Effect of inbreeding on the incidence of retained placenta in Friesian horses

M. Sevinga*, T. Vrijenhoek†, J. W. Hesselink‡, H. W. Barkema§, and A. F. Groen†

*Veterinary Practice Mid-Fryslân, Akkrum, The Netherlands; †Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands; ‡Groningen University Hospital, Groningen, The Netherlands; §Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Canada

ABSTRACT: This study was motivated by the hypothesis that the incidence of retained placenta (RP) in Friesian horses is associated with inbreeding. The objectives were to 1) calculate the inbreeding rate in the total registered Friesian horse population; 2) study the association of the inbreeding coefficient of the foal and the mare with the incidence of RP; and 3) study the heritability of RP in Friesian mares after normal foalings. Data from the total registered Friesian horse population from 1879 to 2000 (52,392 individuals) were collected from the registration files of the Friesian Horse Studbook. In 1999 and 2000, 495 parturitions in 436 mares were studied. From 1979 to 2000, the inbreeding rate of the total population was 1.9% per generation. The regression coefficients for the regression of the incidence of RP on inbreeding coefficients of the foal and the mare were 0.12 ± 0.052 and −0.016 ± 0.019, respectively. Mean heritability estimates of RP as a foal trait and as a mare trait were 0.046 ± 0.088 and 0.105 ± 0.123, respectively. It was concluded that, in order to avoid a further increase in the incidence of RP in Friesian mares, a decrease in the inbreeding rate by increasing the effective breeding population is required. Furthermore, the findings indicate that the high incidence of RP in Friesian horses is at least partly a result of inbreeding.

Since the origin of the breed, the number of Friesian horses has declined during several critical periods (Osinga, 2000). The increased agricultural mechanization in the early 1900s caused a dramatic decrease in interest in Friesian horses. In 1917, only three stallions were registered. A second bottleneck occurred in the late 1960s, when approximately 1,000 horses and 500 matings were registered. The present-day number of approximately 30,000 registered horses originates from this small population. This history suggests the occurrence of inbreeding and genetic drift (Nicholas, 1996). These phenomena may cause a decrease of the level of heterozygosity in a population, which may lead to inbreeding depression.

We hypothesize that the incidence of RP in Friesian horses is associated with inbreeding. Therefore, the objectives of the present study were to 1) calculate the rate of inbreeding in the total registered Friesian horse population; 2) study the association of the inbreeding coefficient of the foal and the mare with the incidence of RP; and 3) estimate the heritability of RP.

Materials and Methods

Total Friesian Horse Population

Data from the total registered Friesian horse population from 1879 to 2000 were collected from the registra-
tion files of the Friesian Horse Studbook. Individual inbreeding coefficients and a sequential ID file were generated using Pedigree Viewer (http://www.personal.unie.edu.au/~bkinghor/pedigree.htm). Average inbreeding coefficients for each year (Ft), and rate of inbreeding ((Ft − Ft-1)/(1 − Ft)) were calculated (Falconer, 1989). The base population was defined as consisting of parents of unknown descent with an inbreeding coefficient of 0.0. The effective population size (Ne) was calculated as Ne = 1/2ΔF (Falconer, 1989), where ΔF is inbreeding trend per generation. All four selection paths were included when estimating the generation interval used for calculating the inbreeding trend per generation.

Study Population for Inbreeding and Retained Placenta

Before the foaling season of 1999 and 2000, owners (n = 198) of Friesian brood mares (Equus caballus) in 12 veterinary practices in the Dutch provinces of Fryslân and Noord-Brabant (n = 11 and n = 1, respectively) were willing and able to participate in this study. Participating owners agreed to register all parturitions of their Friesian mares and to not treat RP within 3 h after delivery of the foal. After at least 3 h following delivery, the mares with RP were, if necessary, treated systemically with oxytocin or oxytocin dissolved in a calcium-magnesium-borogluconate solution (Sevinga et al., 2002a), and the placenta was removed manually when this treatment was not successful. In case of dystocia, mares were excluded from the study.

To classify the mares with and without RP, the time of delivery of the foal and the time of expulsion and/or removal of the placenta were registered. Retained placenta was defined as a failure to expel all or part of the fetal membranes for at least 3 h after delivery. The registered data were checked for completeness and adequacy, such as certainty about the time of expulsion of the fetal membranes and the interval between the delivery of the foal and the initiation of treatment.

Statistical Analyses

Regression analysis was performed using the AS-REML software package (http://www.vsn-intl.com/ASReml/). Regression coefficients of the incidence of RP on the inbreeding coefficient of the foal (foal regression), as well as that of the mare (mare regression), were estimated separately. Based on preliminary analyses, the following regression models were used:

- **Foal regression:** \( y = s + se + ysp + bF_f + e \)
- **Mare regression:** \( sm + se + ysp + bF_m + e \)

where s represents the sire effect, sm is the effect of the maternal grandsire, se is the sex of the foal, ysp is the interaction effect of year, season, and veterinary practice, b is the regression coefficient of the occurrence of RP on the inbreeding coefficient, \( F_f \) is the inbreeding coefficient of the foal, \( F_m \) is the inbreeding coefficient of the mare, and e is the environmental effect.

Logistic regression was performed in order to account for the binary character of RP. The following mixed sire model was derived from both regression models:

\[ y = Xb + Z_1s + Z_2ysp + e \]

where the vector y consists of all observations with \( yi = 1 \) or without \( yi = 0 \) the occurrence of RP; vector b represents one fixed effect, sex of the foal, with two classes (male and female); and vector s contains random sire effects, with 52 classes (sires) in the case of foal regression and 69 classes (maternal grandsires) in the case of mare regression. The variance of s is defined as \( \sigma^2_s \), where A is the matrix with additive genetic relationships between sires. The matrix A is not assumed to be identical to I, the identity matrix, because pedigree information from two generations was included. Therefore not all sires are unrelated. The vector ysp contained random effects of year × season year × practice (72 combinations). The variance of ysp is defined as \( \sigma^2_{ysp} \). Vector e refers to the residual effects, with variance \( \sigma^2_e \). The incidence matrices X, Z1, Z2 relate observations to corresponding sex, sire and ysp effect levels. Calculated variances were used to estimate heritability, using the following formula:

\[ h^2 = 4 \frac{\sigma^2_p}{\sigma^2_p} \]

where \( h^2 \) is the heritability of RP, \( \sigma^2_p \) is the estimated sire variance, and \( \sigma^2_e \) is the total phenotypic variance. The total phenotypic variance is made up of the sire variance (\( \sigma^2_p \)), the year, year × season year × practice variance (\( \sigma^2_{ysp} \)), and the error variance (\( \sigma^2_e \)).

Results

Total Friesian Horse Population

The total Friesian horse population comprised 52,392 individuals, with 21,991 stallions, 547 (2.5%) of which have been used as sires. For every individual, the entire pedigree was registered. Mean total number of offspring per sire was 92. In 1979 and 2000, mean number of offspring per sire was 15 and 54, being 3.4 and 1.4%, respectively, of the total annual offspring. Three stallions produced more than 1,000 total offspring. The base population comprised 1,938 individuals. The number of parturitions per year is shown in Figure 1, and was 4,178 in 1999 and 3,722 in 2000.

Mean inbreeding coefficients of the foals born in 1999 and 2000 were 0.156 ± 0.019 and 0.157 ± 0.018, respectively. As shown in Figure 2, before 1940, the inbreeding coefficient increased only moderately. However, from 1940 to 1979, the inbreeding coefficient showed a substantial, though not constant, increase from 0.04 to approximately 0.12. The inbreeding rate from 1976 to
2000 is shown in Figure 3, and was on average 0.002/yr. With a mean generation interval of 9.6 yr, the inbreeding rate was 0.019 per generation. The Ne was 27 individuals.

**Study Population for Inbreeding and Retained Placenta**

After correcting for inadequate data, 495 observations in 436 mares were included in the study. Numbers of sires and dams' sires were 52 and 69, respectively. Mean number of offspring per sire was 9.5, with 10 sires accounting for 50% of the foals. Two stallions were sire and dam's sire as well. Incidence of RP between sires and dam’s sires ranged from 29.4 to 72.0% and from 42.1% to 66.7%, respectively. The generation interval was 9 yr (mean age of sires 9.2, and of dams 8.7 yr). Of 495 normal parturitions in 436 mares, 267 cases of RP (53.9% with a 95% confidence interval of 49.5 to 58.4%) were observed. Mean inbreeding coefficients of the foals and mares were 0.158 ± 0.018, and 0.145 ± 0.023, respectively. The slopes of the regressions of the incidence of RP on inbreeding of the foal and of the mare were equal to 0.12 ± 0.052 and −0.016 ± 0.019, respectively. Indications of the slopes of the regressions of the incidence of RP on the inbreeding coefficient of the foal and of the mare are shown in Figure 4a and 4b, respectively. These figures were created by grouping the data according to inbreeding coefficient (as indicated in the figures). For each group, the incidence of RP was calculated and plotted against the inbreeding coefficient. Mean heritability estimates of RP as a foal trait and as a mare trait were 0.046 ± 0.088 and 0.105 ± 0.123, respectively.

**Discussion**

In our study, mean inbreeding coefficients of all foals of the total population born in 1999 and 2000, were 0.156 and 0.157, respectively, which is even higher than the level of inbreeding resulting from half-sib mating (0.125). Inbreeding decreases the level of heterozygosity, leading to a reduction in the potential for improving...
that population by selection (Nicholas, 1996). This consequence may undermine the aims of horse breeders. Rather than the level ($F_t$) and increment of inbreeding ($F_t - F_{t-1}$), the inbreeding rate ($|F_t - F_{t-1}|/(1 - F_t)$) is the essential population parameter (Falconer, 1989). Although detailed knowledge of relevant parameters for putting a constraint on the inbreeding rate is lacking, an acceptable level may be between 0.5 and 1% per generation (Bijma, 2000). From 1979 to 2000, the inbreeding rate of the total population was 1.9% per generation. The effective population size was 27 individuals, predominantly sires. However, the mean number of sires used in this period was 49, which, with equal use of the sires, would have resulted in an inbreeding rate per generation of 1%. Firstly, this shows that only a portion of the stallions contributes substantially to the population, which is also reflected by the finding that, in the studied population, only 10 of 52 sires used accounted for 50% of the offspring. Moreover, with this lower inbreeding rate, risk of losing genetic variance in the population is lower and the expected effects of inbreeding depression may be smaller as well. Therefore, increasing the effective population size would be advantageous.

From 1979 to 2000, the number of offspring per stallion increased from 15 to 54, although the relative proportion per sire decreased from 3.4 to 1.4%. The latter is probably in part a result of the Friesian Horse Studbook providing inbreeding coefficients for new offspring since 1979, and advising against using sire–dam combinations that produce offspring with an inbreeding coefficient higher than 5% (calculated over five generations). However, considering the present inbreeding rate, and the relationship between the incidence of RP and the inbreeding coefficient of the foal, this policy has not been sufficiently successful. Therefore, if this breeding policy remains unchanged, the incidence of RP is likely to increase in the future, provided that there is no selection against RP. However, a change in breeding policy might be counteracted by the relatively small practical consequences of RP in Friesian mares (Sevinga et al., 2002a,b), which, at the same time, make selection against RP unlikely. Likewise, the estimated heritability of RP is relatively low, which would likely result in slow progress due to selection.

The findings of our study show a positive linear relationship between a high incidence of RP and the inbreeding coefficient of the foal in the studied population of Friesian horses. In contrast, the regression coefficient of the incidence of RP on the inbreeding coefficient of the mare was negative, with relatively large standard error. The incidence of RP is therefore assumed to be uninfluenced by the inbreeding coefficient of the mare, and RP could thus be regarded as a foal trait. It is suggested that, in cattle, the maturation and separation process of the placenta in normal at term deliveries could be guided by a maternal × fetal immunological interaction, possibly dependent on (in)compatibility of major histocompatibility complex (MHC) class I products between cow and calf (Joosten et al., 1991). The compatibility of MHC antigens, and thus degree of relationship between cow and calf, might be associated with RP. The probability that a foal inherits a paternal haplotype that is identical to that of the mare is increased in a foal with a relatively high inbreeding coefficient. This might determine the degree to which the mare mounts an immune response against the paternal haplotypes/alleles that are inherited by the foal, resulting in inhibited maturation and a delayed separation and expulsion of the placenta. To study the potential relationship between MHC class I genes and RP, comparative molecular studies on (markers for) MHC class I genes of sires, dams, and foals with regard to RP are indicated. Furthermore, altered placental maturation might lead to, or be a consequence of, histological changes of placental tissues. Therefore, this subject also needs further investigation.

Heritability estimates of RP in the present study were not very precise, which was caused by the relatively small population studied. Regarding the previously reported low incidences of RP in other breeds of horses (2 to 10%) (Vandeplassche et al., 1971; Provencher et al., 1988), a more precise estimate of heritability might be difficult to obtain.

In conclusion, the findings of the present study show a strong inbreeding rate in the total Friesian horse population by selection (Nicholas, 1996). This consequence may undermine the aims of horse breeders.
population over the last decades. The present findings indicate that the high incidence of RP in Friesian horses is, at least partly, a result of inbreeding. Reduction of the inbreeding rate by increasing the effective breeding population is required in order to avoid a further increase in the incidence of RP in Friesian mares.

Implications

The findings of our study support our hypothesis that the incidence of retained placenta in Friesian horses is associated with inbreeding. Due to this association, combined with the determined strong inbreeding trend over the last decades, a further increase of the high incidence of retained placenta is to be expected, unless appropriate measures are taken. Reduction of the inbreeding trend is required, which can be achieved by increasing the effective breeding population. A substantial increase of the number of sires used would be the most effective measure. Emphasizing the usefulness of sire/dam combinations that produce offspring with relatively low inbreeding coefficients should be part of the breeding policy. Because the estimated heritability is low, selection against retained placenta would likely result in slow progress.

Literature Cited


