NEONATAL or HEMOLYTIC ICTERUS (NI)

Death of foals in relation to their blood groups

PREFACE

Regardless of what tests are performed or what research is done, there is always a minimal risk that an unpleasant surprise may occur when a foal is born. Birth is a biological event where not everything will go according to mathematical rules. The chance of such a surprise is low, because there are diagnostic tests available to reduce the risk of Neonatal Icterus after birth.

At the present time, not much attention is being paid to research in the area of Neonatal Icterus.

It is a pity, because some issues will never be solved. This is dealing with the phenomenon of agglutinating antibodies and for the mares producing almost unlimited, non-specific antibodies against blood group factors. The duty of breeders ,veterinarians and anybody else involved with horse breeding will be to keep collecting accurate data and to report rare cases to the registries.

It is necessary to supply full data of all events and questions surrounding NI . This includes names of the animals and the registration and studbook numbers. For the mares, it is good to know if there has been an earlier suspicion of NI in the past. If there is such a history, it is important to know the data from the sires in these cases.

The aim of this brochure is to underline the problems around NI. I hope that reading these pages will answer some questions.

I wish to thank Laurie Kasparek from the Friesian FHANA (USA) for her translation of this brochure into understandable English.

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INTRODUCTION

The Latin name, Neonatal Icterus, translates to: Destruction of red blood cells in the new born foal. Instead of the full name, the abbreviation, NI, will be used. It really is a catastrophe when a healthy, newborn foal dies within a couple of hours or days as a result of NI.

Nevertheless, this happens to several horse breeders every year, repeatedly. The questions that arise in such an event have a lot of similarities. The information in this brochure is mainly focused on answering those questions.

The content is particularly important to two categories of readers:

1. The Horse Breeders. This paper seeks to help all breeders come to a better understanding of the background of NI.

2. The veterinarians. This paper is a summary of the items in this field.

THE ORIGIN of the DATA

Not much recently gathered information is available in literature. Not much research is performed nowadays. Much of the existing data is from the archives of Dr Van Haeringen Laboratorium BV at Wageningen, The Netherlands.

Furthermore, the facts known and shared by breeders and veterinarians has been an important source of knowledge. Informal talks with colleagues in various meetings and Conferences has been very useful. A short list of scientific publications is included.

BACKGROUND OF NI

NI can be noticed as a series of steps during pregnancy and after birth:

1. Problems start as a result of incompatible, non-matching blood groups between the mare and her unborn foal.

2. The mare may respond by producing antibodies against one or more of those blood group factors.

3. When the foal starts drinking the mares milk (colostrum) those antibodies enter the gut of the foal.

4. In the first days of life the membranes of the gut are in a condition that these antibodies can pass from the gut into the blood of the foal.

5. A severe reaction begins in the blood of the foal, finally resulting in the destruction of the red blood cells, which results in a dangerous anemia.

6. The waste products of these destroyed cells effect that the surface in the mouth and around the eyes, turning them yellow (icterus).

BLOOD GROUPS

Blood group factors are complex chemical structures in the membrane of red blood cells.

Since 1960, research has been performed on the blood groups of horses. The results have focused primarily on parentage verification. Until approximately 1995, this research was ongoing.

After the mid nineties blood groups have been used less for that purpose, because DNA technologies became the tool for this goal.

NI would not exist if all horses had identical blood groups. However this is not the reality. It appears that at least 34 different factors are in existence, divided into 7 independent blood group systems. Those blood group factors do have particular qualities (antigens). Those antigens can call for a defense resulting in the production of antibodies. This only happens if the antigens enter a body of an individual which is lacking similar antigens.

When the blood group factors of the foal are in close contact with the mare's blood circulation, the mare may produce antibodies against those factor antigens which are not identical with her own factors.

In horses two types of antibodies occur.

1.The *agglutinins* , where the red cells are clotting. These antibodies seem to not be dangerous for the foal.

2.The *hemolysins* ,where the negative effects are very harmful, because a contact between the blood group factors (the antigen) and these antibodies (lysins) results in the destruction of the membrane of the red blood cells of a foal.

In the process of NI, the most threatening factors are: Aa, Qa and Qc.

Another group of risky factors are Ca, Ka, Pa, Pb and Ua. However the number of cases

of NI due to these factors is very low, compared to the former. During 2001- 2006 more than 100 cases have been tested. The results are presented in table 1.

Table 1. Tests in 2001-2006.

Negative: no titer or 1:8 as a maximum ; Positive: titer from 1:16 and more

Total number of sera	Negative	Anti Aa	Anti Qa	Anti Qc	Miscellaneous
123	15	76	11	14	7

DEVELOPMENT OF NI

After fertilization, the development of the blood groups of the foals is starting. Fifty percent of the bloodgroup factors is originating from the mare; these are identical with the mares own system and will never be a problem in the development of NI.

The second half is from the stallions origin. These **MIGHT** be important, in as far as they are different from the mares blood groups.

Figures 1-3 show a schematic overview .

Figure 1. Probability of developing NI when the mare is negative and the stallion is positive. Illustration based on blood group factor Aa.

Mare Aa negative x Stallion Aa positive.

Foal Aa positive

In this case NI can be developed, because the blood groups of mare and foal are not identical.

Figure 2. Probability of developing NI when both the mare and the stallion are negative for blood group factor Aa.

Mare Aa negative x Stallion Aa negative

Foal Aa negative

In this case NI will not be developed, because neither mare nor foal possesses the factor Aa.

Figure 3. Probability of developing NI when both the mare and the stallion are positive for blood group factor Aa

Mare Aa positive x Stallion Aa positive

Foal Aa positive.

No development of NI . The mare and the foal do have the identical factor Aa.

Sometimes during pregnancy red blood cells enter the circulation of the mare. How this happens is not clear. It hardly ever affects the health of the foal. Obviously, some wounds occur because after the birth a thorough inspection of the placenta of the mare can show signs of scars. This is an indication that during pregnancy the placenta has been injured at least once. It is almost common knowledge that bleedings occur during pregnancy, however the mechanism is unknown. Maybe by the hooves of the foal? Almost never is such an injury threatening to the unborn foal, but, inconspicuously, the process of NI has begun.

In such events, when a mare comes in close contact with unknown blood group factors (Figure 1), the immune system of the mare can start the production of antibodies. This has to be considered a normal process due to an effective immune system. Healthy mares ought to have a well functioning immune response, because they have to protect themselves against diseases and they have to transfer the antibodies to the foals as well. This transfer does not happen during pregnancy, but just after birth, when the foal starts to drink.

The amount of antibodies (titer) against blood group factors in the mares varies, depending on several reasons:

1. The time of pregnancy when the injury occurs. If this happens very late in gestation, the mare will not have the opportunity to build a high titer.
2. The frequency of NI births. In the event that the mare has given birth to more foals suffering from NI, the titer will increase during each gravidity. Generally speaking, a titer 1: 16 a fortnight before parturition is a risk. However titers 1: 5000 have been noticed as well.

Those antibodies do not pass through the placenta from mare to foal and, therefore, they are not important, as long as the foal is not yet born. Antibodies will stay in the mares blood.

After birth the situation immediately turns into a dangerous one. The production of colostrum in the udder also means a transfer of antibodies from the mares blood into her colostrum.

The newborn foal starts drinking and the antibodies enter the gut. In the very earliest days of life, the membranes of the gut have the ability to pass along bigger molecules from the gut into the blood stream. Antibodies against diseases are welcome because they are necessary for the protection of the foal against diseases.

The drama starts when antibodies against the blood group factors meet the antigens in the red cell membranes of the foals. Such a reaction will result in the destruction of the membrane (lysis). The waste products which are the result of this destruction are the reason for the yellow color in the eyes and around the mouth and nose of the foal.

THE PROCESS OF NI

The severity of the situation mainly depends on two components:

1. The vitality of the foal. The healthier and the stronger the foal, the more colostrum is drunk and the more antibodies are consumed.
2. The titer in the colostrum. This titer is similar to the titer in the mares blood. In cases with a high titer, the foal will receive a large dose of antibodies.

In cases where the foal is strong and the titer is high, the damage will be very severe and the foal can die within 24 hours.

With a lazy foal and lower titers, the symptoms will show later after birth and the process, if untreated, may take some days.

MISUNDERSTANDINGS

Over the years several persistent misunderstandings have been observed.

1. NI will not occur in mares giving birth to the first foal.

   Several cases are known where these group of mares have lost their foals due to NI.

   This misunderstanding is persistent because this incorrect information was based on data from some older scientific books.

2. Foals suffering from NI can be reintroduced to the mares after three days.

   A number of foals have died due to NI after a preliminary recovery, because they were allowed to drink the mares milk after three days. This is very sad and a tragedy. It has been generally accepted that antibodies will not pass the membranes of the foals gut 48- 72 hours after birth. It was also accepted that the quantity of antibodies in the colostrum should be very low and harmless after this time. However this appeared not to be the truth in all animals.

   Therefore our advice:

   SAFETY FIRST: A PERIOD OF FIVE DAYS SEPARATION HAS TO BE THE RULE.

3. A mare at the age of ten years or more who has not shown NI in the past will not do so in the future.

   This is incorrect. There are situations where mares did not have the chance to develop NI because the blood group factors from the mare have not been incompatible with those from the stallions used so far.

RULES AND EXCEPTIONS

Almost every rule has exceptions. This certainly is true in NI.

1. In a few cases of NI, investigation revealed that incompatible blood group factors could not be the reason.

In South Africa a company tested a new vaccine against Babesiosis. More than 100 horses were in the trial. The vaccine proved to have no effect against the disease, but quite a lot of foals born from vaccinated mares developed NI. The origin has to be looked for in the vaccine's manufactor. Most probably this medium contained, or was contaminated with material from red blood cells of horses.

In the eighties in The Netherlands a similar situation may have occurred. Several cases of NI have been diagnosed, but without the incompatible blood group factors. It has been impossible to trace the origin of this event. More recently such a situation was observed in Poland.

2. Rather recently some particular cases have been noticed in The Netherlands and in Belgium where mares had extremely high titers (lysins and agglutinins) against all the other horses in the panels, except against her own red cells.

The origin is still unclear. A publication will be in VETERINARY RECORD by De Graaf- Roelfsema, et al. This situation is even more complicated because this type of NI appears to be repeated in each foal, no matter which stallion is used. One may consider eliminating these mares from further breeding.

3. Another event which has been observed is that a group of foals develops a severe anemia approximately after the tenth day of life.

Is there any relationship with NI? This is presently unknown . The majority of these foals have died, regardless of preventative therapy and blood transfusion. Tests for the genetic disorder, Severe Combined Immune Deficiency, showed that this factor is not responsible for this problem. Studying this unknown syndrome is difficult because not many cases have been reported.

4. Finally, one case is known where blood groups and antibodies might have been responsible for the death of the foal. However the affected cells were not the red cells, but the white blood cells. To my knowledge only one report is known.

DIAGNOSTICS AND ADVICE TO CHOOSE A STALLION

In some cases the diagnose of NI is rather easy. Yellow membranes in mouth and eyes are clear indicators. However NI is not the only reason for this. Furthermore, it is known that some foals die before becoming yellow. In cases where the NI is less severe, the foal can be weakened more than normal by the anemia , therefore facing the risk of other diseases or trauma.

In any doubtful situation it is advised to confirm the likely diagnose by doing an analysis of the mares blood.

Up until 7 days after birth antibodies can be detected and the titer can be estimated. In order to have the best chance for a correct diagnose, it is a must to have the data from mare and stallion(sire of the foal) available.

ANALYSIS OF THE FOALS BLOOD DOES NOT MAKE ANY SENSE.

In order to give advice on mating, it is absolutely necessary to know the blood groups from mare and stallion; to analyze the factors making the risks. Mares can be tested at a very young age. The best time to test stallions is when they start to be used.

In the past almost every stallion was tested for blood groups as part of parentage verification. Nowadays, DNA is used for parentage testing and the blood groups are not identified automatically.

Only when blood groups are known can breeding advice be given. This is based on the assumption of identifying the lowest risk for developing NI. Sometimes a long list of stallions is necessary to find one or two combinations. This mainly is the case where the mare is lacking all the high-risk factors.

If the blood groups of a stallion are unknown, it is not possible to give a recommendation with regards to the risk of NI. The solution is to test the stallions for blood groups which may become involved in NI.

If a mare with the history of NI is carrying a foal from an untested stallion it is strongly advised to test for antibodies between 2 and 3 weeks before the expected date of birth.

An advise for mating almost always will result in a healthy, non NI foal, but it can never be seen as a guarantee. Biology and life is different from mathematics .

FREQUENTLY ASKED QUESTIONS

Which mares are at risk?

Particularly those mares that are lacking the factors Aa, Qa and Qc. To determine the risk, a laboratory test can be performed. In this test the Blood group factors will be determined.

How big is the risk that a mare really will develop NI?

Sorry, this is not known. It is hard to collect all the necessary data. First of all, all the mares should have been bloodtyped. Secondly all data for mating and AI have to be known, including the bloodtypes from the stallions.

Are there any differences per breed?

Most probably yes. In some breeds NI has been reported more frequently than in other breeds.

In Thoroughbreds, Warmblood Horses and Friesian horses Ni is observed rather frequently.

It is not clear if this means that differences really exist. Not all cases are known due to lack of testing and reporting. The higher either the economic value or the emotional attachments are, the more tests are done. This also depends on the frequency of the risky factors in a population and breed.

These frequencies are variable per breed.

- Thoroughbreds do have a rather low number of animals without Aa and Qa
- In Friesian horses Qa has never been observed. Qc appears to be very important
- In Warmblood breeds the majority of the NI cases is related with Aa .
- In Shetland ponies hardly any NI case is known.

Other important reasons that contribute to the development, or not, of NI are presently unknown. Maybe the size of the foal and the length of the legs is important. Perhaps the bigger the foal - the more risk for injuries in the placenta?

Is NI developed in each pregnancy?

A mare which has developed NI against factor Aa will most probably repeat this very time when she carries a foal with the blood group Aa. On the other hand, a mare with a history of NI against Aa will not develop the antibodies if she carries a foal without the factor Aa. This can be a result of using a different stallion.

Furthermore, not each stallion is homozygous for Aa. If he is heterozygous, only 50% of his foals will have the possibility to create antibodies in the mare. See again figure 1.

What is the risk with mares who survived NI as a foal?

Those mares will not develop NI against the same factor, because they do have the risk factor and are not able to produce antibodies against this factor.

Foals born out of NI mares , going on to grow up without problems, do not possess the risk factor and, therefore, they can develop NI if there is incompatibility with their partner.

Is NI a genetic disorder?

NO. It is absolutely not necessary for a stallion keeper to be ashamed. The problem is genetically specified, but it is absolutely incorrect to speak about a disorder. On the other hand, it appears that some families of mares do have more problems.

Are NI mares of less value?

NO. In many cases it has been demonstrated that they have a very active immune system which will protect them from diseases.

When you sell an NI mare, do you have to tell the story?

This might be a tricky situation; hopefully, not one to be handled by lawyers. As far as I know, no clear jurisprudence exists.

Most likely, the answer to this question depends on the reason why the buyer is willing to purchase the mare. Is she bought mainly for breeding and riding is only incidentally? Then it would seem dishonest not to tell the new owner about the NI history. However, is the new owner only going to use this mare for riding? Then there are less compelling reasons to tell about NI.

How to breed a NI foal ?

The mare is allowed to take care of the foal after birth, but drinking is forbidden! A partition inside the stall can be made with a fence; the two can touch but the foal cannot nurse. It's almost a must that the foal has to receive colostrum, but the source of that colostrum must be another mare. It is advised that the minimum amount will be at least 2 liters during 8-12 hours after birth , but certainly within 24 hours.

Most important are the IgG proteins in the colostrum, because they are the source of the antibodies. It is possible to measure the quantity of IgG in the milk. At least 8 grams per liter is preferred. Following the initial period, artificial substitutes can be administered. Foals appear to drink better from a rather flat pan than from a bottle. Drinking from a bottle is also a higher risk to choke and to develop a pneumonia. The mares have to be milked as frequently and as fully as possible; most mares accept this without much trouble.

In the majority of cases of NI foals, it is not a problem to reacquaint the mare fully to the foal after five days, and the foals will start drinking easily.

Is there a risk if the NI foal is placed on a mare whose own foal has died?

Certainly there is a risk, because it is not always known why the foal from the other mare died! Without testing there is a risk that the other foal died from NI as well .

Is it possible to start storage of Colostrum?

YES. It is possible because colostrum can be frozen and stored in a normal freezer (minus 20˚C). We are not aware of any problem with colostrum stored for more than 12 months. It is preferred to collect the colostrum from older mares which are at the same stable, because those animals usually have the highest amount of antibodies against pathogens in the same environment.

It is easy to test colostrum for the presence of antibodies against blood group factors. An amount of 5 ml will do. One always has to remember that testing is cheaper than losing a foal. Certainly, using colostrum from a mare with a dead foal in her breeding history has to be considered carefully and with trepidation.

Another possibility is to give blood plasma from another horse, preferably from a stallion or a gelding. Even in these events it can be good to test for antibodies; we have seen some of these donors unexpectedly test positive for antibodies against blood group factors. Some substitutes appear to be commercially available at this time.

How long are antibodies present in the mares blood?

It is rare if they can be found present after ten days. After this time period, information about NI can be gathered only from the blood types of dam and sire.

Choosing a stallion for a particular mare. Is this possible?

YES. Therefore it is necessary to know the blood groups from mare and stallion. Many stallions have been tested in the past. This data is available from the testing laboratories, because it is stored in the computer system. However, nowadays DNA tests are commonly used for almost all studbooks. Therefore blood groups are not available from these recent stallions.

Nevertheless, stallion keepers can do a good job in having their stallions tested. A breeder can test his/her mares. Based on the information from both animals, an analysis of the risks can be made.

Do all mares need to be tested for blood groups?

Theoretically this should be the best practice to avoid NI. Breeders who have lost foals to NI are easy to convince. Their colleagues who have not seen this drama are a bit slower!

What happens if a breeder insists on a high risk mating?

The chance of NI will be high. As said before, this does not influence the gravidity. It starts with the colostrum after birth, because this is the source of the antibodies. If this is a reality, one can check the mares blood for antibodies approximately a fortnight before the expected day of birth. If it is demonstrated that there is too high a titer, measures have to be set in motion. This may take several sleepless nights, because the foal must not be allowed to get any chance to nurse!

Safety first: This ought to be for a period of five days!

If the breeder has time and energy to do this job, things can all be fine. However do not underestimate the efforts needed.

Is there a possibility of doing a DNA test for NI?

Not yet. No tests exist at present to determine the blood groups by DNA . A tube of blood is the only possibility.

Are there any explanations for the development and the differences in symptoms of a foal with NI?

Many parts of the body play an active role. Without basic research it will be hard to understand all the mechanisms involved.

It is a sure thing that not all the same effects are seen in all similar circumstances. For example, the function of the gut of the newborn foal in passing the antibodies is not clear. Furthermore there is not enough knowledge about the milk production of the mares. Is the milk production starting in a state of low or high volumes? We do not know how much a healthy foal may consume in the first hours of life.

LITERATURE

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DISCLAIMER

Although the information in this brochure has been gathered and composed as carefully as possible, errors may exist. Always consult with your veterinarian on prevention and follow-up treatments, as related to your own situation and the health of your individual mare.