Effects of inbreeding on semen quality of Friesian stallions

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Abstract

The relationship between inbreeding and semen quality was investigated for 1146 Friesian stallions. Semen of ± 2.5 or ± 3.5 years old stallions was collected in September, October and November of the years 1987-2002. One ejaculate of each stallion was examined on ejaculate volume, sperm cell concentration, % progressively motile sperm and % morphologically normal sperm; TNM value (Total Normal Motile sperm), which is a multiplication of these four traits; and % abnormal acrosomes, which is part of morphology. Inbreeding degree and ancestral decomposition of inbreeding was calculated for each analyzed stallion. 26 Ancestors were analyzed to investigate if inbreeding on specific ancestors can influence semen quality.

Semen quantity and quality was lower for younger stallions, % abnormal acrosomes increased over the years and morphological quality was lowest in November. Moderate to high heritability estimates for the analyzed traits suggest that semen quality is determined substantially by the genotype of a stallion. If stricter selection on semen quality can improve reproductive performance of Friesian horses, depends on the amount of genetic variance and on the effect of improved semen quality on pregnancy and foaling rates. Mean inbreeding, estimated over the entire pedigree, was 15.17 ± 1.75 %. Ejaculate volume was found to increase at higher inbreeding levels. No effect of inbreeding on other semen quality traits was found, which might be due to small variation in inbreeding percentages. Specific inbreeding on 12 of the 26 analyzed ancestors was found to have a significant effect on one or more semen quality traits. Most ancestral effects were found for % abnormal acrosomes. It suggests that further research, with respect to other ancestors and other traits, could provide interesting information about the way in which inbreeding can spread certain abnormalities through a population.

Keywords: Friesian stallions, semen quality, heritability, inbreeding, ancestors

1 Introduction

The Friesian Horse Studbook has been registering Friesian horses since 1879. Currently, the total population size consists of more than 40,000 animals, but the active breeding population contains only 88 approved breeding stallions and about 7,000 mares (www.kfps.nl). Only a few new stallions are selected for breeding each year. Before Friesian stallions can be registered for breeding, they must pass a breeding evaluation test, which includes tests of their conformation, performance and reproduction capacity. Because a stallion usually mates many mares, stallion fertility is an important factor of the overall success of a breeding program (van Buiten *et al*., 2003). During the test period of the current study, the required TNM value (number of Total Normal Motile sperm) was 600 for 3-year-old stallions and 1,000 for older animals. Further requirement was that the percentage progressively motile and morphologically normal sperm should be at least 50%. The average semen quality of Friesian stallions is low, compared to other breeds, e.g. Dutch Warmblood (Parlevliet *et al.*, 1994). Almost 50% of the Friesian stallions submitted for breeding soundness examination are excluded for breeding because of insufficient semen quality.

One of the reasons for the relatively poor fertility of Friesian horses could be inbreeding. There is continuing concern that the narrow genetic base and high inbreeding rates in the Friesian horse population may limit genetic progress and increase the frequency of heritable disorders. There have been two genetic bottlenecks in the pedigree of the Friesian horse, around 1913 and in the 1960's, when the breeding population was very small (Sevinga *et al.,* 2004). After the 1960's, the tide has turned and the Friesian horse gained in popularity. However, when a bottleneck is present in the pedigree, the effective number of founders is decreased (Boichard *et al*., 1997). Furthermore, some ancestors have a much larger genetic contribution to the current Friesian horse population than others, which might have consequences for the spreading of certain characteristics through the population. There is an unbalanced use of reproductive animals, especially of stallions. Besides, the Friesian Horse Studbook is a strictly closed population. Given this situation, it is hard to maintain genetic diversity. The current level of inbreeding in Friesian horses is high. From 1979-2000, the average increase in inbreeding was 1.9% per generation (Sevinga *et al.,* 2004), where an increase in inbreeding between 0.5 and 1.0% is assumed to be acceptable (Bijma, 2000).

Breeding in a closed population, and additionally selection on desirable traits, normally results in a certain degree of inbreeding and thereby increases the degree of homozygosity in that population (van Eldik *et al*., 2006). Inbreeding is widely believed to have detrimental effects on reproduction. Recessive or partially recessive deleterious effects of alleles seem to be the most important source of inbreeding depression (Charlesworth and Charlesworth, 1999). Increased levels of inbreeding were found to have adverse effects on several parameters of sperm production and quality of Shetland pony stallions (van Eldik *et al.,* 2006). However, the effect of inbreeding on male reproduction traits of the highly inbred Friesian horse population is not clear. In a previous study (Orsini, unpublished), higher inbreeding rates were found to have a significantly detrimental effect on morphological quality, but not on motility and TNM. In the study of Orsini, however, inbreeding was probably underestimated, because coefficients were calculated over five generations and the Friesian horse population was already highly inbred five generations ago. Furthermore, it could be that ranking of stallions according to inbreeding estimated over five generations is different from ranking according to inbreeding estimated over the entire pedigree.

Inbreeding computation is very sensitive to pedigree length and quality (Boichard *et al*., 1997). Therefore, it was hypothesized that the use of inbreeding coefficients calculated over the entire known pedigree may lead to more elucidate effects of inbreeding on reproduction traits of Friesian stallions. First aim of the current study was therefore to investigate whether inbreeding degree affects semen quality traits of Friesian stallions. Furthermore, it was hypothesized that certain ancestors of the Friesian horse population are responsible for semen quality in the way that higher inbreeding on specific ancestors can have positive or negative effects. Therefore, the second aim was to investigate if a higher inbreeding degree of stallions on particular ancestors can have influence on their semen quality traits. Third aim was to estimate genetic parameters for semen quality, because those were not yet available for Friesian stallions and they were needed to correct for genetic trends in the analyses of inbreeding effects.

2 Material and methods

2.1 Stallions and semen evaluation

As part of the selection procedure of young breeding stallions, semen quality is routinely examined at the Department of Equine Sciences of Utrecht University. Ejaculates analyzed in the current study were collected during the years 1987-2002. The examined stallions had not been subjected to earlier inspections or selection procedures, but were admitted by their owners to be suitable breeding stallions. Stallion age at the time of semen examination ranged from 24 to 47 months. Most stallions were about 2.5 years old. The analyzed ejaculates were collected in the months September, October and November. In these months, semen was evaluated of the stallions that, if semen quality appeared to be sufficient, were submitted to the first inspection round of the studbook in January of the next year. Semen quality of in total 1146 stallions was analyzed. These stallions descended from 95 sires and 92 dam sires. The number of offspring present in the dataset ranged from 1 to 63 per sire and from 1 to 76 per dam sire.

Two semen ejaculates of each stallion were collected, using an artificial vagina. The interval between collection of the first and second ejaculate was approximately one hour. During routine laboratory examination, the ejaculates were examined on volume, sperm cell concentration, motility and morphology. After measuring the gel-free volume of the ejaculate, a semen sample was taken and diluted approximately 1:1 with a skim-milk extender prior to motility estimation under a microscope equipped with a heated stage (38℃). O ther samples were used to determine sperm cell concentration and morphology. Concentration was estimated with a Burker-Türk counting chamber. Sperm cell morphology was evaluated using slides prepared with eosin-aniline blue stain, to distinguish live and dead spermatozoa. 100 Live spermatozoa were examined on morphological abnormalities, using the Bretschneider table for bull semen (Bretschneider, 1948). The spermatozoa were investigated successively on acrosome, head, midpiece and tail abnormalities. When a cell had more than one abnormality, only the most proximal was recorded. Consequently, the only morphological traits that could be analyzed unconditioned were the percentage of morphological normal cells and the percentage of abnormal acrosomes. Ejaculate

volume was multiplied by sperm cell concentration, percentage of progressively motile and percentage of morphologically normal spermatozoa, to calculate the Total number of Normal, Motile cells (TNM value).

2.2 Statistical analysis

In the current study, only the second ejaculate of all stallions was analyzed. Because of sexual inexperience of most stallions, the first collection was in many cases the actual first ejaculate produced, and therefore, the second ejaculate was considered to give a more reliable indication of true semen quality than the first one. Six semen characteristics were investigated: ejaculate volume, sperm cell concentration, percentage progressively motile sperm and percentage morphologically normal sperm; TNM value, which is a multiplication of these four traits; and the percentage abnormal acrosomes, which is part of morphology. Before analysis, TNM value, ejaculate volume, spermatozoa concentration and percentage abnormal acrosomes were log transformed, to produce a better fit to a normal distribution. Percentage normal motile cells was divided in two classes, <60% or ≥60%, to have sufficient numbers per class. Thus, statistical results inform about which part of the stallions had 60% or more normal motile cells. The percentage of morphologically normal spermatozoa was not transformed.

Because of low numbers of examined stallions per year from 1987 till 1992, these years were grouped in two classes, 1987-1989 and 1990-1992, to get a sufficient number of observations for meaningful statistical analyses. Stallion age was grouped in two age classes: 24-35 months (± 2.5 years) or 36-47 months (± 3.5 years) old. When a stallion appeared to have insufficient semen quality at the age of 2.5, it was sometimes submitted by the owner again at the age of 3.5, with the idea that semen quality improves when a stallion becomes more mature (Dowsett and Knott, 1996). In that case, only the second ejaculate of the last examination (at age 3.5) was analyzed. Of the 318 3.5-years-old animals, 132 were submitted for semen examination for the second time. In total, the last ejaculate of 1146 stallions was analyzed. Table 1, 2 and 3 give an overview of the number of analyzed stallions per year, per month and per age class.

Table 1: Number of examined stallions per year.

Year of examination	*'87-'89*	*'90-'92*	*'93*	*'94*	*'95*	*'96*	*'97*	*'98*	*'99*	*'00*	*'01*	*'02*
# stallions	27	42	27	29	38	86	106	106	136	143	194	212

Table 2: Number of examined stallions per month.

Month of examination	September	October	November
# stallions	332	610	204

Table 3: Number of examined stallions per age class.

Age at examination	2.5-years-old	3.5-years-old
# stallions	828	318

The observations were analyzed using the following linear model:

$$Y_{ijklm} = \mu + year_i + month_j + age_k + b*F_l + animal_m + e_{ijklm}$$

Where:

- Y = dependent variable (log(TNM), log(concentration), log(volume), motility class, % morphological normality or log(% abnormal acrosomes))
- μ = mean
- year = fixed effect of year class i [1987-1989, 1990-1992, 1993, 1994, …, 2002]
- month = fixed effect of month j [September, October, November]
- age = fixed effect of age class k [2.5, 3.5]
- F = inbreeding coefficient
- b = regression coefficient

animal = random animal effect
e = random error

Effects of year class, age class and month were investigated in a preliminary study with SAS (SAS, 2006) and were considered significant at P<0.05. Non-significant fixed effects were in order of significance excluded from the model.

With the current pedigree information, it was possible to estimate inbreeding coefficients back to 1879, when the studbook started registering Friesian horses. The inbreeding coefficient over the entire pedigree of each animal was estimated with PEDIG, a software package developed by Boichard *et al.* (1997). To investigate possible influence of inbreeding on semen quality, inbreeding percentage of the stallions analyzed in the current study was added to the model as linear effect and as class effect to check for non-linearity. A random animal component was added to the model to correct for genetic trend. Four generations of the pedigree were taken into account for setting up the relationship matrix. Genetic analyses were performed using ASReml package (Gilmour *et al.*, 2002). From these analyses, heritability estimates and estimated breeding values for semen quality traits were obtained.

CFC, a software package developed by Sargolzaei *et al.* (2006), was used to calculate the ancestral decomposition of inbreeding for each analyzed stallion. Possible ancestral effects were analyzed for those ancestors that had at least 15 stallions that were more than 0.5 % inbred on that ancestor. This resulted in 26 analyzed ancestors. To avoid confounding between ancestral effects, inbreeding on each ancestor was included separately as class effect in the linear model for each semen quality trait. Also the significant effects of year, age, month and inbreeding were included in these models.

3 Results

3.1 Semen quality parameters

The overall mean values of raw data from the 1146 analyzed ejaculates are given in table 4. Correlation coefficients between TNM value and the other analyzed traits were all significant (P<0.05) and were -0.07 for volume, 0.73 for concentration, 0.40 for motility, 0.47 for morphology and -0.22 for acrosome abnormalities.

Table 4: Mean values (N=1146) of semen quality parameters (based on the untransformed data).

Variable	*Mean*	*Std. deviation*	*Minimum*	*Maximum*
TNM value	1815.94	1716.50	0.10	17028.10
Ejaculate volume (ml)	53.49	24.59	10.00	210.00
Sperm cell concentration ($x10^6$/ml)	109.33	115.99	1.00	1128.00
Progressively motile sperm(%)	66.83	12.60	1.00	90.00
Morphologically normal sperm (%)	52.93	15.62	1.00	88.60
Abnormal acrosomes (%)	32.99	19.67	0.00	97.00

3.2 Effect of year, month and age

Year, month and age at time of semen evaluation appeared to have significant (P<0.05) effects on several parameters for semen quality. The obtained least squares means show that there were significant differences between years for ejaculate volume, motility class and % morphological normality, but there was no consistent trend. The percentage abnormal acrosomes, however, increased significantly over the years (appendix 1). The percentage morphologically normal cells was significantly lower, while percentage abnormal acrosomes was higher in November, compared to September and October. TNM value, ejaculate volume, motility and morphology were significantly lower for 2.5-years-old stallions, compared to 3.5-years-old stallions. Sperm cell concentration was not significantly affected by year, month or age (table 5). Least squares means per month and age class are given in table 6 and 7. The interaction between year and month was investigated, but appeared to have no significant effect on any of the analyzed semen characteristics.

Table 5: Probability level and R^2 of year, month and age effects (ns=not significant(P>0.05)).

	TNM value	*Ejaculate volume*	*Sperm cell concentration*	*Motility class*	*% morph. normal sperm*	*% abnormal acrosomes*
R^2	0.013	0.087	-	0.063	0.047	0.104
Year	ns	P=0.0002	ns	P<0.0001	P<0.0001	P<0.0001
Month	ns	ns	ns	ns	P=0.0016	P=0.0001
Age	P<0.0001	P<0.0001	ns	P=0.0054	P=0.0018	ns

Table 6: Least squares means (± standard error) per age class. (For TNM and volume, numbers before the brackets are back transformed means, between brackets lsmeans ± std. error of log transformed data.)

Age	*TNM value*	*Ejaculate volume*	*Motility class*	*% morph. normal sperm*
2.5	1032.25 (3.01 ± 0.02)	46.43 (1.67 ± 0.01)	0.81 (± 0.01) *	51.76 (± 0.67)
3.5	1395.45 (3.14 ± 0.03)	59.42 (1.77 ± 0.01)	0.88 (± 0.02) *	54.95 (± 0.97)

**respectively 81% and 88% of the stallions had ≥60% progressively motile sperm.*

Table 7: Least squares means (± standard error) per month. (For % abnormal acrosomes,, numbers before the brackets are back transformed means, between brackets lsmeans ± std. error of log transformed data.)

Month	*% morph. normal sperm*	*% abnormal acrosomes*
Sep	55.41 (± 0.98)	22.76 (1.36 ± 0.02)
Oct	54.30 (± 0.80)	23.92 (1.38 ± 0.01)
Nov	50.36 (± 1.14)	29.16 (1.46 ± 0.02)

3.3 Heritability

To estimate heritabilities for semen quality traits, an animal model was carried out for each trait, correcting for significant effects of year, month, age and inbreeding. As shown in table 8, the animal model resulted in moderate heritability estimates for TNM value (0.21), ejaculate volume (0.20), sperm cell concentration (0.22) and percentage progressively motile sperm (0.26). Heritability estimates for percentage morphologically normal cells and percentage abnormal acrosomes were high (0.45 and 0.62 respectively).

Table 8: Heritability estimates (± standard error).

Trait	*Heritability estimate (h^2)*
TNM value	0.21 (± 0.07)
Ejaculate volume	0.20 (± 0.07)
Sperm cell concentration	0.22 (± 0.07)
Motility class	0.26 (± 0.08)
Morphologically normal sperm	0.45 (± 0.10)
Abnormal acrosomes	0.62 (± 0.11)

3.4 Inbreeding

Table 9 gives mean values of inbreeding percentages of the stallions analyzed in the current study, estimated over five generations and over the entire pedigree. The generation interval of the Friesian horse population is estimated on 9.6 years by Sevinga *et al.* (2004), which means that about 12 generations are taken into account when inbreeding is estimated over the entire pedigree. Most of the stallions with a relatively high inbreeding percentage estimated over five generations had also a high inbreeding percentage estimated over the entire pedigree, but there were a number of exceptions (appendix 3). The correlation coefficient between the two percentages was 0.75. The development of inbreeding in the Friesian horse population since 1937 is shown in figure 1.

Table 9: Mean values of estimated inbreeding percentages of 1146 stallions.

Inbreeding percentage (%)	*Mean*	*Std. deviation*	*Minimum*	*Maximum*
Over five generations	3.16	1.54	0.20	10.74
Over entire pedigree	15.17	1.75	10.13	22.06

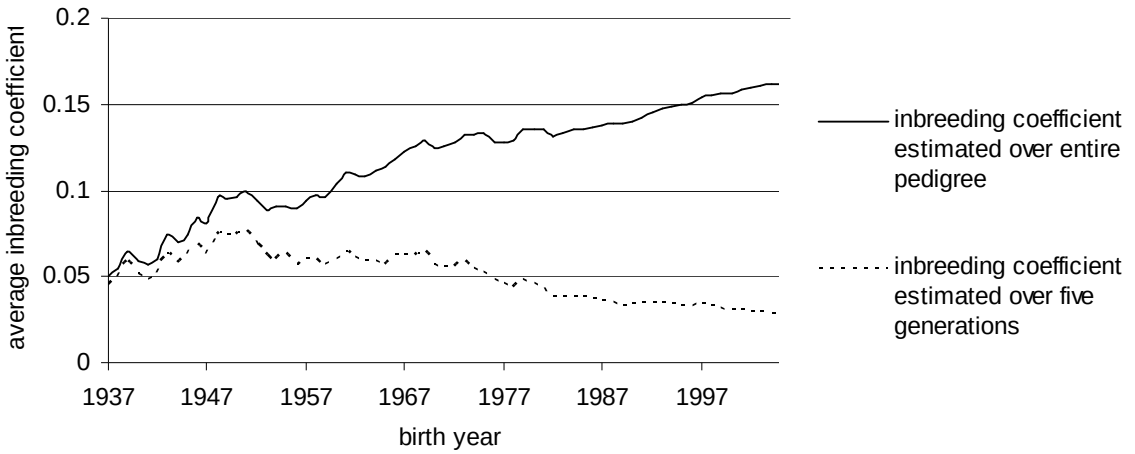

Figure 1: Average inbreeding coefficients of the Friesian horse population per birth year (1937-2006).

Analyses of the linear effect of inbreeding showed that a higher inbreeding level resulted in a higher ejaculate volume (P=0.008). For the other semen quality traits, no significant effects of inbreeding were traced. Also when inbreeding was treated as a class effect, with classification according to table 10, no effect of inbreeding on these traits was found. The least squares means per inbreeding class are shown in appendix 4.

Table 10: number of stallions per inbreeding class (inbreeding percentage estimated over entire pedigree).

Inbreeding percentage	10.1-12.5%	12.5-13.5%	13.5-14.5%	14.5-15.5%	15.5-16.5%	16.5-17.5%	17.5-18.5%	18.5-22.1%
Inbreeding class	10-12	13	14	15	16	17	18	19-22
# stallions	63	125	248	236	209	157	64	44

3.5 Ancestral effects

Possible effects of inbreeding on semen characteristics was analyzed for 26 ancestors, in this study named A-Z. Eight of these ancestors were born before 1925, nine between 1925 en 1960 and nine after 1960. The youngest analyzed ancestor was born in 1978. Since groups of stallions have the same inbreeding loops for a particular ancestor (e.g. the ancestor is two times the great-grandfather of a number of animals), inbreeding level is, especially for the more recent ancestors, not a continuous variable. Consequently, inbreeding on these ancestors could not be analyzed as a linear effect. Therefore, a classification of the inbreeding percentages was made for each ancestor. Inbreeding on 12 of these 26 ancestors was found to have a significant influence on one or more semen quality traits of the stallions in the current dataset. Table 11 A-Z shows the results per ancestor. An overview of the ancestors that have a positive or negative effect on parameters for semen quality is given in table 12.

Table 11 A-Z: Least squares means (± standard error) per ancestor per inbreeding class for significantly affected semen characteristics, with # observations (stallions) per inbreeding class. (For TNM, volume, concentration and % abnormal acrosomes, numbers before the brackets are back transformed means, between brackets lsmeans ± std. error of log transformed data.)

Ancestor A			Lsmeans (± std error)	
Inbreeding %	Class	#	TNM value	Motility class
0.5-0.75%	1	212	1459.84 (3.16 ± 0.04)	0.89 (± 0.03)
0.75-0.8%	2	286	1223.08 (3.09 ± 0.03)	0.87 (± 0.02)
0.8-0.85%	3	378	1205.81 (3.08 ± 0.03)	0.84 (± 0.02)
>0.85%	4	269	1003.57 (3.00 ± 0.03)	0.80 (± 0.02)

Ancestor E			Lsmeans (± std error)
Inbreeding %	Class	#	% abnormal acrosomes
<0.5%	0	148	28.34 (1.45 ± 0.03)
0.5-0.6%	1	232	25.21 (1.40 ± 0.02)
0.6-0.7%	2	273	25.44 (1.41 ± 0.02)
0.7-0.8%	3	228	23.96 (1.38 ± 0.02)
>0.8%	4	264	23.44 (1.37 ± 0.02)

Ancestor G			Lsmeans (± std error)
Inbreeding %	Class	#	Ejaculate volume
<0.5%	0	212	58.87 (1.77 ± 0.01)
0.5-0.7%	1	237	53.85 (1.73 ± 0.01)
0.7-0.9%	2	212	52.34 (1.72 ± 0.01)
0.9-1.1%	3	238	52.09 (1.72 ± 0.01)
>1.1%	4	246	47.98 (1.68 ± 0.01)

Ancestor I			Lsmeans (± std error)	
Inbreeding %	Class	#	% morph. normal cells	% abnormal acrosomes
<0.5%	0	777	54.30 (± 0.74)	24.21 (1.38 ± 0.01)
≥0.5%	1	368	51.79 (± 0.91)	26.65 (1.43 ± 0.02)

Ancestor J			Lsmeans (± std error)
Inbreeding %	Class	#	% abnormal acrosomes
<0.65%	0	866	25.17 (1.40 ± 0.01)
0.65-0.75%	1	174	26.96 (1.43 ± 0.02)
>0.75%	2	105	21.46 (1.33 ± 0.03)

Ancestor K			Lsmeans (± std error)			
Inbreeding %	Class	#	TNM value	(P=0.06) Sperm cell concentration	% morph. normal cells	% abnormal acrosomes
<0.5%	0	868	1270.99 (3.10 ± 0.02)	73.66 (1.87 ± 0.01)	53.99 (± 0.69)	24.44 (1.39 ± 0.01)
0.5-0.65%	1	161	1100.68 (3.04 ± 0.04)	66.22 (1.82 ± 0.03)	53.64 (± 1.32)	25.14 (1.40 ± 0.02)
>0.65%	2	116	888.18 (2.95 ± 0.05)	59.90 (1.77 ± 0.04)	48.33 (± 1.51)	30.42 (1.48 ± 0.03)

Ancestor M			Lsmeans (± std error)
Inbreeding %	Class	#	Sperm cell concentration
<0.5%	0	1004	72.89 (1.86 ± 0.01)
≥0.5%	1	141	59.29 (1.77 ± 0.04)

Ancestor O			Lsmeans (± std error)
Inbreeding %	Class	#	% morph. normal cells
<0.5%	0	1074	53.59 (± 0.66)
≥0.5%	1	71	48.26 (± 1.96)

Ancestor P			Lsmeans (± std error)
Inbreeding %	Class	#	Ejaculate volume
<0.5%	0	1077	52.16 (1.72 ± 0.01)
0.5-0.7%	1	36	51.40 (1.71 ± 0.03)
>0.7%	2	32	65.34 (1.82 ± 0.03)

Ancestor S			Lsmeans (± std error)
Inbreeding %	Class	#	% abnormal acrosomes
<0.5%	0	1113	25.29 (1.40 ± 0.01)
≥0.5%	1	32	19.01 (1.28 ± 0.05)

Ancestor W			Lsmeans (± std error)
Inbreeding %	Class	#	TNM value
<0.5%	0	1120	1181.15 (3.07 ± 0.02)
≥0.5%	1	25	2396.75 (3.38 ± 0.10)

Ancestor Z			Lsmeans (± std error)
Inbreeding %	Class	#	% abnormal acrosomes
<0.5%	0	1128	25.18 (1.40 ± 0.01)
≥0.5%	1	17	17.33 (1.24 ± 0.07)

Table 12: overview of ancestors with significantly (P<0.05) positive (+) or negative (-) influence on semen characteristics.

Ancestor	*TNM value*	*Ejaculate volume*	*Sperm cell concentration*	*% progress. motile sperm*	*% morph. normal sperm*	*% abnormal acrosomes*
A	-			-		
E						+
G		-				
I					-	-
J						+
K	-				-	-
M			-			
O					-	
P		+				
S						+
W	+					
Z						+

Inbreeding on ancestor K appeared to have the most detrimental effect on semen quality. When inbreeding on this animal exceeds 0.65%, TNM value, percentage morphologically normal cells and percentage abnormal acrosomes is significantly worse and also sperm cell concentration tends to decrease (P=0.06). Most ancestral effects were traced for percentage abnormal acrosomes. Four of the analyzed ancestors were found to have a positive influence; an increased inbreeding percentage on those ancestors resulted in less abnormalities. Inbreeding on two ancestors was found to have a negative influence on the amount of acrosome abnormalities, and both had also a detrimental effect on overall morphological quality. Ancestors which detrimentally affected certain semen quality traits when inbreeding on that ancestor was higher, were also found to have negative estimated breeding values for those traits, and the other way around.

4 Discussion

4.1 Introduction

Aim of this study was to investigate the genetic influence on semen quality in Friesian stallions. Genetic influence can be thought of as additive genetic variance of a trait, but also as the effect of inbreeding on a trait. Inbreeding in general leads to an increase of homozygosity and can result in inbreeding depression for certain traits, which reflects that deleterious genes are segregating in the population. This estimates an average effect of inbreeding on the outcome of a certain trait, but it could also be that a certain ancestor is segregating deleterious alleles. Estimating the effect of inbreeding on a particular ancestor would provide such information. Analysis of variance was used to estimate the influence of these three types of genetic phenomena (heritability, general inbreeding and particular inbreeding) on semen quality in Friesian stallions. In the next paragraphs, these topics will be discussed, after a short discussion about some environmental influences.

4.2 Effects of year, month and age

Sperm production and quality are affected by many environmental and animal factors. Several studies reported significant differences between first and second ejaculates collected at routine semen evaluation of stallions (Van Eldik *et al.*, 2006; Sieme *et al.*, 2004). Experimental results, presented by Jasko *et al.* (1991) and in a review of Malmgren (1997), describe substantial variation between ejaculates of individual stallions, caused by factors such as season, collection technique and frequency of collection. Also Janett *et al*. (2003) reported that season significantly influences semen quality traits. Results from the current study showed a decreased morphological quality in November, compared to September and October, which is again an indication that semen quality of a stallion is not constant throughout the year. Climatic conditions might also be an explanation for differences between years. Ejaculate volume, percentage of progressively motile sperm and percentage of morphologically normal sperm differed significantly between years of examination, but showed no consistent trend. Because the number of stallions was properly distributed, it is not likely that year effect is confounded with month. In the current study, stallions of 2.5 years old had in general poorer semen quality than 3.5-years-old animals. Differences between age classes were also reported by Aurich *et al.* (2003) and by Dowsett and Knott, (1996), who found lower values for 2-year-old stallions, compared with older ones, for total sperm number and ejaculate volume, and a higher percentage of sperm abnormalities. It is assumed that the ejaculates analyzed in the current study give a reasonable indication, but it should be possible to obtain more representative numbers of what semen quality would be at daily sperm output of mature stallions during the breeding season.

Acrosome abnormalities are often seen in the sperm of Friesian stallions (table 4). The increasing number of acrosome abnormalities, found in the current study (appendix 1.4), could be an indication that there is a decreasing tendency in overall morphological quality of Friesian stallion semen. Unfortunately, with the available data it was not possible to investigate this properly. When more abnormal acrosomes are recorded, it could be that less other abnormalities are scored, because only the most proximal abnormality per sperm cell is registered. This could be the reason that no change is seen in the percentage morphologically normal spermatozoa.

4.3 Heritability

A large range in heritability estimates has been reported for semen characteristics of bulls (Mathevon *et al*., 1998) and boars (Smital *et al.*, 2005). By contrast, there is not much literature available about heritability of stallion semen. Parlevliet *et al*., (1994) found a significant sire effect ($P<0.05$) for volume, motility and sperm concentration of Dutch Warmblood stallions, which gave a first indication that those factors are heritable in horses. In a study of Van Eldik *et al.* (2006), heritability estimates for Shetland pony semen characteristics were 0.23 (± 0.18) for TNM, 0.57 (± 0.24) for volume, 0.24 (± 0.16) for concentration, 0.46 (± 0.23) for progressive motility and 0.13 (± 0.20) for morphology. The large standard errors were probably caused by the relatively small dataset (285 stallions). In the current study, a sire model resulted in somewhat lower estimates for

heritability than an animal model. Appendix 2 compares the breeding values of all sires estimated with a sire model and with an animal model.

The moderate to high heritability estimates obtained in the current study, combined with the large phenotypic variation present for semen quality parameters, indicate that selection could be a promising tool to improve semen quality in Friesians. At the moment, almost half of the submitted Friesian stallions fail to meet the minimum requirements for semen quality. When the percentage that passes semen quality examination would increase, there are more possibilities to select stallions on other traits, e.g. conformation and gaits.

4.4 Inbreeding

Figure 1 shows that the inbreeding coefficient estimated over five generations is decreasing. Probably, this is due to the efforts of the studbook, which provides inbreeding percentages estimated over five generations to the breeders and strongly advices not to mate horses when inbreeding percentage of the foal will exceed 5%.

Inbreeding is often discussed to impair fertility, but until now, only a very few studies have been performed with respect to inbreeding effects on stallion semen. Aurich *et al.* (2003) genotyped 12 microsattelite markers of 110 Noriker draught horse stallions to determine heterozygosity, and did not found a relationship between heterozygosity and semen parameters. Van Eldik *et al*. (2006) examined two ejaculates of 285 Shetland pony stallions on sperm quantity and quality. These stallions were grouped with respect to their estimated inbreeding coefficients, calculated over six generations of pedigree. The level of inbreeding ranged from 0 to 25 % (mean 3 ± 4.6 %) and appeared to have a significant effect on some parameters of semen production and sperm quality. In particular, increased inbreeding percentage was correlated with lower percentages of progressively motile and morphologically normal sperm. The negative effect of inbreeding on motility and morphology was evident at inbreeding coefficients of 2-5% and became increasingly obvious at higher inbreeding levels. Furthermore, ejaculate volume of Shetland ponies increased when inbreeding level was higher. This was also seen in Friesians in the current study. Yet, no biological explanation for this phenomenon has been found. However, ejaculate volume is not assumed to be strongly correlated with fertility, so it is not expected that higher inbreeding in this way can compromise fertility.

There was a limited range in inbreeding percentage of the animals analyzed in the current study. Furthermore, the lowest inbreeding percentage was already 10.13%. This could be reasons that, except from the small effect on ejaculate volume, no significant influence of inbreeding on semen quality traits was found. Therefore, it would be interesting to know the semen quality of some Friesian stallions with a lower inbreeding degree than the animals in the current study. Populations seem to differ in sensitivity to inbreeding depression (as reviewed in Oliehoek, 1999), which might be a reason that inbreeding was found to have a detrimental effect on semen quality traits in Shetland ponies (Van Eldik *et al*., 2006), but that these effects are not seen in Friesian horses.

4.5 Ancestral effects

The effect of inbreeding on particular ancestors has been studied in birth cohorts to describe the epidemiology of dichotomous genetic disorders, to estimate the risk of an inherited disease (Man *et al*., 2007; Ubbink *et al.*, 1998). Semen quality parameters, however, are not likely to be dichotomous characteristics, but are expected to involve multiple genes. No literature was found about this kind of pedigree analysis for continuous traits. Inbreeding increases homozygosity and thereby attends fixation of recessive deleterious alleles. This is a potential mechanism by which not only inbreeding in general, but also inbreeding on a particular ancestor might affect semen quality in stallions. It would be helpful for breeding management to know to which extend deleterious genes are spread throughout the population, because it will inform about the likely incidence of certain disorders. For this purpose, the ancestral sources of such genes has to be known.

Because of the very unequal representation of ancestors in the pedigrees of the analyzed stallions, it was hard to analyze and compare their effect on semen quality traits. When taking inbreeding classes smaller, standard errors became too large and differences were not significant

anymore, due to the large data range of the analyzed parameters for semen quality. Furthermore, interaction between ancestral effects may play a role, but was not investigated. There might be other and better methods (e.g. segregation analysis; Jarvik, 1998) to investigate ancestral effects on quantitative traits in a large population, but the current research already clearly showed that there are differences between ancestors. In the current study, inbreeding on only 26 ancestors was analyzed for effects on semen quality. These results indicate that further research, with respect to other influential ancestors as well as to other traits, could provide interesting information about the way in which inbreeding can spread certain abnormalities through a population. It is not recommended to avoid the ancestors which were associated in the current study with negative semen quality in the pedigree of potential breeding stallions, but to avoid too much inbreeding on these ancestors. Stallions with poor semen quality will be rejected from breeding anyway, irrespective of their pedigree.

4.6 Additional remarks

Although the selection intensity on semen quality is not very high, with the heritabilities estimated in the current study, one would expect some increasing genetic trend, at least for the amount of morphologically normal cells and a decreasing trend for the amount of abnormal acrosomes. However, it is not known which stallions that are analyzed in the current study have passed the further inspections and tests of the studbook, and are subsequently used for breeding.

Foaling rate is the true index of stallion fertility, but those numbers are retrospective and largely influenced by factors like mare reproductive status and breeding management (Colenbrander *et al*., 2003; Langlouis and Blouin). It is yet not clear to which extent semen quality parameters are reliable predictors of fertility or if the low foaling rate observed in Friesian horses is a more direct consequence of the high inbreeding level. It is agreed that stallions with really poor semen quality will fail to produce many offspring. However, good semen quality is not always a guarantee for adequate reproductive performance. Van Buiten *et al.* (1999) concluded that the non-return rate at 28 days is a valuable parameter for the assessment of stallion fertility. Unfortunately, the true relationship between semen quality and pregnancy and foaling rates has not been determined yet.

5 Conclusions

- Heritability estimates for semen quality parameters were 0.21 for TNM value, 0.20 for ejaculate volume, 0.22 for sperm cell concentration, 0.26 for motility class, 0.45 for % morphologically normal sperm and 0.62 for % abnormal acrosomes.
- Except for ejaculate volume, higher inbreeding levels did not affect semen quality of Friesian stallions.
- Significant effects of inbreeding on particular ancestors were found for the traits TNM value, ejaculate volume, sperm cell concentration, motility class, % morphologically normal sperm cells and % abnormal acrosomes.

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Appendices

Appendix 1: least squares means per year for ejaculate volume (back transformed), motility class, % morphologically normal cells and % abnormal acrosomes (back transformed).

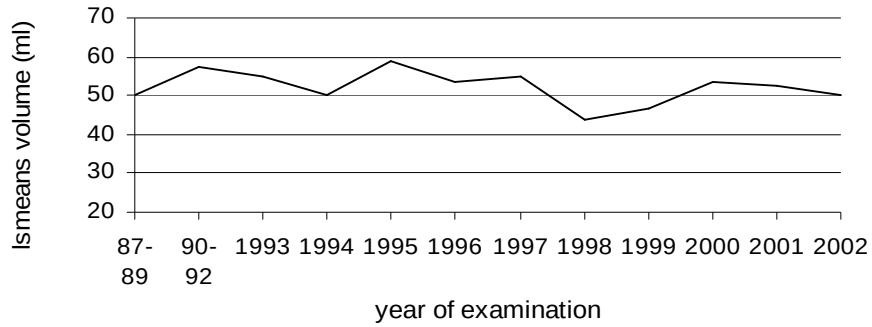

Figure 1.1: Least squares means of ejaculate volume per year.

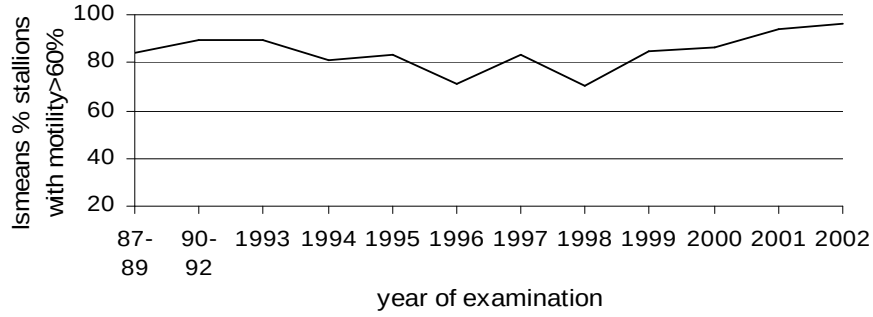

Figure 1.2: Least squares means of motility class per year.

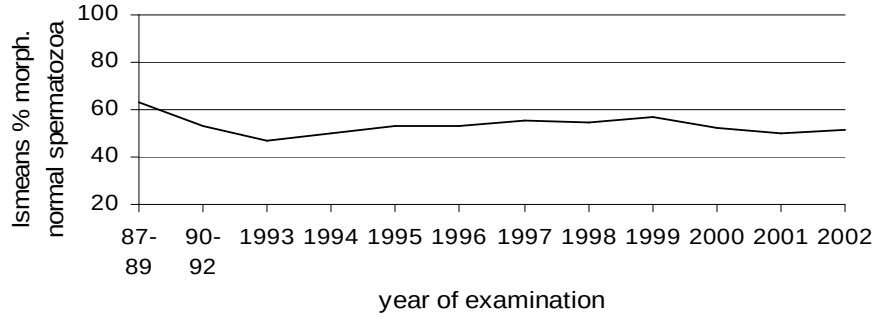

Figure 1.3: Least squares means of percentage morphologically normal cells per year.

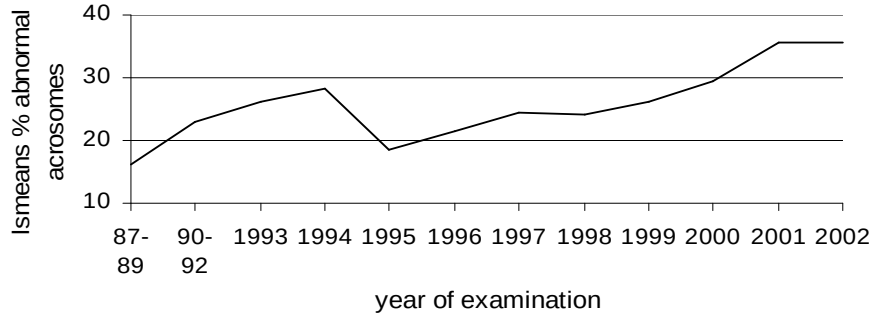

Figure 1.4: Least squares means of percentage abnormal acrosomes per year.

Appendix 2: relation between estimated breeding values of sire model and animal model.
These figures compare breeding values of the 95 sires of the stallions that were examined on semen quality in the current study. On the x-axis the breeding values estimated with a sire model; on the y-axis the breeding values of these sires estimated with an animal model.

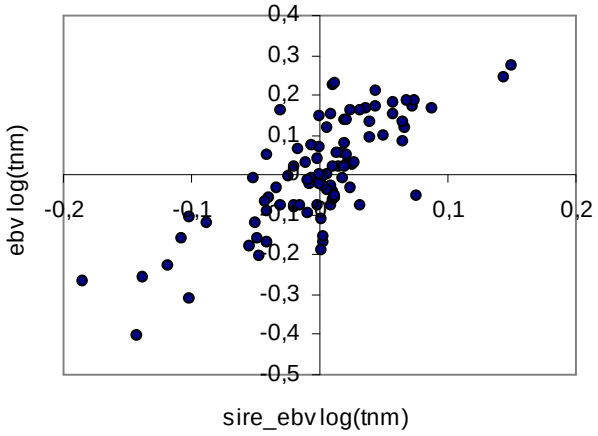

Figure 2.1: Log(TNM) estimated breeding values of sires.

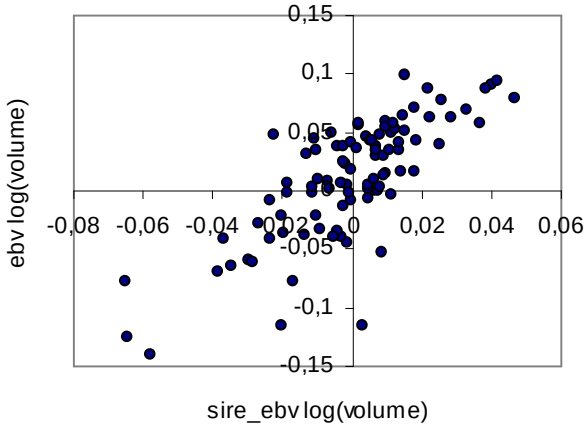

Figure 2.2: Log(ejaculate volume) estimated breeding values of sires.

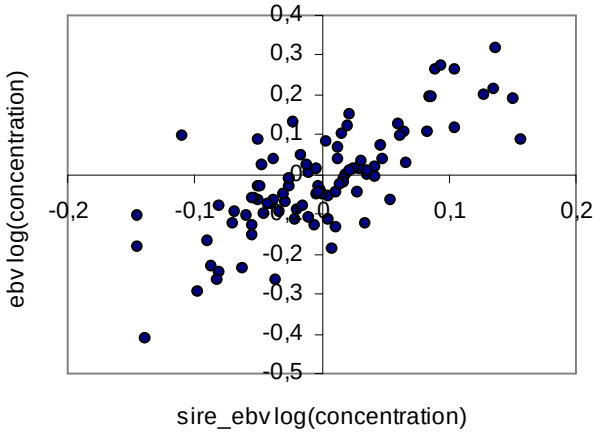

Figure 2.3: Log(concentration) estimated breeding values of sires.

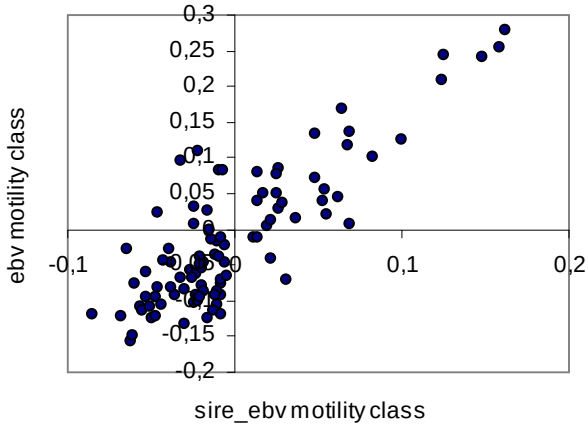

Figure 2.4: Motility class estimated breeding values of sires.

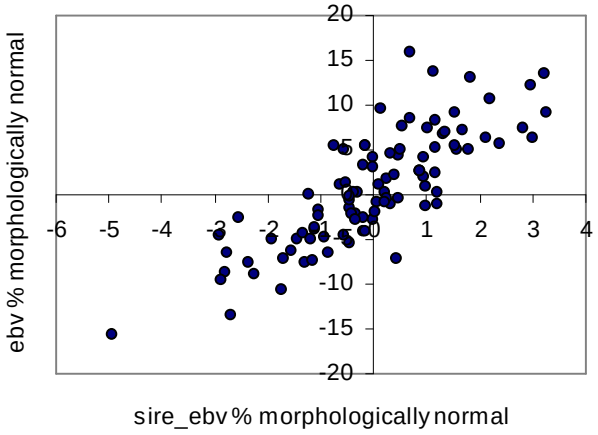

Figure 2.5 : % morphologically normal spermatozoa estimated breeding values of sires.

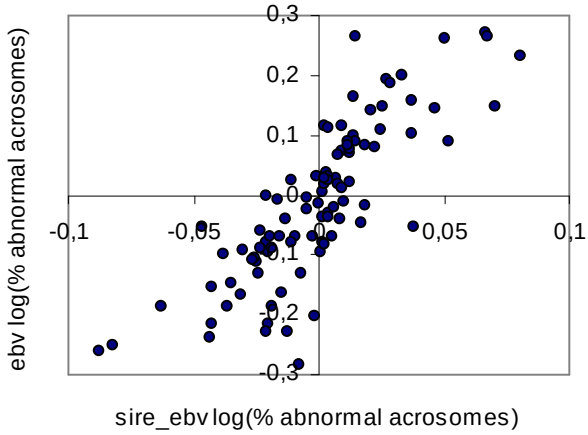

Figure 2.6: Log(% abnormal acrosomes) estimated breeding values of sires.

Appendix 3:inbreeding percentage of the stallions that were examined on semen quality in the current study (N=1146).

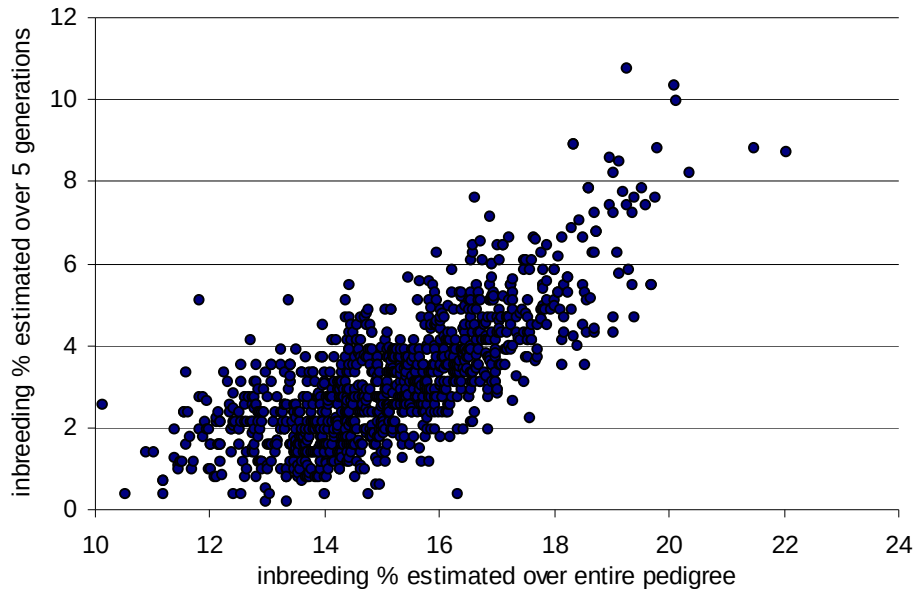

Figure 3.1: Relation between inbreeding % estimated over 5 generations and inbreeding % estimated over entire pedigree (correlation coefficient=0.75).

Appendix 4: least squares means per inbreeding class and probability levels for inbreeding effect.

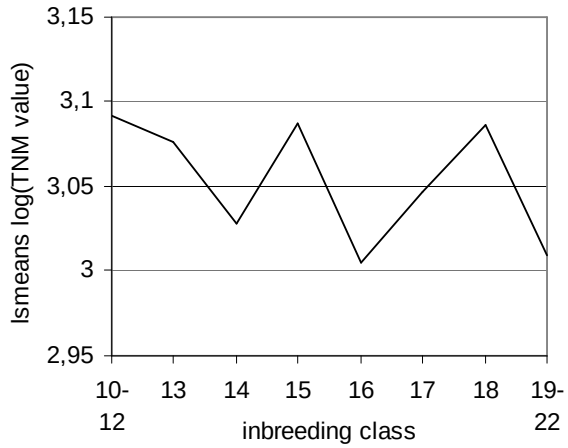

Figure 4.1: Lsmeans log(TNM) per inbreeding class (P=0.7494).

Figure 4.2: Lsmeans log(ejaculate volume) per inbreeding class (P=0.0218).

Figure 4.3: Lsmeans log(concentration) per inbreeding class (P=0.4428).

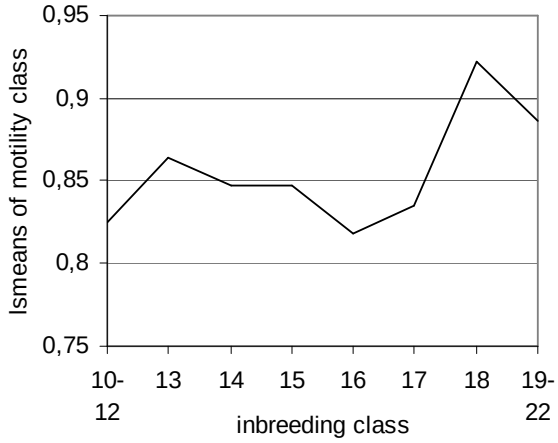

Figure 4.4: Lsmeans motility class per inbreeding class (P=0.4606).

Figure 4.5: Lsmeans % morphologically normal spermatozoa per inbreeding class (P=0.4760).

Figure 4.6: Lsmeans log(% abnormal acrosomes) per inbreeding class (P=0.5473).