

Study of the relationship between birth weight, weight gain in the neonatal period, inbreeding and parity and neonatal mortality in a population of australian cattle dog breed

Estudo da relação entre peso ao nascer, ganho de peso no período neonatal, inbreeding e paridade e a mortalidade neonatal em uma população de cães da raça australian cattle dog

Estudio de la relación entre peso al nacimiento, aumento de peso en el período neonatal, consagración y paridad y mortalidad neonatal en una población de perros de la raza ganadero australiano

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Abstract

Breeding purebred dogs presents numerous challenges requiring the implementation of actions to ensure reproductive success. The aim of this study was to describe, through quantitative parameters, the relationship between birth weight (PN), weight gain (GPD) and inbreeding coefficient (CoI) with mortality observed in the neonatal period in the Australian Cattle Dog breed. A prospective, longitudinal study was carried out in a kennel registered to CBKC/FCI in the year 2020. Puppies had their vitality evaluated and were weighed daily until 45 days, with the heterogeneity of PN, GPD and CoI analyzed for each litter. Quartiles for the variables: PN, GPD and CoI were calculated and generalized linear models fitted using the R® system, with neonatal mortality as the binary outcome variable. Pup sex and primiparous occurrence were introduced as random effects. Multicollinearity was estimated between the predictors using Cramer's method and the median area under the receiver operating characteristic curve (AUROC). The mean number of pups per litter was 5.88 ± 1.93 , mean BW was 258.98 ± 47.19 g. The highest odds ratio for mortality is found in the intermediate quartiles, and the fact that the mother is primiparous ($p0.0014$). The relationship between mortality in the neonatal period and primiparous mothers ($AUROC \geq 0.7$) was validated in the population studied.

Keywords: Puppies; Growth curve; Litter; Canine; Neonate care.

Resumo

A criação de cães de raça pura apresenta inúmeros desafios exigindo a implementação de ações para garantir o sucesso reprodutivo. O objetivo deste estudo foi descrever através de parâmetros quantitativos a relação entre peso ao nascer (PN), ganho de peso (GPD) e coeficiente de inbreeding (CoI) com a mortalidade observada no período neonatal na raça Australian Cattle Dog. Estudo prospectivo, longitudinal, foi realizado em um canil registrado a CBKC/FCI no ano de 2020. Filhotes tiveram a vitalidade avaliada e foram pesados diariamente até os 45 dias, tendo a heterogeneidade do PN, GPD e CoI analisados para cada ninhada. Quartis para as variáveis: PN, GPD e CoI foram calculados e modelos lineares generalizados ajustados usando sistema R®, com mortalidade neonatal como variável binária de desfecho. Sexo do filhote e ocorrência de primípara foram introduzidas como efeitos aleatórios. A multicolinearidade foi estimada entre os preditores usando o método de Cramer e a área mediana sob a curva característica de operação do receptor (AUROC). O número médio de filhotes por ninhada foi de $5,88 \pm 1,93$, PN médio foi de $258,98 \pm 47,19$ g. A maior razão de chance de mortalidade encontra-se nos quartis intermediários, e o fato

da mãe ser primípara ($p0,0014$). A relação entre mortalidade no período neonatal e mães primíparas ($AUROC \geq 0,7$) foi validada na população estudada.

Palavras-chave: Filhotes; Curva de crescimento; Ninhada; Canino; Cuidado neonatal.

Resumen

La crianza de perros de pura raza presenta numerosos desafíos que requieren la implementación de acciones para asegurar el éxito reproductivo. El objetivo de este estudio fue describir, a través de parámetros cuantitativos, la relación entre el peso al nacer (PN), la ganancia de peso (GPD) y el coeficiente de consanguinidad (CoI) con la mortalidad observada en el período neonatal en la raza Ganadero Australiano. Se realizó un estudio longitudinal prospectivo en un criadero registrado en CBKC/FCI en el año 2020. Se evaluó la vitalidad de los cachorros y se pesaron diariamente hasta los 45 días, analizándose la heterogeneidad de PN, GPD y CoI para cada camada. Se calcularon los cuartiles para las variables: NP, GPD y CoI y se ajustaron modelos lineales generalizados utilizando el sistema R®, con la mortalidad neonatal como variable de resultado binaria. El sexo de las crías y la aparición de primíparas se introdujeron como efectos aleatorios. Se estimó la multicolinealidad entre los predictores mediante el método de Cramer y la mediana del área bajo la curva característica operativa del receptor (AUROC). El número medio de crías por camada fue de $5,88 \pm 1,93$, el peso corporal medio fue de $258,98 \pm 47,19g$. La razón de probabilidad más alta de mortalidad se encuentra en los cuartiles intermedios y el hecho de que la madre sea primípara ($p0,0014$). Se validó la relación entre mortalidad en el período neonatal y madres primíparas ($AUROC \geq 0.7$) en la población estudiada.

Palabras clave: Cachorros; Curva de crecimiento; Camada; Canino; Cuidado neonatal.

1. Introduction

Australian Cattle Dog (ACD) is a medium-sized, active and rustic breed selected in Australia to assist in the management of cattle. The cattle breed belongs to group 1 of the International Cynological Federation which also includes sheepdogs and different breeds with different sizes. The resistance to travel long journeys, rusticity and ability to build strong bonds with owners made the breed popular on other continents as well as expanding its participation in other activities, inserting ACD into urban environments as well. Conservation breeding must not only strive for the maintenance of morphological and functional characteristics but also care for the health and well-being of the animals.

Thus, the zootechnical management of the pack, the choice and selection of parents, as well as the adoption of preventive and curative measures when necessary, are routine activities in the creation of purebred dogs (Münnich, 2008). The development of tools and coordinated efforts in favor of animal welfare while collaborating to reduce neonatal losses (even today, they remain impacting on reproductive success in numerous pure breeds in the canine species), contributes to the creation of conservation because the success reproduction in the canine species involves numerous stages that even precede the birth of the puppies and continue intense until the weaning period.

The period between birth and weaning includes important physiological adjustments that often require specific measures so that critical phases can be overcome (Grundy, 2006). In addition to pregnancy, delivery and the fetal-neonatal transition period being challenging, the adequate growth and development of puppies depends on the conduction of intrinsic and extrinsic factors. In addition to zootechnical knowledge and adequate sanitary and environmental management, the need to prospect more in-depth data capable of reflecting the genetic basis of the herd, including more recently specific molecular markers for the different breeds, should not be neglected.

The inbreeding coefficient allows estimating the loss of genetic diversity and increase in homozygosity in a given population (Marelli *et al.*, 2020), and can be analyzed in different ways. Estimating the inbreeding of litters, prospecting vulnerability through the analysis of molecular markers in the herd, recording birth weight as well as drawing up growth curves capable of showing weight gain during the neonatal period are actions easily implemented in the routines of dog breeders of purebred, constituting an important instrument for the diligent reduction of risks and losses in the first stages of life. Conducting properly planned crosses, monitoring pregnancy and delivery and identifying vulnerable pups are control measures seeking to reverse risk situations, contributing to the conservation of pure breeds (Münnich & Kuchenmeister, 2014;

Souza *et al.*, 2017; Pereira *et al.*, 2019; Marelli *et al.*, 2020). The aim of this study is to describe through quantitative parameters the relationship between birth weight, weight gain and the inbreeding coefficient, with the mortality observed in the neonatal period in litters of an ACD kennel.

2. Methodology

The study was conducted in a prospective, longitudinal manner, including 17 litters of ACD born in 2020, in a kennel located in the city of Pelotas, registered in the CBKC/FCI system. The parents received uniform handling, super premium feed and periodic endo and ectoparasite control and during the gestational period, abdominal ultrasound examinations were performed at 30 and 55 days after mating for monitoring and review of the fetuses. The vitality of the pups was analyzed using the modified APGAR test (Vassalo *et al.*, 2015; Veronesi *et al.*, 2016) at 1 and 60 minutes after birth.

Litters whose parturition was normal, without interventions and whose pups presented APGAR scores between 7 and 10 were included in the study. The parturition was monitored and the puppies were weighed soon after birth, using a portable digital scale with three-point accuracy. Birth weight heterogeneity was estimated for each litter by calculating the coefficient of variation (Mugnier *et al.*, 2019; Milligan, B. N., Fraser, D., & Kramer, D. L., 2002).

The weighing were repeated daily, and a daily monitoring routine was established, keeping the weighing time uniform until the 45th day of life. For the purpose of calculating the daily weight gain (DWG) of the pups, the formula was used: $GPD = (Final\ weight - Initial\ weight)/45$.

Inbreeding was estimated through the inbreeding coefficient (CoI) of each litter, calculated using the algorithm available on the Sistema Pet® website (ENCANTU, 2022), and based on the data available in the pedigree of the parents. Neonatal mortality was monitored and recorded during the 45-day study period.

The 17 litters were grouped according to the number of pups born and the quartiles were calculated for the variables: a) birth weight (BW), b) Daily weight gain (GPD) and c) inbreeding coefficient (CoI). To deal with unbalanced data and the random sampling approach, generalized linear mixed models were fitted using the R® system, with neonatal mortality as the binary outcome variable. Furthermore, explanatory variables were introduced into the models only if the missing values represented less than more than 15% of the data. The fixed effects introduced in the models were: PN, GPD, neonatal mortality rate (TNM) and CoI. Pup sex and primiparous occurrence were introduced as random effects, and the parameters were introduced into the model.

The results were combined into balanced subsets of data using the median, and in this way, p-values, odds ratio and its 95% CI, the mortality rate, as well as the 95% CI and the prevalence ratio were obtained. for each of the parameters evaluated. Multicollinearity was estimated among the predictors using Cramer's method and the median area under the receiver operating characteristic curve (AUROC) was used to assess the models' ability to differentiate pups that died during the neonatal period (birth to 45 days) and those that survive, according to the relationship with each of the variables involved in the model.

3. Results and Discussion

In all, 17 litters born in 2020 were monitored, totaling 100 puppies, 54 males and 46 females. The mean number of pups per litter was 5.88 ± 1.93 , with litters ranging from 2 to 10 pups and the mean weight of pups at birth was $258.98 \pm 47.19g$. The age of the mothers ranged from 18 to 70 months, with 8 being primiparous.

In Australia, the breed's country of origin, ACD have a high number of records (Shariflou *et al.*, 2011). The breed has also grown in popularity in Brazil where these dogs contribute both as cattle and sport dogs and as pets. In the population studied, the inbreeding coefficients of the crosses ranged from zero to 14.1 (Table 1). Inbreeding coefficient of 3.23% was

obtained in a study based on the genealogy of the ACD breed in Italy. The assertions that inbreeding results in open populations tend to be lower than those observed in restricted populations corroborate the results obtained in our study (Cicarelli, J., Macchioni, F., & Cecchi, F. 2021).

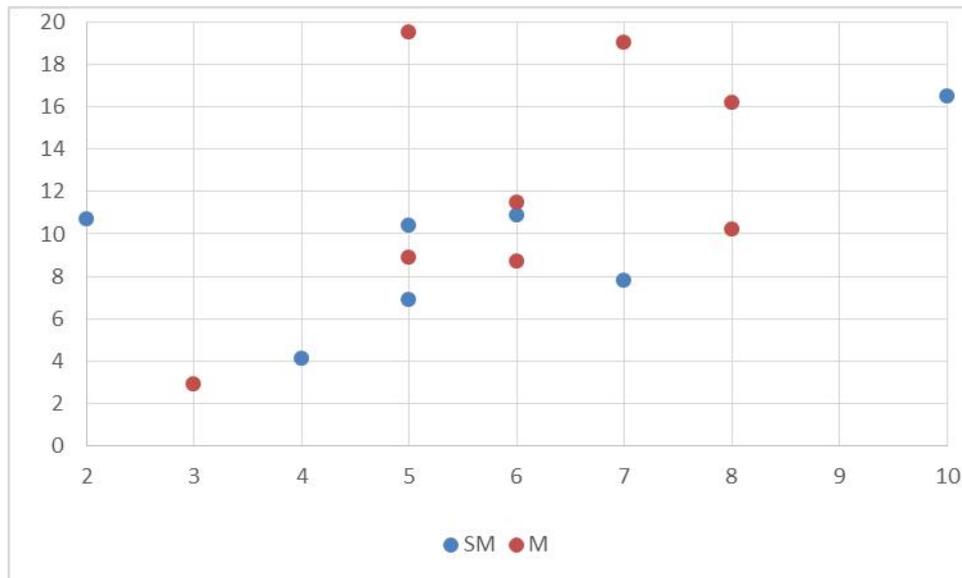
Table 1. Quartile values of groups classified by number of pups born, used to classify 100 pups from 17 litters for three parameters: birth weight, daily weight gain in the first 45 days and inbreeding coefficient.

Litter	Number of pups	Birth Weight (g)			GPD (g)			Inbreeding (%)		
		Q1	Q2	Q3	Q1	Q2	Q3	Q1	Q2	Q3
Two pups	2	238,0	266,5	295,5	57,0	60,1	63,2	2,34	2,34	2,34
Three pups	3	225,0	236,0	243,0	50,0	51,5	52,5	12,0	12,0	12,0
Four pups	4	275,3	280,5	286,5	46,4	46,7	46,8	3,91	3,91	3,91
Five pups	20	221,0	279,0	326,0	44,1	60,4	67,1	0,20	0,59	9,25
Six pups	24	247,0	268,5	289,0	52,5	62,5	70,3	0,59	2,93	4,69
Seven pups	21	241,0	248,0	275,2	44,7	60,4	62,6	0,68	2,34	12,99
Eight pups	16	197,0	246,5	270,5	23,0	27,5	45,4	0,00	3,71	7,42
Ten pups	10	250,0	266,5	280,3	43,6	50,6	58,3	14,6	14,6	14,6

GPD- Daily weight gain; Q1=first quartile value, Q2=second quartile value, Q3=third quartile value. Source: Authors (2022)

The heterogeneity of birth weight observed in the evaluated sample ranged from 2.9% in the litter with 3 pups to 19.5% in one of the litters with 5 pups. Most litters studied showed heterogeneity between 5 and 15% (Figure 1). In their study with different polytoxic species, Wootton *et al.* (1983) observed that changes in growth during the intrauterine period resulted in birth weight distributions that did not follow the normal pattern of distribution. Such a discrepancy was observed in 11 of the 48 litters of dogs analyzed and also in approximately 1/3 of the litters in the different species studied by the authors.

Figure 1. Distribution of birth weight heterogeneity in the Australian Cattle Dog in relation to the number of puppies in the litter and losses in the neonatal period. SM - litter without loss, M- litter with loss.



Source: Authors (2022).

Recent studies point to the complexity of the biological analyzes and of the methods of collection of samples necessary in order to identify biochemical and physiological parameters capable of contributing to a better understanding of fetal and perinatal metabolism, which in canines has a relevant impact on the development of conceptuses, weight at birth, weight gain in the neonatal period and consequently neonatal mortality affecting animal welfare in purebred dog breeding (Veronesi *et al.*, 2020; Mila *et al.*, 2015)

Mother parity, pup sex, and litter size were not significant in the birth weight analyzes of Tesi *et al.* (2020) in their study on toy and small breeds. However, the relationship between placental weight and size with placental vascularization and pup birth weight was observed. Factors related to the complex physiology of the gestational period and the fetal-neonatal transition process, in addition to the morphometric differences of the parents, and the characteristics of the cross that gave rise to the litter are some of the factors that may contribute to the variation observed in the studied population.

Bohn and Hoy (2000) concluded with the results of their studies in canines that birth weight should be taken into account as a risk factor for mortality in the first weeks of life. The occurrence of offspring whose weights do not fit the normal distribution is relevant in polytocuous species of mammals, where studies indicate not only a higher risk of mortality for individuals with lower birth weights during the neonatal period, but also the possibility of changes in growth and development after weaning (Wootton *et al.*, 1983).

Mugnier and colleagues (2019) also report the breadth of heterogeneity observed in litters across different canine breeds. Studies in swine and canines point to different hypotheses regarding the occurrence of birth weight heterogeneity and its effects on the percentage of losses during the neonatal period. Such reasons have not yet been fully elucidated and make up a complex scenario whose confrontation requires a set of short, medium and long-term actions in order to reduce perinatal and neonatal losses as well as ensure the well-being of the pups. In our study, although losses were observed in most litters with higher heterogeneity, it was also possible to observe losses in litters in which the variation in birth weight between the offspring of the litter was low or moderate (Figure 1) as for example the selection of the age of the mothers can reduce the prevalence of underweight puppies. Low birth weight pups born into litters with fewer pups require intensified management strategies to limit risks and mortality in the neonatal period (Mugnier *et al.*, 2020).

The weight gain of healthy pups in the neonatal period is also a characteristic influenced by numerous genetic, hormonal, environmental and behavioral factors of the mother. Obtaining growth curves for different canine breeds is a challenging activity and has been the subject of numerous studies that seek to validate ideal growth models. Salt *et al.*, (2017) developed growth curves for numerous dog breeds from 12 weeks of age, grouping them into categories to monitor the growth patterns of dogs of different breeds, while Helmink, Shanks, & Leighton (2000) developed a growth modeling proposal. in the German Shepherd and Labrador Retriever breeds, from frequent weighing in a wide population to through the application of mathematical functions. Trangerud *et al.*, (2007) highlight important factors affecting growth in different phases and the relevance of identifying normal or altered patterns.

In our studies, it was observed that, in addition to the lower values of birth weight, lower daily weight gain was observed in litters of 8 pups (Table 1). Alves (2020) points out that litters with a greater number of pups place high total energy demands on the lactating female and may be more severely affected if milk production is insufficient or reduced, a fact that may have contributed to the lower values. Tesi *et al.*, (2020) did not observe any influence of birth weight on the growth of pups in the first 6 days of life, however lower values were reported in larger litters in a study with small and toy breeds.

Low birth weight was considered relevant in the mortality observed in the first 48 hours of life, while losses between 2 and 21 days were associated with weight gain in the first 48 hours, indicating the importance of uterine growth patterns and colostrum intake in neonatal development (Mila *et al.*, 2015). The effectiveness of food support in low birth weight pups was observed after supplementation for 21 days optimizing weight gain without, however, impacting weaning weight (Boutigny *et al.*, 2016).

The litters were grouped into three groups, $\leq Q1$ = low birth weight, low weight gain and low inbreeding; between Q1 and Q3= average birth weight, average weight gain and average inbreeding and $>Q3$ = high birth weight, high weight gain and high inbreeding. Among the factors included in the model analyzed in the study population, birth weight (p 0.039), inbreeding coefficient (p 0.040), with the highest odds ratio being found in the intermediate groups, and the fact that the mother was primiparous (p 0.014) as related to mortality in the 45-day period (Table 2).

Table 2. Predictive factors for neonatal mortality in 100 pups from 17 litters using generalized linear mixed models. Pup sex and primiparous occurrence were included as random effects in the model.

Factors included in the model	Neonatal mortality			
	p-value	Odds ratio (95% IC)	Prevalence (%)	Mortality rate % (95% IC)
PN	0,039			
<Q1		1(Ref.)	10,5	13,03(0-20)
[Q1-Q3]		1,059(0,88-1,27)	74,7	14,1(0-50)
>Q3		0,76(0,32-1,80)	14,8	7,6(0-20)
GPD (g)	0,381			
<Q1		1(Ref.)	20,0	6,5(0-13)
[Q1-Q3]		1,18(0,78-1,76)	53,7	16,9(0-50)
>Q3		0,85(0,58-1,25)	26,3	9,1(0-20)
CoI	0,040			
<Q1		1(Ref.)	25,0	16(0-50)
[Q1-Q3]		1,05(0,80-1,38)	55,0	12,7(0-40)
>Q3		0,87(0,41-1,85)	20,0	10,0(0-33)
Primiparous	0,014			
Yes		15,93 (4,08-62,09)	95,1	3,44 (0-16,6)
No		0,25 (0,16-0,40)	4,9	26,18(0-50)
Pup sex	0,630			
Male		1,23(0,85-1,80)	59,3	11,46(0-50)
Female		0,78(0,51-1,19)	47,8	14,28(0-50)

PN= Birth weight; GPD = Daily weight gain. Source: Authors (2022).

The literature points out different mean values of inbreeding in breeds and canine populations. In studies carried out in populations of hound dogs of the Bavarian Mountain hound, Hanoverian hound and Tyrolean hound breeds, calculated inbreeding coefficients include medium or high values (ranging from 4.51% to 9.47) where the 10 most important ancestors have genetic contributions above 50% (Voges & Distl, 2009). As noted by the authors, Management in restricted populations can promote an increase in inbreeding over generations. In the Alpine Dachsbrake breed, the inbreeding coefficient of the entire population was 2.25% (Bednarek, E., Sławińska, A., & Mroczkowski, 2018) while in the Tatra Shepherd breed the inbreeding of the population ranged from 6.68 to 6.85%. (Sweklej, Horoszewicz, & Niedziółka, 2020). The increase in the frequency of unfavorable or deleterious alleles as well as the reduction in genetic variability can negatively impact parameters related to fertility and prolificacy (Marelli *et al.*, 2020; Sweklej, Horoszewicz, & Niedziółka, 2020; Sargolzaei & Colleau, 2006; Leroy *et al.*, 2021; Leroy *et al.*, 2015).

Wu *et al.*, (2006) highlight in their review on changes in intrauterine growth in production animals the complexity of controlling fetal development and growth, pointing out the interactions between genetic, epigenetic and environmental factors and maternal maturity and the relevance of considering the intrauterine environment for reproductive success and also for postnatal growth.

Tonnessen *et al.*, (2012) reported that mortality in pups has an increased risk in relation to the mother's age (including stillbirths and losses during the neonatal period) but emphasize that the risk of losses in the perinatal period is lower in females as the order increases of litters, a fact that corroborates the observations in the analyzed population. Studies with piglets showed a negative correlation between litter size and weight during the weaning period and a positive correlation between birth weight and weight during the weaning period, also indicating a superior survival in the group with higher birth weight. When considering survival in the weaning period, parity (less than or equal to 2) and litter size did not show significant effects (Akdag, Arslan & Demir, 2009). Such information, despite not fully explaining the results obtained, corroborate the data obtained in our study, where 95.1 of the observed deaths included litters of first-calf mothers (Table 2).

In the Shetland Sheepdog breed, significant differences in litter size reduction were observed in first, sixth and seventh parity mothers and also in females from 5 years of age (when compared to mothers of 3 years or younger) (Eleryd, 2022).

The analysis of multicollinearity between the predictors using the calculation of Cramer's V coefficient (Table 3), showed strong relationships between the primiparous mother variables and mortality, as well as the inbreeding coefficient and mortality of the population analyzed in the period. The median area under the receiver operating characteristic curve (AUROC) was used to assess the models' ability to differentiate pups that died during the neonatal period and those that survived. According to the relationship between each of the variables analyzed, the validity of the relationship between mortality in the neonatal period and the fact that the mother was primiparous ($AUROC \geq 0.7$) was observed.

Table 3. Cramer's V coefficients and median area under the curve (CI=95%).

Related variables	Cramer's V	ROC Curve (IC=95%)
Mortality x primiparous	0,70	0,85 (0,77-0,93)
Mortality x puppy sex	0,27	0,44 (0,33-0,56)
Mortality x PN	0,60	0,50 (0,38-0,63)
Mortality x GPD	0,08	0,26 (0,16-0,36)
Mortality x CoI	0,90	0,61 (0,46-0,76)

PN= Birth weight ; GPD = daily weight gain; CoI = inbreeding coefficient. Source: Authors (2022).

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The analysis of multicollinearity between the predictors using the calculation of Cramer's V coefficient (Table 4), showed strong relationships between the primiparous mother variables and mortality, as well as the inbreeding coefficient and mortality of the population analyzed in the period. The median area under the receiver operating characteristic curve (AUROC) was used to assess the models' ability to differentiate pups that died during the neonatal period and those that survived.

According to the relationship between each of the variables analyzed, the validity of the relationship between mortality in the neonatal period and the fact that the mother was primiparous ($AUROC \geq 0.7$) was observed.

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Mortality x CoI	0,90	0,61 (0,46-0,76)

PN= Birth weight; GPD = daily weight gain; CoI = inbreeding coefficient. Source: Authors (2022).

In our study, the low number of analyzes can be considered limiting for the validation of the results obtained.

4. Conclusion

The success of reproductive management in purebred dogs must be supported by preventive actions that begin in the planning of crosses and in the careful selection of matrices, both by age and parity, as well as by maternal ability. Simple measures such as weighing and tracking pups through curves should be implemented intensifying attention in large, heterogeneous litters of first-partuition females.

This work brings information from an research area that still now less explored. New studies with other breeds, longer periods, could access developmental interactions and the impact of neonatal phase on breeding, can lead to breed improvement and preservation.

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