has spent an extended period of time with either a donkey dam or horse dam it only makes sense to watch them naturally pair with the same animal as their mother.

One may also assume that a mule and hinny when testing such physiological and haematological parameters would produce the same results considering the genetic backgrounds (*Equus asinus* x *Equus caballus*). The Donkey Sanctuary uses a reference

guideline for their internal work that groups both mules and hinnies together [7]. Granted, many variables can also be found when testing groups of mules and hinnies such as the difference in breeds of horses and donkeys used to produce mules or hinnies.

Temperature, pulse and respiration: When comparing vital signs such as temperature, pulse and respiration, we have found that a mule's temperature is more similar to the horse than the donkey but the hinnies' temperature is closer to that of a horse. A donkey's baseline temperature is around 98.6°F and a horse is approximately 100.5. A mule has been found to be 99.18 and a hinny 98.7. When measuring respiration we have the donkeys to have the lowest respiration rate, followed by the hinny and then the mule. However, there was no significant difference when looking at respiration in this study. When comparing heart rates amongst the equid groups we have found a significant difference amongst the four groups with the hinnies having the lowest heart rate 42.6 beats per minute (bpm) similar to that of the horse at 42.5 bpm compared to the mule at 43.3 and the donkey having the highest at 48. Additional measurements have recently been collected from a group of Paso Fino mules, hinnies, donkeys and horses. The preliminary data also suggested the hinnies had a lower heart rate. One hinny that recorded one of the lower heart rates was used throughout the week in Colombia as a trail mount and was very quiet and easy going on the trail. It maybe possible to suggest that heart rate reflects an equid's attitude or level of reactivity.

The populations of mules and hinnies used in the second study that's represented in tables 1, 2 and 3 were unique considering that they came from similar breeds of horses and donkeys (Spanish or Portuguese, such as the Zamorano-Leonés or Mirandês donkeys or middleweight Iberian type horse) used for both mule and hinny production, the ascendancy of each one of the hybrids was perfectly know and the hybrids were all located in the same geographical area in a healthy status. However, the population of hinnies was also significantly older than the other equid groups due to the fact that the owners of the hinnies were also aging and there was no renewed interest in breeding hinnies for draught purposes. The most recent study conducted in Colombia was also unique in the fact the animals were all of similar Paso Fino genetics and in some cases we have sampled the dam, sire, and multiple brother and sister pairs. This group of animals was also all managed under the same direction. Having such homogeneity in a population of animals is more ideal when testing for blood chemistry or conducted such studies for reference values due to previous differences in climate, management, and nutrition, genetics that may affect the outcome of blood profiles.

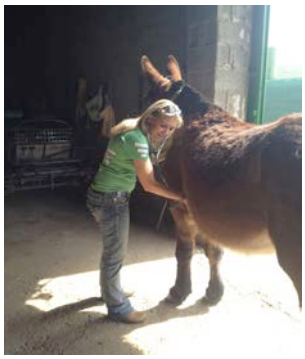

Figure 8. Taking the heart rate of a hinny in Toro, Spain. There's a significant difference found when comparing the heart rate of hinnies, mules, donkeys and horses in Spain and Portugal.

Knowing the vital signs of equids is important when diagnosing various conditions from bacterial infections, disease and colic. A stressed animal or one infected with a bacteria or even undergoing symptoms of colic will exhibit an elevated heart rate and increased respiration. An animal with an infection may show signs of an increased temperature but if you compare a donkey's temperature to that of a horse (98.6 to 100.5) you could easily misdiagnose such conditions and the same is true for the mule and hinny (Mclean et al., 2014).

Table I. Comparing significant differences of the hinny, mule, donkey and horse using the Kruskal-Wallis test (P < 0.05)

Parameter	Hinny	Mule	Donkey	Horse	P-value
Temperature °C	**37.0**	**34.5**	**36.6**	**37.5**	**0.05**
Heart Rate bpm	**42.6**	**43.3**	**50.5**	**42.5**	**0.01**
Respiration br/min	**29.5**	**34.5**	**21**	**34.2**	**0.44**

Biochemical Parameters: Physiological, haematological and biochemical values may vary according to many factors directly animal related (age, breed, gender, genetics, geographical and environmental variations, emotional status, physical activity / use of animal, diet), with many other factors also influencing the results obtained, such as the sampling technique or the laboratory analysis [Caldin et al., 2005, Folch et al., 1997, Greene et al. 2006, Gut et al., 2007, Grondin and Dewitt, McLean et al., 2014]. The number of factors that can vary will certainly increase the difficulty of comparing the present values from other studies conducted worldwide [Gut et al., 2007, McLean and Wang, 2013, Mendoza et al., 2011] but authors believe that the present values and intervals will help to establish reference information for hybrid equids, especially mules and hinnies. We have found differences when comparing all four equid groups as well as comparing mules to hinnies (refer to table II and III). When testing for blood chemistry it's important to realize that most lab standards are in accordance or based on horse values. Therefore, ideally owners should have a blood chemistry and hematocrit profile completed when their mule and or hinny is in a healthy state. For example you will notice there's a difference when looking at primary cell counts such as red and white blood cells for all four equid groups. If you only compared white blood cells to the standard (the horse reference value) then you may assume your hinny is sick or anaemic due to the lower cell count.

A significant difference was reported for blood chemistry parameters: red blood cells ($P = 0.003$), haemoglobin, hematocrit, MCV, and MCH ($P < 0.001$), Phosphorus ($P = 0.04$), Magnesium ($P = 0.01$), Glucose ($P = 0.04$), triglycerides ($P < 0.001$), creatine phosphorous ($P < 0.001$), aspartate aminotransferase ($P < 0.001$), gamma glutamyl transferase ($P = 0.004$), and lactate dehydrogenase ($P = 0.005$). In conclusion, many blood chemistry parameters were found to be significantly different when comparing the four groups of equid: horses, donkeys, mules and hinnies. The hinnies and mules were of similar genetics but differences in blood chemistry were also found in these two populations. Significant differences were found comparing red blood cell (7.3 ± 2.0 hinny, 8.7 ± 1.4 mule, $P = 0.006$), white blood cell (7.3 ± 1.9, 8.7 ± 1.4, $P < 0.006$), VCM (55.6 ± 0.8, 48.2 ± 5.3, $P < 0.001$), and HCM (19.8 ± 2.3, 16.6 ± 1.0, $P < 0.001$).

Figure 9. Comparing all four equid groups' serum can owners and professionals better manage these animals and properly prevent or treat diseases.

Table II. Comparing significant differences of the hinny, mule, donkey and horse using the Kruskal-Wallis test (P < 0.05)

Parameter	Hinny	Mule	Donkey	Horse	P-value
RBC	**7.3± 2.0**	**8.7± 1.4**	**3± 2.1**	**8.4± 2.3**	**0.003**
WBC	**7.3± 1.9**	**8.7± 1.4**	**9.3± 2.1**	**8.4± 2.1**	**0.003**
Haemoglobin	**11.96± 1.1**	**12.61± 2.3**	**10.46± 0.8**	**13.04± 1.8**	**<0.001**
Hematocrit	**34.3± 3.1**	**36.5 ± 5.9**	**31.4± 2.5**	**38.3± 5.1**	**<0.001**
MCV	**55.6± 10.8**	**48.2± 5.3**	**61.5 ± 7.1**	**49.1± 5.3**	**<0.001**
MCH	**19.8± 2.3**	**16.6± 1.7**	**20.5± 2.5**	**16.7± 2.2**	**<0.001**
Eosinophils	**5.9± 4.2**	**3. 3± 4.3**	**4.6± 4.7**	**2.8± 4.0**	**0.024**
Phosphorus	**2.49± 0.8**	**2.80± 0.8**	**2.92± 0.6**	**2.53± 0.5**	**0.04**
Magnesium	**1.55± 0.3**	**1.84± 0.3**	**1.65± 0.4**	**1.46± 0.3**	**0.01**
Bilirrubina	**0.73± 0.2**	**0.97± 0.3**	**0.30± 0.1**	**1.02± 0.5**	**<0.001**
Glucose	**92. 0± 18.9**	**85.2± 8.8**	**82.0± 10.4**	**91.7± 13.0**	**0.04**
Triglycerides	**45± 19**	**55± 18**	**103± 37**	**58± 27**	**< 0.001**
Creatine phosphorous	**255± 125**	**268± 144**	**134± 33**	**379± 324**	**< 0.001**
Aspartate Aminotransferase	**329± 65**	**391± 79**	**324± 67**	**445± 108**	**< 0.001**
Gamma glutamyl transferase	**19.4± 8.9**	**22.8± 10.1**	**31.2± 21.2**	**17.9± 12.6**	**0.004**
Lactate Dehydrogenase	**569± 235**	**646± 224**	**581± 228**	**889± 378**	**0.005**

(McLean et al., 2014)

Table III. Comparing significant differences of the hinny and mule different using the Kruskal-Wallis test (P < 0.05)

Parameter	Hinny	Mule	P-value
Red blood cells	**7.3 ± 2.0**	**8.7 ± 1.4**	**0.006**
White blood cell	**7.3 ± 1.9**	**8.7 ± 1.4**	**< 0.001**
MCV	**55.6 ± 0.8**	**48.2 ± 5.3**	**< 0.001**
MCH	**19.8 ± 2.3**	**16.6 ± 1.0**	**< 0.001**
Eosinophils	**5.9 ± 4.2**	**3.3 ± 4.3**	**0.014**
Magnesium	**1.55 ± 0.31**	**1.84 ± 0.33**	**0.007**
Bilirrubina	**0.73 ± 0.29**	**0.97 ± 0.10**	**0.01**
Creatine	**0.91 ± 0.11**	**1.05 ± 0.23**	**0.02**
Aspartate Aminotransferase	**329 ± 65**	**391± 79**	**0.004**

(McLean et al., 2014)

Conclusion: One would assume that a mule and hinny would exhibit similar or the same values for biochemical parameters McLean et al. (2014) suggests differences among the two hybrid crosses. Baseline values for mules and hinnies are invaluable veterinary science information for those involved in management and disease diagnostics (McLean and Wang, 2013, McLean et al., 2014). Overall, mules and hinnies have many fallacies to overcome but with continued interest from nongovernmental organizations working with working equids abroad and breeders continuing to produce mules and hinnies for recreation and performance purposes, hopefully, research will continue to focus on learning more about the behaviour, anatomical and physiological commonalities and differences of these unique creatures (Figure 10) to decrease the deficit in knowledge about an animal that has served mankind for thousands of years.

Figure 10. Paso Fino mules and hinny from Criadero Villaluz Farm in Colombia. These equids are used for showing, trail riding and helping harvest sugar cane. Blood chemistry and conformation samples were collected from all of these equids that are all closely related. Breeders such as these will continue to help inspire researchers to collect data on mules and hinnies to learn even more about them.

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