Genetics in Friesian Horses

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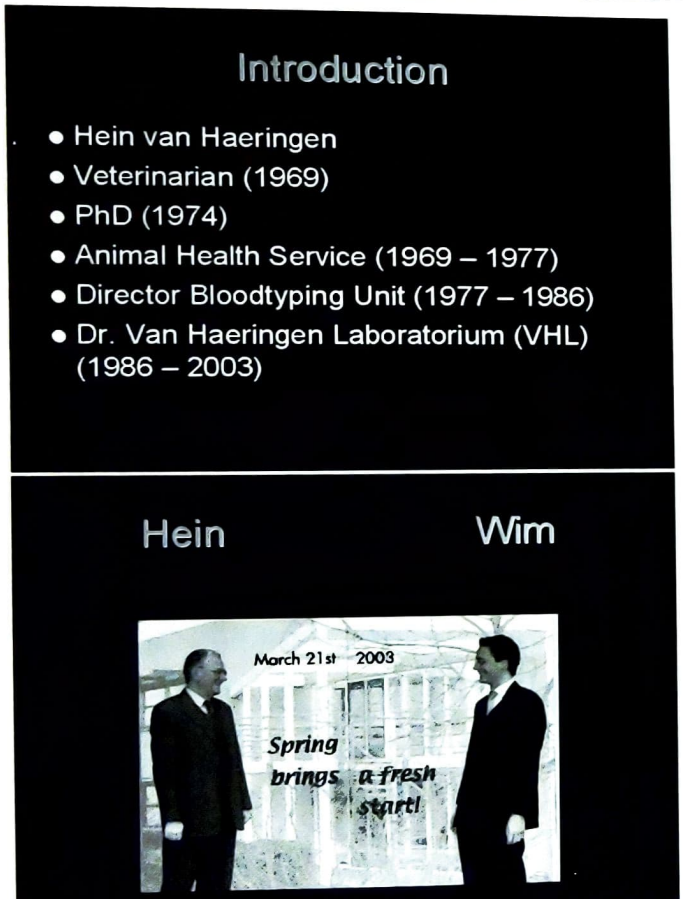
From notes taken by Laurie Kasperek



Dr. Hein van Haeringen was originally destined to be a farmer but plans changed when he attended the University of Utrecht and graduated with a veterinarian degree in 1969. He went back for further education and earned a PhD in 1974 in Bacteriology. He worked in various animal health and blood typing facilities for a time until founding the genetics laboratory that bears his name: the Dr. Van Haeringen Laboratorium (VHL) in Wageningen, The Netherlands.

The VHL employs over 30 scientists and researchers and a new branch has recently been opened in Belgium. The lab services breed registries of many types of livestock, governments, and universities around the world. They hold the DNA data for the KFPS and work closely with the studbook.

In 2003, Hein turned the helm of the VHL over to his son, Wim, who continues the excellence of the lab. Hein has contributed a number of articles to THE FRIESIAN, beginning in 2003 with his first contribution on neonatal isoerythrolysis (NI). He has continued to supply NI information to FHANA, as well as information about the possibilities of starting a genetic database for North American Friesian horses. Hein still travels the world in genetic consultations and FHANA was very pleased to have him as our keynote speaker at the 2008 AGM. The following notes were taken during the presentation by the editor of THE FRIESIAN.

The discussion will cover the differences between the external genetics and the internal genetics of a horse. External genetics is the phenotype of the horse, what is seen on the outside, while the internal genetics is the genotype. Blood typing will be discussed, in particular, parentage verification and NI. DNA basics will be discussed, leading to an explanation of DNA in parentage verification, in genetic disorders, and in the single-gene recessive autosomal and how this plays a role in our horses' genetics. Finally, a discussion on a genetic database will cover the collection of the data, the actual banking of the material and data, and the research that ultimately is the product of a database.

External Genetics (1)

- Start of the Studbook 1879
  Radboud 19 was born Sire Unknown
- Description of the breed
  Selection criteria
  Farm or Show?
  Riding Coaches
  Jumping or Dressage?
- Breeding
  Colour (Black??)
  Height
  Movement
  Hair growth

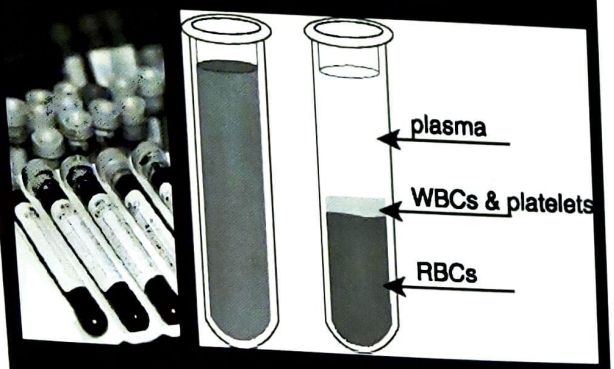
External Genetics (2)

Final goal

To choose the best one according to the goal of that time;

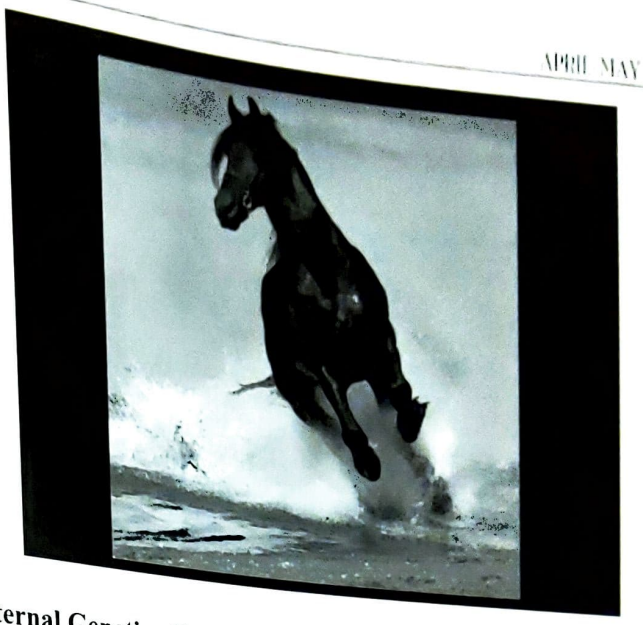
No knowledge to use internal genetic characteristics.

Blood



Internal Genetics

- Understanding genetics and tools to assess variation
  - Cattle were leading
    - Blood typing since 1950
    - Identical Twins
    - Parentage
  - Horses
    - Blood typing since 1970
    - Used for parentage & Studbooks
    - NI – Individual breeders



External Genetics (Phenotype)

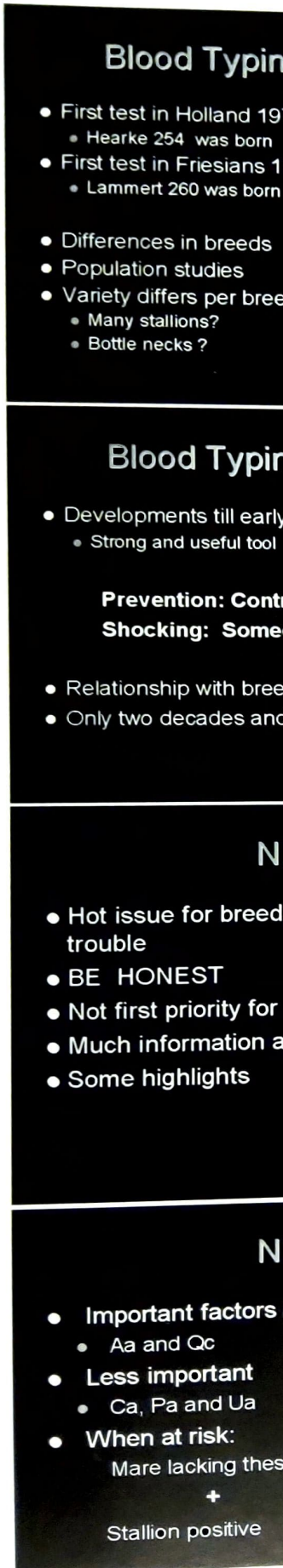
A description of the external genetics of Friesians goes back to the start of the studbook in 1879 when a description of the breed was set down. Radboud 19 was born to an unknown sire. The phenotype of the Friesian is used by the studbook to specify the selection criteria for approved stallions and for the premiums rewarded at inspections. The phenotype also determines the best usage of a particular horse; will the horse be better at farm work or on the show circuit, as a riding horse or a coach horse, or even as a jumping horse or a dressage horse. The phenotype is also used in breeding considerations. The studbook calls for black horses. In 1900 there were other colors in the Friesian horse, but black is the phenotype desired. Breeders also consider the horses' height, hair growth, and movement, all external genetic components.

The goal is always to choose the best phenotype according to the stated goal of the time. It took awhile before we understood the inside of the horse, the genotype. This is fairly recent so the phenotype was the only characteristic that could be used.

Equine blood oxygenates in 30 minutes. After this time the blood in a test tube will have a clear upper layer of plasma, a white layer composed of white blood cells, and a layer of red blood cells on the bottom. The white blood cell layer is the source of DNA for testing.

Internal Genetics (Genotype)

Parentage verification in animals came about first in cattle after the second World War. Researchers were able to identify identical twins through blood typing. For horses, blood typing began in 1970 and was used for parentage verification and the studbooks. It also began to be used by individual breeders for assistance in dealing with NI. The genotype is a tool to assessing the variation in a population.



Blood Typing Horses

Horses were first blood typed in Holland in 1973. This was the year that Hearke 254 (sired by Mark 232) was born. The first test in Friesians was in 1975. This was the year that Lammert 260 (sired by Bjinse 241) was born. There are DNA differences between breeds. For example, Dutch Warmbloods have a larger gene pool than Friesians. Since Friesians have lower numbers of genes in the population, the battery of tests must be extended. This variety between breeds can depend on a number of factors, including how many stallions are in the population and the number and extent of bottlenecks in the breed's history.

Blood typing was used until the early 1990's and it was a strong and useful tool. Prevention and control was possible, but the flip side of this was that it had a shocking component - someone could see my breeding errors!

Is there a relationship between blood groups and breeding characteristics? NO!

Blood typing only lasted for two decades and then it was replaced with DNA.

Neonatal Isoerythrolysis (NI)

Much has been published in THE FRIESIAN already regarding NI, so this will be an overview. NI has become an important and hot issue for breeders who have experienced the problem. Once experienced, it cannot be ignored. BE HONEST. Tell the owner of a mare of the NI risks because the buyer could lose a foal; this is a priority of all breeders and owners. It may not be a first priorit for the studbook, since it is a combination issue in the breedin but every breeder needs to understand and prepare for NI.

There are two active blood groups in Friesians: Aa and Qc. Le important are the blood factors Ca, Pa and Ua. The Qa that is fou in Thoroughbreds doesn't exist in Friesians. A foal is at risk of when a mare is lacking a factor and the stallion is positive for

The University of California at Davis has been doing the test f NI risk factors in the laboratory of Dr. Ann Bowling until he death. Without her leadership the lab stopped this activity and it is now housed in a local private clinic. The majority of NI testing is housed at the University of Kentucky, Lexington, so that is a logical place to have NI testing done. It is not recommended to spread information between 2 or 3 labs, but rather to conduct tests all in one location where possible. The contact at Kentucky is Dr. Kathryn Graves.

NI (3)

- Fairy tales
  - First foal not at risk
  - Introduction of foals to the mares after 3 days
  - Older mares without a previous NI history do not develop NI

Three times 'NO'.

NI (4)

- Comments & Remarks:
  - Origin of the placenta damage — Unknown
  - Titer — When positive?
  - New developments
    - All positive and high titers. De Graaf-Roelfsema et al. Vet Rec 2007 Aug 11;161(6) 202-4
  - Prevention
    - Testing all mares and stallions?
    - Families at risk?
    - Choosing partners

NI is genetics but do not call it a Disorder!

Genetics (1)

- Some statements
  - Genetics is not a fixed situation
  - Changing fast or slow
  - In viruses sometimes going fast
    - Bird flu now in human
      - Not yet spreading from human to human
  - Genetics changes in mammals slower
    - Litter size in pigs
    - Production and Reproduction

Genetics (2)

- Single gene effects
  - One gene, direct effect
  - Chestnut
  - Detection relatively easy
- Complex situations
  - Multiple genes interacting
  - Examples
    - Fertility
    - Height
- More difficult to detect; Step by step

There are a number of fairy tales associated with NI. One of them that is still heard, even among practitioners, is that a first foal is not at risk. This is very definitely false. Even colleagues have discovered this, much to their dismay and disappointment. Another widely accepted fairy tale is that the mare and foal only need to be separated for three days. Again, this has proven false. Why spend three days working hard to separate the mare and foal only to reintroduce them prematurely and have the foal die? It is safe to reintroduce the foal to the mare after FIVE days. And finally, the fairy tale that an older mare without any previous NI history will not develop NI is also false! She can and may indeed develop NI. It can be that the conditions were never just right in her past history to develop this problem before.

The origin of NI is still mostly unknown.

There is confusion regarding the titer results and what has been recommended. A titer before birth of 1:2 is not a positive, but a titer of 1:8 is positive and a muzzle is required.

A new development in NI has been published by DeGraaf-Roelfsema, et. al. They have found an antibody that is killing every red cell and the reasons are not known as yet. There are 7 to 10 documented cases of this.

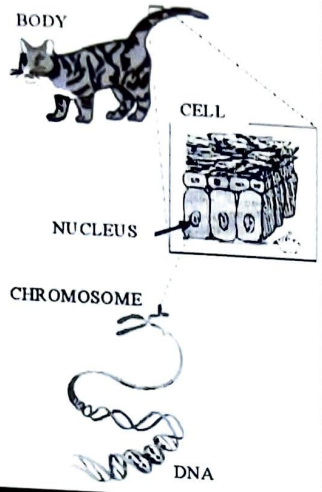
To prevent the birth of NI foals test all stallions and mares. Some families can have a higher frequency of NI, so the prevention is to choose another partner. Remember to test all donors and replacement colostrum prior to using. NI is completely nature - remember that NI mares DO NOT EXIST. NI is genetics but it is not a disorder!

Genetics

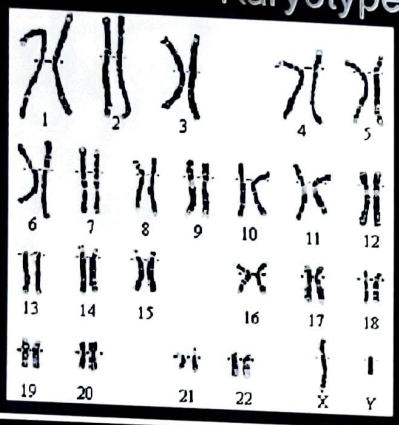
Genetics is not a fixed situation; changes occur at either a fast or a slow rate. Viruses, for example, can change rapidly and one we've all heard of is the bird flu. This has rapidly changed to now be found in humans. Genetic changes in mammals are much slower. These genetic changes are often seen in production and reproduction, such as the number of piglets born in a litter which has slowly increased over time.

Single gene effects can be detected relatively easily. An example of the single gene effect is the chestnut factor that the VHL isolated for the KFPS. On the other hand, multiple genes interact and they are more difficult to detect, taking more time. Examples of multiple genes interacting are fertility and height.

Mutations can occur in one of the DNA blocks - a portion can become missing, lost, flipped, and so on. Recent studies exploring sex linked infertility have found that the chromosomes are changed; pieces are switched or another chromosome is present that normally wouldn't be there.



Karyotypes



Human	23 pair
Cat	19 pair
Horse	32 pair
Cattle	30 pair
Goat	30 pair
Dog	39 pair

DNA Background (1)

- Important for animal breeding
  - Parentage verification
    - First test in Holland 1994 (Reitse 272) GRADUS 356 was born
    - Better conclusions than blood typing: more errors detected
    - Number of markers to be used depends on variety of markers within a breed
  - Population genetics
    - Differences between breeds
  - Marker Assisted Selection
  - Detection of mutations

DNA Background (2)

- DNA is the key to produce amino acids and proteins
  - If DNA is rearranged = MUTATION
  - Consequence: not the correct/ usual amino acids & proteins
  - Direct result: abnormalities
- Do not panic!
  - Not each mutation shows an effect
  - Not each abnormality is unpleasant
- Part of selection is based on mutations considered as positive

Chromosome - DNA

- Chromosome based on long chains of DNA
- DNA built of 4 blocks (A, T, G and C)
- DNA sequence: ATCGGTCGTAATGG

DNA Background

The slides show that the structure of a chromosome is long chains of DNA. DNA is built of 4 building blocks called A, T, G, and C. These four blocks, when arranged in sequences, form amino acids and proteins that comprise DNA. Specific numbers of paired chromosomes form the karyotype of every animal (left).

DNA is a better tool than blood typing and it has become very important for breeding. DNA in parentage verification was first used in Holland in 1994, the year that Gradus 356 (Reitse 272) was born. DNA uses more markers and better conclusions can be made than with blood typing. All countries use the same set of markers. A particular DNA marker could be used to come up with a better horse (but the first question is "What is a better horse?").

Mutations can be detected through DNA investigations. If one block of the sequence is missing than the correct amino acids and proteins are missing. Not every mutation will show an effect, though, and not every abnormality is unpleasant. Mutations are used all the time in selection but maybe the breeder didn't even know this fact! Mutations can indeed be positive.

Not all mutations genetically behave identically. Dominant mutations are normally lethal, resulting in abortion or still birth.

DNA Background (3)

- Do all mutations genetically behave identical?
  - Dominant
    - Many are lethal
      - Abortion or Stillbirth
      - No spreading in population
  - Sex related
    - More chance for males = Single copy X/Y chromosome
      - Colourblind in men
    - X chromosome
      - Only females = definitely X chromosome
  - Single gene = autosomal recessive:
    - NOT on X or Y chromosome

DNA Background (4)

- Single gene autosomal recessive
  - Most frequent cause of genetic disorders
  - Both parents carrier of a the same mutation
  - Parents mostly without symptoms
  - Mendelian Inheritance Offspring:
    - 25 % show disorder (affected)
    - 50% are carrier of the mutation
    - 25% free of the mutation

Sex related mutations tend to have a higher chance of occurring in males since a single copy of either the X or the Y chromosome is all that is needed. An example of this is color blindness in humans. A single gene autosomal mutation is not on the sex chromosomes and most disorders are due to this form. Two parents are needed, both are carriers, and the parents tend to not show outward symptoms.

Non Genetic Factors

- NOT ALL ABNORMALITIES ARE GENETIC:
  - Toxicology = Poisoning
    - All kind of abnormalities depending on the age of the embryo
    - Mostly one farm or region
    - Many newborns affected
  - Infections
    - Virus
    - Mostly a specific effect
    - Depending on the age of embryo
    - History of mares with a disease
    - Rhinopneumonia Virus
  - Reason unknown
    - Most frustrating
    - Research may decrease the numbers within this group

Not all abnormalities are genetic! The two slides below - Non Genetic Factors and Genetic Disorders (1) - show the usual differences between these two types of abnormalities.

Genetic Disorders (1)

- Spread over farms
- Starts with a few cases
- Exactly similar defects
- Sometimes lethal (Abortion or Stillbirth)
- Not always visible at birth

Genetic Disorders (2)

- Spreading of a single gene
  - Less than 50% ever to be seen
  - Depends (Abortion / stillbirth)
- Gender of the first mutation carrier
  - Gelding — end of the story
  - Mare — slow and not very many
  - Stallion — can go fast!

The spreading of a single gene disorder will be seen in less than 50% of those who could have the mutation. This depends partly on what number in the population may have aborted or been still born due to the mutation. At the outset of the mutation, the gender of the first carrier will play a role. Obviously, if this first carrier is a gelding his entire life, it is the end of the story. If the first carrier is a mare, it will be spread but in a slow manner. However, if the first carrier is a stallion, the mutation can be spread very fast.

Genetic Disorders (3)

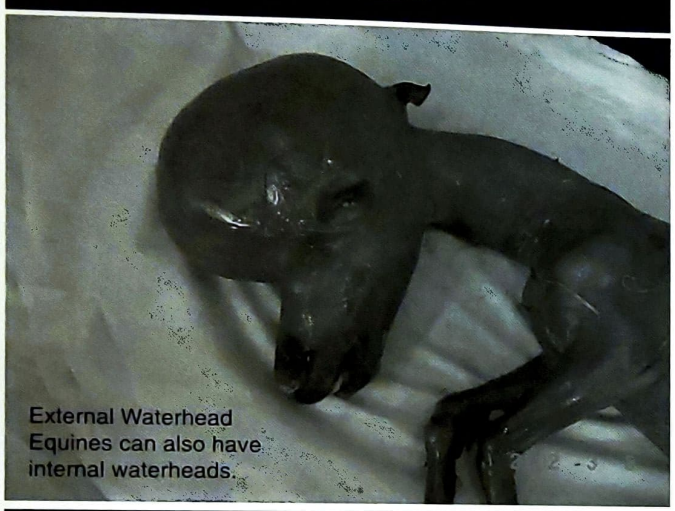
- Detection of a single gene
  - Only if TWO carriers are producing a foal
    - 25% affected foals
    - Six or seven generations after start of the mutation

Single gene disorders only occur when two carriers produce a foal and the chance of the foal being affected is 25%. The chance of the foal being a carrier is 50%. It takes 6 to 7 generations after the onset of a mutation for it to be seen!

Which genetic disorders are in Friesians? DO NOT TRUST THE LIST BELOW. The only one for sure is dwarfism.

Genetic Disorders (4)

- Which ones in Friesian?
  - Dwarfism
    - Back W et al 2008 Equine Vet J.
  - Water head
  - White hairs — Definition 1951
    - President Mr Anema:
    - White spotting: if there are any black hairs between the white ones.
    - Star: no black hairs between the white hairs
  - Aorta rupture — Inheritance?
  - Mega esophagus — Inheritance?
  - Fertility — Inheritance?
  - OCD — Inheritance?



Chestnut

- Most probably another story.
  - Technically a mutation, but not a disorder
  - Not a mutation in Friesians, but a natural coat colour variant from other breeds (decades ago)
  - Parentage verification!
  - First diagnostic test in Friesians 1999
    Wobke 403 (Fetse 349)
    Wikke 404 (Ritse 322)
- Data Bank of laboratories such as VHL very useful
- Many old stallions can be tested

Be honest
Once a label is always a suspect

Be careful
Not every abnormality is genetically

Waterhead has still to be proven with a larger sample size before it can be stated as a single gene recessive autosomal. Aorta rupture does exist, but it is not yet known if it is genetic. I have read and studied the paper on mega-esophagus but the study was located close to Friesland where the sample would tend toward predominantly Friesians, not a random sample. In addition, the symptoms in all the referenced cases do not appear to be completely identical in their presentation. More studies would need to be conducted before any conclusion can be drawn. Fertility issues will take a long time to investigate and resolve.

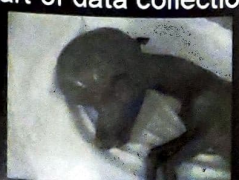
The chestnut factor is not a new mutation, it is a natural coat color. Had parentage verification been in practice in the early history of the studbook, than chestnut would not exist now.

Single Gene Dominant
In this scenerio only one copy of the gene is necessary to have an affected animal, the gene coming from either parent. A recently published work out of the University of Minnesota describes such a one - PSSM (Polysaccharide Storage Myopathy). This is also known as Monday Morning Sickness and the Friesian is known to have PSSM. Ninety percent of PSSM cases are due to a mutation and Minnesota has a patented DNA test available. There has been a muscle biopsy test previously. Afflicted horses have low appetites, reluctance to move in work, inability to often backup, poor muscle development, etc. Most PSSM cases respond to a change in diet that is away from high sugars since this is a sugar storage problem. There are two types of PSSM, as the new literature points out: the less severe type where it is inherited from one parent and the more severe type where it is inherited from both parents. With the DNA test, all owners can test horses and make the proper diet and management changes before this disorder becomes a problem. In addition, the DNA tests will allow researchers to determine the extent of PSSM in the Friesian population.

It is important to be HONEST and to be CAREFUL. Once a label is placed on a horse it is there forever. It needs to be true. Remember: not every abnormality is genetic! In order to be sure the proper data is collected, the breeder must take a photograph and report only the facts of the case. Do provide all necessary information and an official necropsy report to the studbook. In return, the studbooks should make the process a positive one for the breeders in recognition of their time and trouble.

Data Collection (1)

- Information is needed
- How? Who? When? What to do?
  - Breeder first identification
  - Studbook coordination
- Description prior to start of data collection



To the left is a slide with the items that breeders should record and send to the studbook. This data becomes part of a Databank. Remember - BE VERY CAREFUL NOT TO MAKE A STALLION A SUSPECT, but stick to reporting the facts.

Studbooks, in turn, register the facts reported in a confidential manner. They also need to be circumspect about the data collected, keeping it safe until research has produced results.

Studbooks and breeders do not have a conflict of interest in this data collection. The studbook is in a unique position to know things such as the pedigrees and offspring, while breeders are in a unique position to know details of health, fertility, and veterinary history.

Data Collection (2)

- Breeder
  - FACTS and only FACTS
    - Fairy tales are not important
  - Sire? Dam? Parentage verification!!!
  - Abortion what age?
  - Stillbirth weight
  - Partus normal?
  - First foal of a mare?
  - Gender?
  - Post Mortem diagnosis
  - Inform the Studbook

Be careful to make a stallion a suspect

Data Collection (3)

- Studbooks
  - Register the facts
  - SILENCE please! Once a label…..
  - Keep it Confidential in a small group until at least two proven cases
  - Costs?

Invite the breeders to send information

Databank (1)

- Data Collection
  - Conflict of Interest Breeder versus Studbook ? NO
  - Studbook knows much
    - Pedigree
    - Premiums
    - Registered offspring
  - Breeders know more
    - Health
    - Effect of treatments
    - Character
    - Fertility
    - Vitality

Databank (2)

- To be registered
  - Date of birth
  - First foal of the mare?
  - Abortion? When?
  - Stillbirth: When? Weight? Abnormalities if any?
  - Gender
  - Reg number + 3 generations pedigree
  - All diseases Who is the vet?
  - Fertility
  - Character
  - Reason of Death
  - Sold to..

Do NOT trust your memory! Make records per animal!

Databank (3)

- Which animals to include
  - All abnormalities
  - All stallions
  - Mares? All?

Databank (4)

- Storage
  - BLOOD is preferred
    - 10 ml EDTA
  - Abnormal or dead : Ear tips
    - Frozen
  - One place? or split samples?
  - Needs a perfect registration of samples
  - How long?
  - Costs

Blood is preferred for the databank and only 10 ml is required. If the animal is deceased, the tips of the ears contain cartilage that has a lot of immature cells, making them a good source of DNA. Take only the end 3 cm for freezing. An important question to be decided prior to setting up a databank is whether all samples will be stored at one location or whether samples will be divided and stored in two or more locations. Multiple storage locations keep the samples safe in the event of a power failure or natural disaster in one of the storage locations. It is a risk to store all samples in one location.

Another important question to answer is who pays for what portions of the costs of storage, tests, and research?

Databank (5)

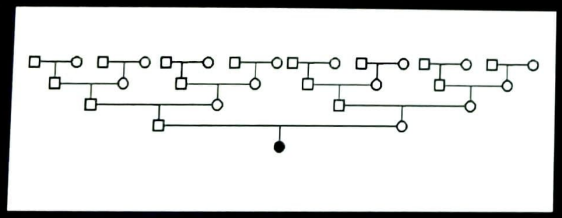
- Ownership
  - Who is the owner of the stored material
  - Breeders should not be!
  - Legally approved documents

Applications (1)

- **Examination & Interpretation**
  - Pedigree information in case of abnormalities
  - Which test? And where?
  - Everybody wants to know about his neighbour's trouble
  - Who decides? President?
  - How many cases you need?
  - When?
  - Costs?
  - Who is the owner of the results?

Pedigrees (2)

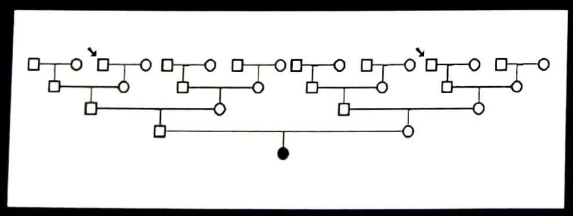
- **Expanded pedigree analysis reveals true mode of inheritance**



- **Identical ancestors?**

Pedigrees (3)

- **Yes, see arrow**



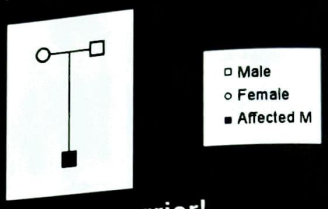
- **But...**

Applications

The databank requires legal documents outlining the ownership of the data. This should be the studbook/registry in order for research to be conducted. Documents should spell out the constraints of the use of the data - who has the right to make decisions? The president? A committee? This is very important to legally define because everyone wants to know what is happening at their neighbors! How many cases are needed before research can begin? The number is 10 to 12 cases. Who owns the results? This should be the studbook/registry but the members should have access to the results of research.

Pedigrees (1)

- **Autosomal Recessive?**



- **If true, both parents carrier!**

Pedigrees

If a disorder is autosomal recessive then both parents are indeed carriers. Be careful though - don't stop analyzing the pedigree if you have a common ancestor in the fourth generation. Sometimes, or more likely, often times, you need to go back to 6 or 7 generations to find the real source of the disorder. Such a starting point of a new mutation is called a FOUNDER. In 7 generations there are 256 parents to look at, so it can be hard to determine which is the real founder.

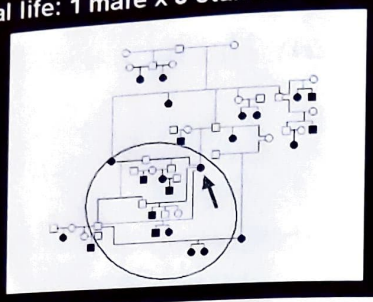
The good news is that some DNA tests will be available in 5 more years or less, such as dwarfism. The horse genome may not be completed due to the expenses involved, but enough information has been found to work on these tests.

Pedigrees (4)

- **But:**
  - **Also previous generations may have other shared ancestors,**
  - **Has parentage been verified?**
  - **Every pedigree in Friesians goes back to the bottleneck,**
  - **Be careful when sticking a label to suspected horses!**

Pedigrees (5)

- Real life: 1 mare x 3 stallions



Applications (2)

- Research and developments
  - Carriers of some genetic disease can be recognized
  - Tests will be available one or two years?
- Important to know how to handle carriers (policy)
  - Killing?
  - Out of breeding?
  - Use the information to choose the partners
  - NO carriers x Carriers
  - Carriers x Non Carriers:
    - Can be done. No new victims
    - But 50% new carriers
  - Next generations: avoid new stallions being a carrier
  - Hunting & selection based on one gene is a risk!
- Maybe other positive genes are closely linked to the undesired gene!

Future (1)

- Dreaming about the long term future
  - Sexing of semen
  - Selection of stallions
    - Now 200 starting and 5 OK?
  - Vitality
    = Condition
    = Health

Future (2)

- Possible and Probable
  - Gaulder (2008):
    - Everything will be possible
      but
      Not all is probable
- Theoretically there will the possibility of a 100% perfect horse
  but
  I think this is not probable that this will ever be the reality

Future (3)

- Genetics is still 'on the move'
  - New Mutations will occur
- Breeders are also 'on the move'
  - Ideas about the breeding policy will change
  - The ideal horse will always be a dream

Concluding Remarks

- Breeders are important
- Studbooks are important
- Genetics is important
- What is the role of the geneticist?

At this time the policy of the KFPS is NOT to eliminate or destroy known carriers. This could be one management policy, but the problem with "hunting" for one gene to eliminate is that, in doing so, one may also eliminate desirable genes that are next to this one, near it, or associated with it. We could lose important traits we want to keep along with the disorders. Additional ways to control these genes are in Applications (2). We will see more disorders; genetics is not stagnant and neither are breeders. Breeders will always be striving for the perfect horse, but that is not reality.

Geneticists have a role to work with the breeders and the studbooks to assist them to be happy with their Friesians through the breeding of healthy horses.

Only as long as they understand breeders and will assist them to be

Happy with their FRIESIANS