

# Inbreeding on litter size of German Spitz dogs

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**ABSTRACT** - The objective of the present study was to assess the association between the inbreeding coefficient (F) of German Spitz dogs (litter, sires, and dams) and number of live newborn dogs for this breed. Records of dams and sires of a breeding system were used to calculate the F of 105 litters and their sires and dams and the number of live newborn dogs. The analysis performed through the GLM procedure showed a negative influence of F of litter and mother on litter size. This influence was investigated through models that considered linear and quadratic influences. Although the model that considered quadratic effect of F of the litter achieved the best adjustment, only linear coefficients were significant in both analyses. According to these results, the studied sample of German Spitz dogs exhibits inbreeding depression for litter size, which is an important information for breeders and professionals that assist in dog breeding. In addition to all the known effects of inbreeding on canine health, the results indicate that monitoring inbreeding through F is important for the reproductive success of the breed.

**Keywords:** canine genetics, inbreeding depression, reproductive traits

## 1. Introduction

Dog breeding systems have become important due to the growth of pet market. According to the Instituto Brasileiro de Geografia e Estatística (IBGE, 2013), Brazil has 52.2 million of households with dogs. The Confederação Brasileira de Cinofilia (CBKC) annually registers 143,000 pure-breed dogs, and one of the most registered breeds is the Dwarf German Spitz, with 21,937 records in 2020, which is placed first in terms of registrations (CBKC, 2020). Most breeders have focused, besides morphology, also on best results for fertility to supply the market demand for puppies, searching for large numbers of newborns in each litter. However, unfortunately, data registered by CBKC does not contain any kind of phenotypes, as litter size, which makes investigations with big samples difficult.

Besides breeds, several factors affect prolificacy, including dam size (Bergström et al., 2006), birth season (Gavrilovic et al., 2008; Leroy et al., 2015), and age and health of parents, especially the mother (Borge et al., 2011; Wang et al., 2014; Schrack et al., 2017; Keid et al., 2017; Kendall et al., 2017). However, variations in litter size can be found even when evaluating similar animals for these factors, and the heritability for this trait varies between 5.9 and 10.1% (Leroy et al., 2015).

Some studies have evaluated the effect of inbreeding on litter size (Leroy et al., 2015; Schrack et al., 2017). The magnitude of inbreeding is determined through the inbreeding coefficient (F), which can

be calculated through analysis of pedigree data that are recorded by institutions such as the CBKC. Falconer and Mackay (1996) defined inbreeding depression as the reduction of the mean phenotypic value shown by a given trait in relation to inbreeding. After this, several data showed this phenomenon on production phenotypes (Norén et al., 2016; Mokhtari et al., 2014; Pereira et al., 2016; Perez et al., 2017; Silva et al., 2019). Although inbreeding depression has been found for long time in these animals, its existence in pets has been less investigated.

The relation between inbreeding and decreases in reproductive success has been found in different species, including some canine breeds. The correlation varies among breeds, from no correlation in Entlebucher Mountain (Schrack et al., 2017), Irish Wolfhound (Urfer et al., 2009), and Basset Hound (Leroy et al., 2015) to a strong one in Leonberger (Leroy et al., 2015), and no scientific work has considered it for German Spitz dogs. Thus, the objective of the present study was to assess the association between F of German Spitz dogs (litter, sires, and dams) and number of live newborn dogs for this breed.

## 2. Material and Methods

The data used were from a private database of a dog kennel registered in the Kennel Clube do Rio Grande do Sul (CBKC/FCI) in Porto Alegre, RS, Brazil. Total litter size, with number of live newborn and stillborn dogs in each birth was collected, from litters born from June to December 2017. However, once only five litters had stillborns, the trait studied in this work was number of live newborn dogs. Besides size litter, we also collected birth date, age of the father and mother at birth, and pedigree information for three generations. The analysis was carried out using data of 105 litters, whose general description is presented in Table 1. The mean litter size of the populational sample was  $3.65 \pm 1.72$ . The age of parents varied from 365 to 3,039 days for males, and from 327 to 2,951 days for females. Litters were born from June through February. Therefore, this period was divided as winter (June to September), with 39 litters born, and spring/summer (October to February), with 66 litters (Table 1).

Sire, dam, and litter F were calculated using pedigree records of dams and sires, using the program Breeders Assistant for Dogs 5 (Tenset Pedigree Software Products), which performs Wright's inbreeding coefficient. This parameter was calculated from records of three generations for the father and mother and four generations for litters.

To assess the influence of each variable (birth month, birth season, age of father, age of mother, and F of mother and litter) on litter size, the minimum squares analysis was performed through the GLM (General Linear Models) procedure of SAS (Statistical Analysis System, version 9.3). Available variables were tested based on influences already commonly detected in literature.

**Table 1** - Data description of 105 litters that composed the database

Trait	Mean	Standard deviation	Minimum	Maximum
Litter size	3.65	1.72	1.00	8.00
Age of sires (days)	1,749	594.6	365	3,039
Age of dams (days)	1,785	596.6	327	2,951
Number of litters per birth season	n			%
Winter	39			37.14
Spring and summer	66			62.86
		Number of matings		
		Minimum	Maximum	
Number of sires	9	2	43	
Number of dams	85	1	2	

Based on these results, litter size was assessed using the GLM, considering the following effects and models:

$$Y_{ijkl} = \mu + D_i + S_j + E_k + b_{d1}(FD_i) + b_{l1}(FL_l) + e_{ijkl};$$

$$Y_{ijkl} = \mu + D_i + S_j + E_k + b_{d1}(FD_i) + b_{d2}(FD_i)^2 + b_{l1}(FL_l) + b_{l2}(FL_l)^2 + e_{ijkl},$$

in which  $Y_{ijkl}$  = litter size  $l$  belonging to dam  $i$  and sire  $j$  with calving at station  $k$ ;  $\mu$  = average litter size;  $D_i$  = random effect of dam  $i$ ;  $S_j$  = random effect of sire  $j$ ;  $E_k$  = fixed effect of birth season  $k$ ;  $b_{d1}$  = regression coefficient associated with the linear effect of inbreeding coefficient of dam  $i$ ;  $b_{d2}$  = regression coefficient associated with the quadratic effect of inbreeding coefficient of dam  $i$ ;  $b_{l1}$  = regression coefficient associated with the linear effect of inbreeding coefficient of litter  $l$ ;  $b_{l2}$  = regression coefficient associated with the quadratic effect of inbreeding coefficient of litter  $l$ ;  $FD_i$  = inbreeding coefficient of dam  $i$ ;  $FL_l$  = inbreeding coefficient of litter  $l$ ; and  $e_{ijkl}$  = residual random error associated with the  $Y_{ijkl}$  observation, assuming NID  $(0, \sigma_e^2)$ .

The quality of fit was carried out through comparison tests between non-nested models and penalties according to the number of parameters to be estimated. The following criteria were used: Akaike's information criterion ( $AIC = -2\log L + 2p$ ), in which  $p$  is the number of parameters in the model and  $-2\log L$  is the log-likelihood function); and Schwarz's Bayesian information criterion ( $BIC = -2\log L + p\log(\lambda)$ ), in which  $\log(\lambda)$  is the natural logarithm of the sample size - (or dimension of  $y$ ). The model with the lowest value, in the two criteria, was considered to have the best fit.

### 3. Results

The mean  $F$  of litters ( $FL$ ) was 1.21%, with 75% of litters presenting  $F = 0$ , calculated for four generations. Among litters that presented inbreeding, five had  $F$  higher than 10%, reaching 12.7%. Regarding the inbreeding of dams ( $FD$ ) for three generations, the mean  $F$  was 1.64%, with 82.9% of females presenting  $F = 0$ . Among mothers that presented inbreeding, five had  $F$  higher than 10%, with a female reaching an  $F$  of 31.3%. No father had  $F$  above zero, evaluated for three generations (Table 2). Spearman's correlation between  $FL$  and  $FD$  was not significant ( $\rho = -0.018$ ;  $P = 0.854$ ), demonstrating that high female inbreeding is not related to high litter inbreeding.

The variance analysis showed that age of parents did not significantly influence litter size (Table 3). Through this analysis, it is possible to note that only season of the year in which the litter was born (winter or spring/summer) and  $FL$  and  $FD$  were significant ( $P < 0.01$ ).

The analysis performed through the GLM procedure (Table 4) demonstrated a harmful (or negative) influence of  $FL$  and  $FD$  on litter size. For  $FL$ , although the best fit was obtained using the model containing the linear and quadratic terms, which presented the lowest AIC and BIC, only linear coefficient was significant. Litters with  $F = 0$  had, on average, 3.12 more newborn dogs than litters with  $F = 12.7\%$  ( $P = 0.0069$ ; Table 4 and Figure 1).

Regarding  $FD$ , only the model with the linear term showed significant effect for number of live newborn dogs ( $P = 0.006$ ; Table 4 and Figure 2). Females with  $F = 0$  had, on average, 2.72 more newborn dogs than females with  $F = 31.3\%$ . This analysis could not be done for fathers, since all of them had  $F = 0$ .

**Table 2 - Inbreeding coefficients of the studied sample**

	Mean	Median	CI 95%	Minimum	Maximum
FS (%)	0	0	0	0	0
FD (%)	1.64	0	0.63-1.79	0	31.3
FL (%)	1.21	0	0.72-2.56	0	12.7

FS - inbreeding coefficient of sires; FD - inbreeding coefficient of dams; FL - inbreeding coefficient of litter; CI - confidence interval.

**Table 3** - Summary of analysis of variance for litter size

	Df	Mean square	F	P
Birth season <sup>1</sup>	1	16.973	7.57	0.0071
Birth month <sup>2</sup>	1	4.941	2.20	0.1411
Age of father	1	6.503	2.90	0.0919
Age of mother	1	1.364	0.61	0.4374
FL	1	38.727	17.26	<0.0001
FD	1	16.575	7.39	0.0078

FD - inbreeding coefficient of dams; FL - inbreeding coefficient of litter.

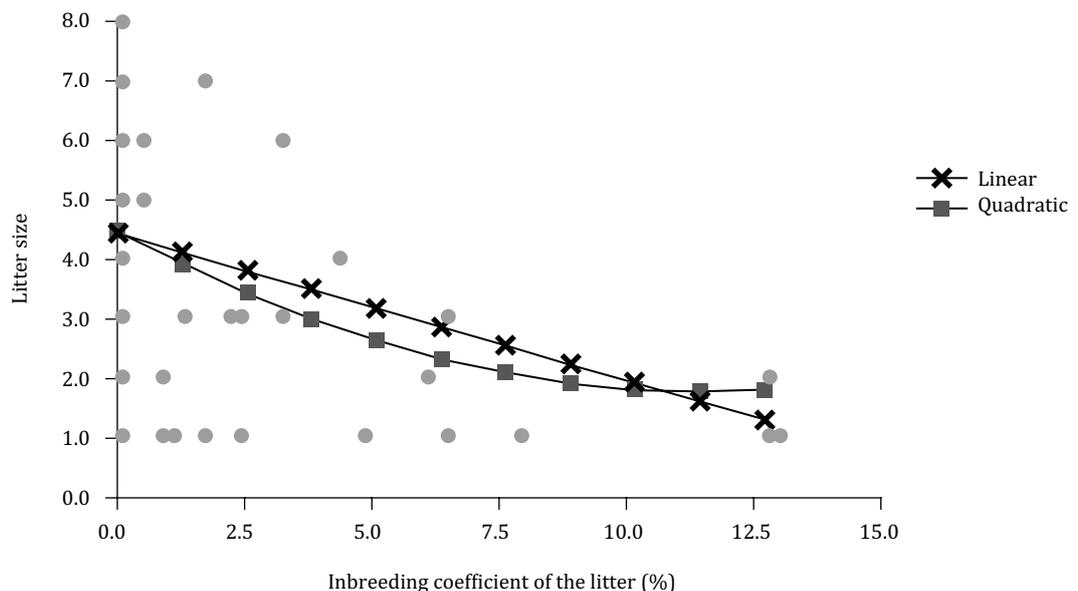
<sup>1</sup> Winter vs spring/summer.

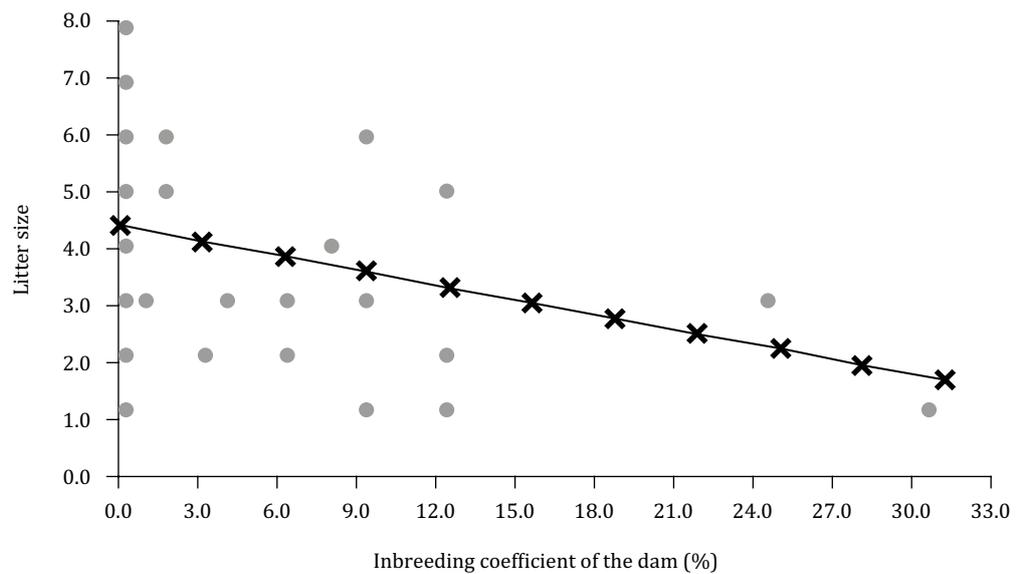
<sup>2</sup> June to February.

**Table 4** - Regression coefficients from a Generalized Linear Model for litter size in German Spitz dogs

Variable	Parameter	Model	
		Linear	Quadratic
FL	$\beta_1$ (SE)	-24.543 (4.951)	-46.938 (17.006)
	P	<0.001	0.0069
	$\beta_2$ (SE)	-	203.12 (147.70)
	P	-	0.172
FD	$\beta_1$ (SE)	-8.699 (3.111)	-11.999 (7.538)
	P	0.006	0.115
	$\beta_2$ (SE)	-	15.056 (30.939)
	P	-	0.628
	BIC	371.1	348.3
	AIC	373.1	350.3

FD - inbreeding coefficient of dams; FL - inbreeding coefficient of litter;  $\beta_1$  and  $\beta_2$  - coefficients of regression; P - P-value of significance of coefficient of regression; SE - standard error; BIC - Bayesian Information Criterion; AIC - Akaike Information Criterion.

**Figure 1** - Effect of inbreeding depression of the inbreeding coefficient of litters on litter size of German Spitz dogs.



**Figure 2** - Effect of inbreeding depression of the inbreeding coefficient of dams on litter size of German Spitz dogs obtained through linear model.

#### 4. Discussion

Litter size is an important trait in dog breeding that is affected by several factors. Although studies have shown effects of inbreeding on number of live newborn dogs for some canine breeds (Gresky et al., 2005; Leroy et al., 2015), in general, the breeder does not have enough information to evaluate the risks, because they are often unaware of the level of inbreeding of their breeders. In cases where this parameter is known, it is often disregarded, with the mating of animals with extremely high inbreeding levels (there is no limitation), as they prioritize the choice of breeders only by the external physical aspects (example: morphology and coat color). Thus, studies showing the occurrence of inbreeding depression for prolificacy provide scientific basis for professionals working in dog breeding to increase the focus on controlling inbreeding in kennels (subpopulations). Although using a limited sample size, our study cautions for a strong effect of high levels of puppy inbreeding leading to a decrease in litter size in the population. This pioneering study with a population of German Spitz also indicates that inbreeding depression on this trait occurs both related to maternal inbreeding and to the litter itself, and that the latter is much more pronounced.

In addition, studies on canines have always carried out this analysis using linear regression models, with the use of models other than the linear being common to explain the relationship of inbreeding with different traits of interest in other species. Some examples include analyses performed by Pereira et al. (2016) for Zebu cattle, Mokhtari et al. (2014) for sheep, and Malhado et al. (2013) for Buffaloes.

However, some studies have shown that the linear model is not always indicated to detect inbreeding depression, mainly when  $F$  is high (higher than 20%) (Carrillo and Siewerdt, 2010; Malhado et al., 2013). Ralls et al. (1998) reported that when  $F$  is low to moderate, the linear and exponential models are equally adequate.

Silva et al. (2019) evaluated a sample with low  $F$  (mean of 0.17%) and found that the linear model is not the most adequate to describe inbreeding depression for productive traits in dairy cattle. However, the fit of the best model should be evaluated for each phenotype of each different species.

The results showed that the two models evaluated can be used similarly to describe inbreeding depression related to FL, which presented a low inbreeding level (mean = 1.21%, maximum = 12.7%; Table 2). However, only the linear coefficient was adequate ( $P < 0.01$ ; Table 4) to describe

the inbreeding depression of litter size considering the inbreeding of dams (mean = 1.64%, maximum = 31.3%; Table 2).

According to Carolino and Gama (2008), the linear relation between  $F$  and both reproduction and production traits is based on partial dominance and overdominance theories, which consider the heterozygosis loss and increases in deleterious recessive homozygote frequency. However, according to Charlesworth and Charlesworth (1999), a non-linear effect of inbreeding depression would indicate the involvement of epistasis. As our data demonstrate similar influences of  $FL$  on litter size (Table 4), it is possible to infer about the occurrence of complex genetic influences on litter size in this subpopulation, based on the sum of partial dominance effects and overdominance and epistatic interactions. On the other hand, the inbreeding depression related to mothers of litters is possibly explained only by the influences of effects of partial dominance and overdominance, since the quadratic model did not present significant regression coefficients ( $P > 0.05$ ) (Table 4).

Some studies detected inbreeding depression on number of newborn dogs, but none of them investigated this detrimental effect for German Spitz dogs. The relation between  $FL$  and number of live newborn dogs was evaluated by Leroy et al. (2015), who published results for seven French dog breeds (Bernese Mountain Dog, Basset Hound, Cairn Terrier, Epagneul Breton, German Shepherd, Leonberger, and West Highland White Terrier). They found a negative effect for increases in  $FL$  and number of newborn dogs for all breeds, except Basset Hound; thus, this relation is not similar for all breeds and can be weaker, stronger, or inexistent depending on the breed. Moreover, the negative effect of inbreeding on litter size was higher for Leonberger and lower for West Highland Terrier. While the values of linear model coefficients estimated by those authors ranged from  $-3.80$  to  $-1.32$  for these various breeds, our value was higher ( $-24.543$ ), indicating the occurrence of a more expressive inbreeding depression in the German Spitz subpopulation evaluated (Table 4). On the other side, this big difference can be an artefact due to the shallow pedigree studied and to the small sample size, showing our data cannot be generalized to the breed as a whole.

Another three breeds were studied for these traits, and negative effects of inbreeding depression was found for number of stillborn puppies per litter for Dachshund dogs (Gresky et al., 2005), but not for Entlebucher Mountain (Schrack et al., 2017) and Irish Wolfhound (Urfer et al., 2009) dogs. Considering the emergence of genomic studies for dogs, this influence was also found for Golden Retrievers, through the relations with genomic  $F$  from runs of homozygosity (Chu et al., 2019).

A negative influence of  $FD$  on litter size was found for our small population of German Spitz dogs, with an estimated coefficient of  $-8.699$  (Table 4). Similar relation was also found for other breeds, such as Bernese Mountain Dog, Cairn Terrier, German Shepherd, West Highland Terrier (Leroy et al., 2015), Entlebucher Mountain (Schrack et al., 2017), Irish Wolfhound (Urfer et al., 2009), and Dachshund (Gresky et al., 2005). However, inbreeding depression related to  $FD$  is also not similar among all breeds, with estimated coefficients varying from  $-14.95$  to  $-1.18$ . Besides, this effect was not found in the study of Leroy et al. (2015) for three (Leonberger, Epagneul Breton, and Basset Hound) of the seven breeds evaluated.

It was not possible to investigate the effect of inbreeding of fathers on litter size. Nevertheless, studies indicate that few breeds have significant inbreeding depression, as in the case of Epagneul Breton (Leroy et al., 2015), West Highland Terrier (Leroy et al., 2015), Dachshund (Gresky et al., 2005), and Entlebuch Mountain (Schrack et al., 2017) breeds.

It is possible that different factors are associated to the variation in magnitude of inbreeding depression on litter size in the different studies cited. A possible first factor may be related to the type of inbreeding coefficient being evaluated, since the estimates of inbreeding depression vary a lot, even within the same breed, or if inbreeding is being evaluated for the litter itself or for the litter's mother or father. The effect of inbreeding of dams and litters are more common and expressive since many studies found negative influence for different breeds. An increase in  $F$  of male animals was correlated to litter size for few breeds. A high inbreeding affects the reproductive success due to decreases in number of viable gametes, as shown for felines (Pukazhenthhi et al., 2006), equines (van

Eldik et al., 2006), and bovines (Maximini et al., 2011; Dorado et al., 2015). Decreases in number of viable male and female gametes affect litter size differently and are decisive in the case of oocytes and less important in the case of number of spermatozoa, once oocytes number determine the embryo number, and spermatozoa number does not; this can be the reason for the low number of studies that identified inbreeding depression on litter size for sires.

The hypothetical genetic basis subjacent to decreases in litter size are different when the litter has inbreeding (FL) or when FD is high. Highly inbred newborn dogs are the ones that probably survive within the litters with high F values. The other siblings did not complete their embryo development, since homozygosis of deleterious recessive alleles causes embryo death, as shown for swine animals (Derks et al., 2019).

High FD affects litter size due to homozygosis in genes related to fertility, as reported for bovine animals (Martikainen et al., 2018) and humans (Olubunmi et al., 2019). This is due to problems in oocyte maturation or to the hindered capacity of intrauterine growing of embryos.

A second factor that may be related to the variation in the intensity of inbreeding depression in the studies is the difference in genetic diversity between breeds, which will not always be related to inbreeding estimates from pedigree data. Breeds whose populations have had decreases in the gene pool for many generations, but that have undergone matings aimed at decreasing inbreeding in more recent generations, will have low estimated F values; however, due to their history, they will still have low genetic diversity. In these populations, many animals will have several homozygous loci, even if the F value estimated through the pedigree is low. Thus, it is possible that, without a genomic assessment of the number of homozygous loci, some breeds are showing low inbreeding values in many animals and that for this reason, the intensity of inbreeding depression seems statistically weaker or even non-existent. Studies have shown large variation in genetic diversity among different dog breeds through genomic data and pedigree records (Lewis et al., 2015; Dreger et al., 2016; Wang et al., 2017), but none of them addressed this issue until now. Unfortunately, German Shepherd was the only canine breed evaluated for parameters of diversity in Brazil (Bignardi and Santana Júnior, 2019), and results for diversity for German Spitz are not available neither in Brazil nor for other populations.

## 5. Conclusions

German Spitz dogs have reached large reproductive growth in the world. Studies on number of newborn dogs analyzing the relation of inbreeding of mothers, fathers, and litters with number of newborn dogs per litter are important to assist breeders and professionals in dog breeding. This is the first study that presents such data for a sample from this breed.

Data presented here show the strong influence of puppies' inbreeding on litter size and the influence of dam inbreeding on this trait in this subpopulation. On the other hand, the results alert us to the possibility that the harmful effect of litter inbreeding on litter size does not occur linearly, as is commonly expected and described in several studies and breeds.

Therefore, the present study still demonstrates the need to offer more information to breeders and professionals regarding the importance of mating unrelated animals and, mainly, the importance of not prioritizing the use of breeding dogs with high inbreeding, unlike common practice by many dog breeders. Monitoring the level of inbreeding in animals can help also obtain larger litters and increase the chances of producing newborn dogs without genetic diseases, generating success and satisfaction in breeding quality with a focus on animal welfare.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: F.M. Andrade. Formal analysis: F.M. Andrade, G. Krebs, G.L. Feltes and J.A. Cobuci. Investigation: F.M. Andrade. Methodology: F.M. Andrade and J.A. Cobuci. Project administration: F.M. Andrade. Software: G. Krebs. Supervision: J.A. Cobuci. Writing-original draft: M.M. Silva.

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