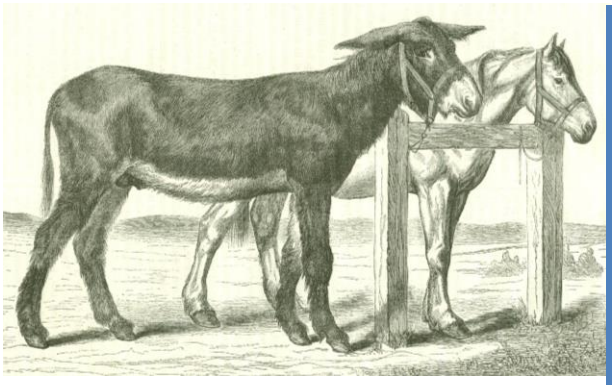
EFTBA Veterinary Newsletter 30



Races asine et chevaline de Poitou *(Bonheur)*

The maternal grandsire effect - wishful thinking or reality?

April 2019

. For centuries, breeders wondered whether the maternal grandsire may have an effect on the offspring.

. To answer such questions, geneticists investigated the parent-of-origin identification of genes in reciprocal hybrids (mules an hinnies).

. The results of this research allow to explain that some genes skip a generation.

Welcome to EFTBA's veterinary newsletter

Dear Breeder,

This is normally a great time of year for breeders as with each new foal born, we wonder if this is the one which will put our name in lights; as being a champion, or a big sale horse (for those in the commercial sector). We then worry if it will fulfil its genetic plan of being a successful, correct and sound race horse.
To this end, Hanspeter Meier will explain how it works.

In the latest edition of the EFTBA Veterinary Newsletter Hanspeter laid out in plain language how the genetic map will or should guide a new born thoroughbred through its young life, how it develops to reach its potential. There are many mile stones, pitfalls, which may or may not be encountered that are beyond the control of breeders or guardians, including safe birth, biosecurity issues, scour, lameness as well as external factors such as poor husbandry and horsemanship, all of which we hope to avoid with our stock.

In the current climate of world racing, our sport, our racing and associated way of life has never been under so much threat. The developments at Santa Anita, welfare issues, taxational funding, or lack thereof, changing of jockey rules, medication issues to name but a few. In many cases those in the position of power are not fully understanding of our industry, when drafting and implementing legislation, which can impinge our industry and livelihood negatively. These are some of the issues we should discuss at the upcoming EFTBA 2019 AGM.

In the face of Brexit, I led a group of concerned breeders, including members of the EFTBA Executive and Paul Marie Gadot of France Galop to Brussels on 22nd February 2019, during which EU Lobbyist Michael Treacy had arranged a series of strategic meetings with EU Commissioner Phil Hogan and senior EU officials who were part of the BREXIT negotiation team. It proved to be a beneficial delegation in terms of highlighting the concerns of European breeders and offering possible solutions in terms of free movement of High Health Horses. Commissioner Hogan, along with Dr. Alf-Eckbert Fussel, EU Commission's Directorate General on Animal Health as well as David Bruck, Donal O'Donnell, Tom Tynan and Bernard Van Goethe have a good understanding of what is required and seemed willing to foster and advance these requirements to facilitate continued movement and trade of bloodstock after the UK exits from the EU.

"Many thanks to Mrs. Eva-Maria Bucher-Haefner, Moyglare Stud Farm, for her valued sponsorship of this newsletter."

Please be reassured your federation is working to stay on top of these and other issues challenging our industry. If there are any questions you would like raised in advance of the AGM, please do not hesitate to contact me. I look forward to welcoming you to Kildare, Ireland on 24th May 2019.

Best regards

Joe Hernon

Chairman, EFTBA

Editorial

In our last newsletter, we looked at results of genetic studies in our time and possible connections with traditional views on possible peculiarities of horse-breeding. There, our subjects were the mitochondrial DNA and the dam-line in pedigrees. In this issue now, we discuss the role of paternally expressed genes in the placenta of the mare. Is there an influence of the maternal grandsire on the offspring? Are the modern genetic findings in accordance with views of our ancestors on this subject? Let's have a look at some publications on these questions in earlier times and scientific discoveries of today.

Dr Hanspeter Meier

EFTBA veterinary advisor & Newsletter editor

Introduction

Six years ago, **Wang and co-workers** from Cornell University (NY) published a very comprehensive study with the title **"Paternally expressed genes predominate in the placenta"** and referred to earlier studies with mouse embryos which had allowed the discovery that the paternal genome – by means of **genomic imprinting** – has a major influence on placental development.

Of course, these scientists were above all interested to find out whether the phenomenon of genomic imprinting also exists in other mammals – and it certainly was a brilliant idea to select equids for the research in this field. With them, one is able to investigate **reciprocal hybrids** of the horse and the donkey - **mules and hinnies**. Here, genotypic differences allow the **parent-of-origin identification** of most expressed genes.

Using this approach, the scientists identified a core group of 15 ancient imprinted genes, of which 10 were paternally expressed (Wang et al., 2013).

Genomic imprinting is an epigenetic phenomenon that causes genes to be expressed in a parent-of-origin-specific manner. Forms of genomic imprinting have been demonstrated in fungi, plants and animals. There are about 150 imprinted genes known in the mouse and about half that in humans.

Genomic imprinting is an **inheritance process** independent of the classical Mendelian inheritance. It is an epigenetic process without altering the genetic sequence. These epigenetic marks are established ("imprinted") in the germ-line (sperm or egg cells) of the parents and are maintained through mitotic cell divisions in the somatic cells of an organism (Wikipedia).

May these discoveries now help us breeders to explain the "**maternal grandsire effect**" where traits may only be seen in later generations? – But first, let us review some endeavors in investigating a possible role of the broodmare sire in the history of horse breeding and the Thoroughbred.

The "maternal grandsire effect" in the history of breeding

Presumptions of individual hereditary influences by the dam or the sire, most probably are as old as horse breeding itself. For instance, in the 18th century already, **Prizelius** (1777) expressed the opinion that the stallion contributes more to the procreation of a foal than the mare *("Die Erfahrung lehret es, dass der Bescheler mehr zur Zeugung eines Füllens beyträgt als eine Stute")*. And - most interestingly - he also added the remark that a foal exceptionally may present itself like an ancestor further back *("… wobey jedoch, wiewohl selten, eine Ausnahme statt findet, indem ein Füllen auf das dritte Glied zurück schlagen kann"*). Although Prizelius just mentioned personal experience(s), the history tells us that his remarks had a great influence on many breeders.

Comparable notes e.g. are reported by **Sir Charles Leicester** in his book **'Bloodstock Breeding'**; he had heard breeders of hunters in England and Ireland aver that if they mated their broodmares to a Shire or other heavy horse in the first instance, the subsequent foals by thoroughbred sires would be up to more weight than if they had not followed this course. According to Sir Charles, this attitude was so ingrained amongst farmer breeders of hunters in earlier times that a Thoroughbred sire as a first mate was rarely tried (Wright, 1983).

At the beginning of the 19th, such considerations were known under the conception "telegony".

> **Telegony** was a theory in heredity, holding that offspring can inherit the characteristics of a previous mate of the female parent; thus the offspring of a female might partake of traits of a previous sexual partner.
> Telegony was a very popular theory in the first half of the 19th century among biologists, but was largely dismissed with the arrival of Mendelian genetics (1866).
> Experiments in the late 19th century on several species failed to provide evidence that offspring would inherit any character from their mother's previous mates. However, in 2014 the evolutionary ecologists A.J. Crean and colleagues reported a similar phenomenon – passing environmental (non-genetic) traits in a species of fly *(Telostylinus angusticollis*) (Wikipedia).

The most famous case of this alleged theory was the following: In about 1815, **Lord Morton** mated a quagga stallion (a quagga is of the zebra species) to a mare of seven/eights Arabian blood with the result that she foaled a hybrid quagga/horse. A picture of the mare's produce by the quagga and by the Arab sire was painted by Agasse and found its way into the Royal College of Surgeons' Museum (Fig. 1). It shows the animal to be of undoubted Arab type with no detectable sign of quagga conformation - but the peculiar quagga-like stripes are plainly discernible.

Fig. 1 Lord Morton's Quagga-Arab-cross
by Jacques Laurent Agasse
(Courtesy of the Royal College of Surgeons)
(Wikipedia)

He then sold the mare to Sir Gore Ougeley who put her to an Arab stallion. From this mating she produced a bay foal with a black dorsal line and a few dark (quagga like) stripes on its forehand and legs. To a subsequent mating to the same horse, she produced another similarly marked foal. On discovering this, Lord Morton sent details to the Royal Society, whereupon many learned members of this institution accepted the facts as indisputable proof of the existence of telegony (Wright, 1983).

However, other experts weren't convinced of such a simple explanation and later, Sir Charles Leicester mentioned photographs of two different pony foals with similar but more abundant stripes. Neither of the dams of these two pony foals had ever been near a quagga or zebra. The fact is that in some breeds of horses – and Sir Charles believes especially amongst Norwegian ponies – these zebra stripes are sometimes seen. In his opinion, it was pure coincidence that the peculiarity showed itself in the two horses bred from Lord Morton's mare (Wright, 1983).

Since those far-off days there have been innumerable attempts to produce a similar result both by crossing zebras etc., with mares and by other hybrid matings but all have failed. It was around the years 1894-95, when Scottish zoologist, **James Cossar Ewart** began his **Penicuik Experiments** in animal breeding on his private experimental farm where he conducted many pioneering investigations in genetics. However, the quagga species had become extinct by that time, wherefore he decided to use a Burchell's zebra stallion which served several different breeds of mares (West Highland, Shetland, Iceland, Irish and other Ponies and Thoroughbreds) which produced striped hybrid foals (Fig. 2).

Fig. 2 James Cossar Ewart and one of his
striped hybrid foals, called "Tartan Cuddies"

Their conclusion was: *"It thus appears that the evidence in support of the belief that Lord Morton's mare was "infected" by the quagga is at the best far from satisfactory"* (Ewart & Lord Arthur Cecil, 1899).

An investigation by means of reciprocal crosses (in principle the same method which has been used by Wang et al., 2013) was already chosen by **John Hammond and A. Walton** in Cambridge in 1938. They also wanted to know more about the parent-of-origin specific manner of inheritance and studied **the intra-uterine growth of the fetus in Shire- and Shetland Pony-mares**
The results of these crosses showed that the progeny foaled by the Shetland dam grew to resemble more closely the Shetland breed; but the progeny foaled by the Shire dam grew to resemble the Shire breed (Fig. 3).

In their book **'Genetics of the Horse'**, **Jones and Bogart** (1971) referred to the work of Hammond and Walton in the chapter 'Maternal Inheritance'. By using crosses between breeds of horses that contained some drastic differences, Hammond and Walton showed that the foal inherited more of its dam's size characteristics than its sire's (Fig. 3). This allowed the statement that the chromosomes are not the only important element determining inheritance and did shed light on epigenetic factors. Jones and Bogart (1971) did agree that each parent contributed equally to the diploid autosome complement of an embryo and that they only differ in contribution with respect to the sex chromosomes. They meant that there is a big difference between spermatozoon and egg, as the first is almost entirely void of cytoplasmatic substances, in contrast to the egg which also contains a mitochondrion. They thought that the cytoplasm contains some structures or substances which affect the overall conformation and function of the horse's body (Jones and Bogart, 1971).

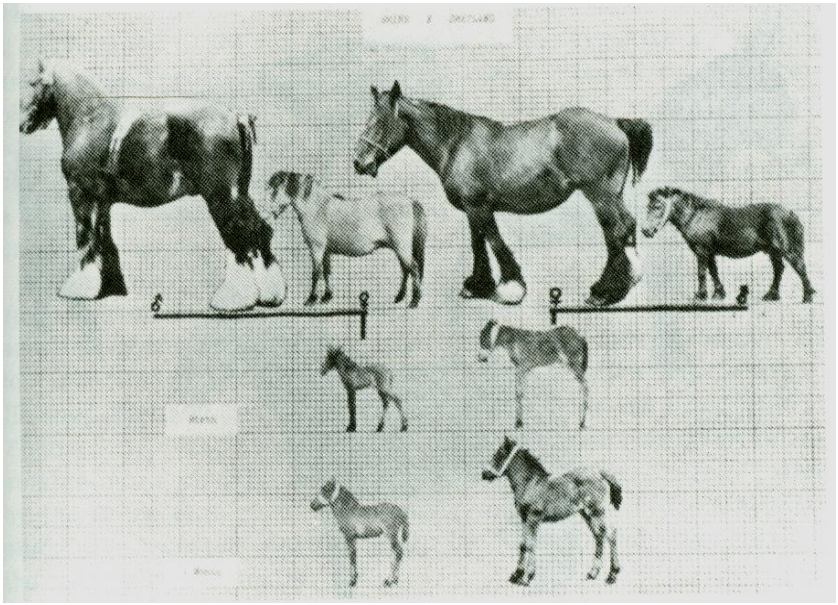


Fig. 3 Hammond's and Walton's reciprocal crosses between Shires and Shetland Ponies. The progeny from the Shire mares were three times the size of those from the Shetland mares (Jones and Bogart, 1971)

Research on pregnancies of horse, donkey, mule and hinny also occurred in the 1960s in Newmarket. The article **'Factors influencing Pregnant Mare Serum Gonadotrophin Production'** was published by **W.R. Allen** in 1969. This research primarily concentrated on the hormone 'Pregnant Mare Serum Gonadotropin' (PMSG), which is first detected in the serum of mares between days 35 and 41 of gestation. Maximum levels occur at about day 60 and the hormone often disappears from the serum by day 130. PMSG is produced by uterine structures known as endometrial cups which form in a circle around the developing foetal sac. These areas are only present in the pregnant animal; they are maximally developed by about day 70 and then degenerate rapidly. Allen (1969) noticed now that the **concentrations of PMSG** in mares which had been mated to a **jack donkey** were **much lower** than in mares carrying a horse foetus. Thus, the paternal genotype appeared to have a dominant influence on PMSG (today 'eCG') production and the placental development.

The subject of the broodmare sire was also dealt with by **Matthew Binns and Tony Morris** (2010). They mentioned it in the chapter **'Nicks and Broodmare Sires'** and among the several different types of 'nick', they consider the sire-broodmare nick as the only one where there is at least a close enough relationship between the individuals involved for certain gene combinations to be reproduced in a fair proportion of the offspring. Their opinion is founded by also referring to the studies on mice in the mid-1980s in England, that it did matter from which parent an individual animal inherited different forms of a gene: genes which were transmitted in a "switched-off" (inactivated) form from one sex, whilst the other sex passed on an active form of the gene - the phenomenon of 'genomic imprinting'.
Such a positive statement of these great experts certainly motivates us to occupy ourselves with this phenomenon. Especially as they added that nicking remains an interesting idea that could become verified with increasing amounts of molecular genetic information on Thoroughbreds in the future.

Paternally expressed genes predominate in the placenta

The presumptions of Binns and Morris (2010) became reality very soon, with the study of **Wang and co-workers** at Cornell University (Ithaca NY) in 2013. These scientists referred both to the discovery of genomic imprinting through studies of manipulated mouse embryos (in 1984 & 1986) but also to the work of Allen (1969 - by now 50 years ago!) on equine Chorionic Gonadotropin (eCG - the new expression for the former 'PMSG') in the horse, the donkey and their hybrids. There, the peak serum concentrations of eCG were markedly higher when the sire was a horse than in pregnancies in which the sire was a donkey.

Wang et al. (2013) consider the results of Allen (1969) as consistent with the action of paternally expressed imprinted genes in mice 15 years later and their own results.

They also followed the approach of Allen (1969) by using hybrids and used the method of RNA sequencing to placental tissues (trophoblast) of mules and hinnies. This combination allowed the parent-of-origin identification of most expressed genes. They identified a core group of 15 ancient imprinted genes, of which 10 were paternally expressed.
Additional 78 candidate imprinted genes also showed paternal bias. These genes displayed expression in the placenta but not in the fetus. Some showed individual variability which results in a unique epigenetic signature for each placenta. This variation presents a central importance of the placenta and the opportunity for natural selection generally.

In the case of reciprocal horse-donkey hybrids, the mule (donkey father) and hinny (horse father) differ physiologically and in temperament, despite sharing nuclear genomes, leading to speculation that these phenotypic differences might be attributable to the action of imprinted genes. Evidence suggests that genome-wide methylation does **not** undergo any organizational alteration in interspecific hybrids of placental mammals; therefore, the mule and hinny may provide a model for the identification of further imprinted genes.

Wang concluded that their results confirm what past findings suggested and that the methods used may also help breeders. Doug Antczak (one of the co-authors) added: ***"This discovery explains what breeders call the maternal grandsire effect. Some genes skip a generation and only express in grandchildren if their carrier was a certain sex. We've developed a new approach that can identify imprinted genes that may be linked to racehorse traits and which could help breeders' decision-making."*** *(Hodes and Shtessel, 2013)*.

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Mules and hinnies as hybrids are once more fantastic helpers for genetic studies in our times. However, mules already had been most reliable companions for us for centuries (photo: Schmid, Valais 1944)

If there is any aera you would like covered in these very informative newsletters you should contact Kerry on kryan@itba.ie and she will forward your request on.

Joe Hernon, Chairman EFTBA

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