A BREED-SPECIFIC APPROACH OF BIRTH WEIGHT AS A RISK FACTOR FOR NEONATAL MORTALITY IN THE CANINE SPECIES

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SUMMARY

In numerous species, low birth weight is an important risk factor for neonatal mortality. In the canine species, definition of a low birth weight is complex due to the huge interbreed variability in its size. To identify puppies at higher risk of neonatal death, data from 6,694 puppies (27 breeds, 1,202 litters and 75 French breeding kennels) were collected and analysed. A generalised linear mixed model was fitted and birth weight and size of the breeding kennel were identified as important risk factors for neonatal death. Then, breed specific thresholds for birth weight (associated with higher risk of neonatal death) were identified in 12 breeds using receiver operating characteristics (ROC) analysis, ranging from 100 to 500 g (area under the ROC ≥0.70; sensitivity ≥75%; specificity: 45–68%). The results allow the identification of puppies at higher risk of neonatal death and thus potentially increased productivity in a dog breeding kennel.

INTRODUCTION

Despite progress in veterinary medicine, mortality rates from birth to weaning remains high in the canine species with around one puppy out of five dying before the age of two months (Gill, 2001; Indrebø et al., 2007; Chastant-Maillard et al., 2017). To improve breeding performances, better understanding regarding factors influencing neonatal and paediatric mortality is essential.

In numerous species such as human, porcine and bovine, low birth weight is considered as a major risk factor for neonatal mortality (Wu et al., 2006; Fix, 2010). Also in dogs, low-birth-weight newborns are at a higher risk of death, with a risk of mortality multiplied by 12 compared with normal-birth-weight puppies (Groppetti et al., 2015; Mila et al., 2015). However, studies conducted in dogs focused on only few breeds or analyses were performed at breed size level only (Nielen et al., 2001; Indrebø et al., 2007; Fiszdon & Kowalczyk, 2009). Nevertheless, selective breeding of the domestic dog (*Canis familiaris*) has induced the differentiation of 344 varied breeds (Fédération Cynologique Internationale, 2018) showing the greatest morphological variability of any land mammal with an adult body weight ranging from 500 g in miniature breeds like Chihuahua to more than 100 kg in giant

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ones (e.g., Mastiff) (Boyko et al., 2010). Therefore, breed-specific information on birth weight is necessary.

The aims of this study were i) to identify risk factors of neonatal mortality in puppies, including the impact of birth weight in a large canine purebred population issued from a large number of kennels, and ii) to determine breed-specific cut-off values for birth weight, allowing for the identification of at-risk puppies in order to provide them with more nursing and medical care.

MATERIALS AND METHODS

Statistical analyses were performed using R software (R Core Team, 2016) (version 3.3.2) with add-on packages "lme4" (Bates et al., 2015) and "pROC" (Robin et al., 2011). Results with p-values <0.05 were considered to be significant and statistical uncertainty was assessed by calculating exact 95% binomial confidence intervals (CI).

Data on puppies and their litter were collected through a questionnaire administered to French dog breeders from 2015 to 2017. Only breeds with at least 100 individuals in the dataset were analysed.

Explanatory variables were selected based on their biological relevance and introduced in the models only if missing values represented less than 15% of the data. A generalised linear mixed model was fitted with neonatal mortality as a binary outcome variable. Neonatal mortality rates included all of the deaths of live-born puppies on or before 21 days after birth. The fixed-effects introduced in the model were birth weight, presence of stillborn in the litter, sex, litter size (total number of born alive puppies) and breeding kennel size. The size of breeding kennel was categorised into three groups: "Small" for breeding kennels producing less than 10 puppies per year; "Medium" for 10 to 50 puppies per year and "Large" with more than 50 puppies per year. Statistically significant interactions were found between breed and litter size, breed and birth weight (all $p < 0.001$, Kruskal-Wallis rank sum test). Consequently, breed effect was introduced by classifying these two parameters using breed-specific quartiles. Litter size was categorised into three groups according to quartiles calculated at the litter level (group 1: ≤Quartile 1 (Q1); group 2: between Q1 and Q3; group 3: >Q3). Birth weight was categorised into four groups according to quartiles calculated at the puppy level (group 1: ≤ Q1; group 2: between Q1 and Q2; group 3: between Q2 and Q3; group 4: >Q3). Breeding kennel and dam were introduced as random effects to deal with the non-independence of puppies sharing the same breeding kennel and the same mother.

To deal with imbalanced data, the random under sampling approach described by Chan and Stolfo (2001) and Weiss (2004) was used, and a generalised linear mixed model was fitted with all selected variables and validated for each sub-dataset using the holdout method. Results were combined across balanced sub-datasets using the median. Indeed, p-values, odds ratio and their 95%CI were obtained for each parameter. Moreover, the median area under the receiver operating characteristic curve (AUROC) was used to assess the ability of the model to differentiate between puppies which die during neonatal period and those which do not.

In a second step, ROC curves were used to identify optimal cut-off values for birth weight. The effectiveness of the parameter to discriminate between puppies dying before three weeks of age and the ones which were still alive at the end of neonatal period was assessed using the

AUROC. If the AUROC was greater than or equal to 0.70, the cut-off value was determined separately for each breed on maximised Youden's J statistic (J=Se+Sp−1) with a sensitivity greater than or equal to 75% in order to minimise the false negative rate (Greiner et al., 2000; Hajian-Tilaki, 2013).

RESULTS

Study population

A total of 6,694 live-born puppies from 27 breeds, 1,202 litters and 75 French breeding kennels were included (Table 1). Litters were born between 1994 and 2017 with 82% of the litters born over the last 10 years (from 2007 to 2017). Among the 27 breeds included, 12 were part of the 20 most represented breeds in France according to the French Kennel Club (Société Centrale Canine, 2018). Median number of puppies included per breed was 155, ranging from 104 to 1,846 puppies. The global mean litter size at birth was 6.3 (standard deviation, SD: 2.7) puppies and 28.4% of the litters contained at least one stillborn (285/1002). Sex ratio was 1 (3,313 males and 3,314 females). Almost a quarter of the puppies were born in large breeding kennels (4845/6526). Average birth weight ranged from 119.6 (SD: 25.6) g for Chihuahua to 630.3 (SD: 112.1) g for Newfoundland. Birth weight coefficients of variation within the same breed-size puppies were 26%, 26%, 20% and 22% for respectively small, medium, large and giant breed puppies (adult body weight <10 kg, 10–25 kg, 26–45 kg and >45 kg).

Puppy mortality risk factors

A total of 9% (604/6,694; 95%CI: 8.3–9.7) of live-born puppies died during neonatal period (Table 1). Among all tested fixed effects parameters, neonatal mortality was influenced by birth weight ($p<0.001$) and size of breeding kennel ($p=0.014$) (Table 2). Neonatal mortality was significantly higher in puppies from the first quartile according to their birth weight compared with other quartiles: Q1: 17.8% (95%CI: 16–19.7) vs. Q2: 8.5% (95%CI: 7.2–9.9), Q3: 5.5% (95%CI: 4.4–6.7) and Q4: 3.7% (95%CI: 2.8–4.8). Moreover, the neonatal mortality rate was significantly higher in large (10.8%–95%CI: 9.9–11.7) and small (5%–95%CI: 3.9–6.4) breeding kennels compared to medium breeding kennels (2.3%–95%CI: 1.1–4.1). Finally, puppies with birth weight lower than the first quartile born in large breeding kennels were more at risk of neonatal death compared to puppies from other categories: mortality rate respectively at 20.7% (95%CI: 18.5–23) and 6.3% (95%CI: 5.6–7) (Fig. 1). Variances of random effects parameters were respectively 1.25 (SD: 1.12) and 0.67 (SD: 0.82) for dam and breeding kennel. The model studied presented a good discriminatory power with a median AUROC of 0.80 (range: 0.77–0.83).

Table 1. Summary of the breed-specific descriptive statistics (n=6,694)

Breed	Number of puppies included	Mean litter size at birth (SD)	Mean birth weight (SD), g	Neonatal mortality (%)
Alaskan Malamute	104	6.1 (±1.9)	562.5 (±93.3)	0.0
Australian Shepherd Dog	420	7 (±1.8)	363 (±82)	5.7
Beagle	124	6.4 (±1.6)	309 (±50.4)	7.3
Berner Sennenhund	265	6.7 (±2.7)	490.1 (±77.6)	9.8
Bichon Frise	107	5.6 (±2.2)	189 (±37.5)	21.5
Boxer	123	6.7 (±1.5)	464 (±71.7)	9.8
Cavalier King Charles Spaniel	155	4.8 (±2.2)	225.4 (±39.7)	13.5
Chihuahua	157	2.8 (±1)	119.6 (±25.6)	1.3
Cocker Spaniel	477	5.3 (±2)	266.1 (±64.1)	11.7
Coton de Tulear	159	4.4 (±1.6)	187.9 (±35.5)	3.1
Dachshund	152	3.6 (±1.6)	184 (±36.5)	7.2
English Bulldog	123	5.4 (±2)	315.9 (±68.1)	11.4
French Bulldog	111	5.1 (±2.4)	237.6 (±42.6)	15.3
German Shepherd Dog	197	6.5 (±2.7)	506.2 (±93.8)	10.2
Golden Retriever	483	7.2 (±2.7)	395.4 (±71.7)	8.1
Jack Russell Terrier	122	3.6 (±1.7)	202.1 (±36.2)	9.8
Labrador Retriever	1,846	7.3 (±2.6)	410.2 (±69.7)	6.2
Leonberger	216	7.9 (±3.8)	516.7 (±104.1)	10.2
Lhasa Apso	153	4.5 (±1.8)	187.5 (±40)	12.4
Maltese	178	4.9 (±1.5)	164.7 (±35.6)	13.5
Newfoundland	163	5.4 (±2.2)	630.3 (±112.1)	4.3
Pomeranian	117	3.4 (±1.5)	152.1 (±40)	17.1
Rottweiler	111	7.6 (±2)	403.8 (±58.6)	18.9
Shih Tzu	225	4.8 (±2.1)	176.4 (±27.9)	19.1
White Swiss Shepherd Dog	114	6.5 (±2.4)	473.4 (±80.7)	6.1
West Highland White Terrier	164	4.2 (±1.5)	196.3 (±37.5)	16.5
Yorkshire Terrier	128	4.3 (±1.8)	142.3 (±30.9)	7.0
All breeds included	6,694	6.3 (±2.7)	345.4 (±142.1)	9.0

Table 2. Fixed effects parameters of neonatal mortality model

Risk factor	P-value	Odds ratio (95%CI)
Size of breeding kennel	0.014	
Small		1 (Ref.)
Medium		0.18 (0.03–1.08)
Large		2.25 (0.78–7.35)
Presence of stillborn in the litter	0.407	
No		1 (Ref.)
Yes		0.84 (0.52–1.35)
Litter size	0.479	
Small		1 (Ref.)
Medium		1.05 (0.64–1.72)
Large		1.47 (0.66–3.27)
Birth weight	<0.001	
Q1		1 (Ref.)
Q2		0.34 (0.2–0.56)
Q3		0.18 (0.1–0.33)
Q4		0.08 (0.04–0.17)
Sex	0.225	
Female		1 (Ref.)
Male		1.29 (0.86–1.93)

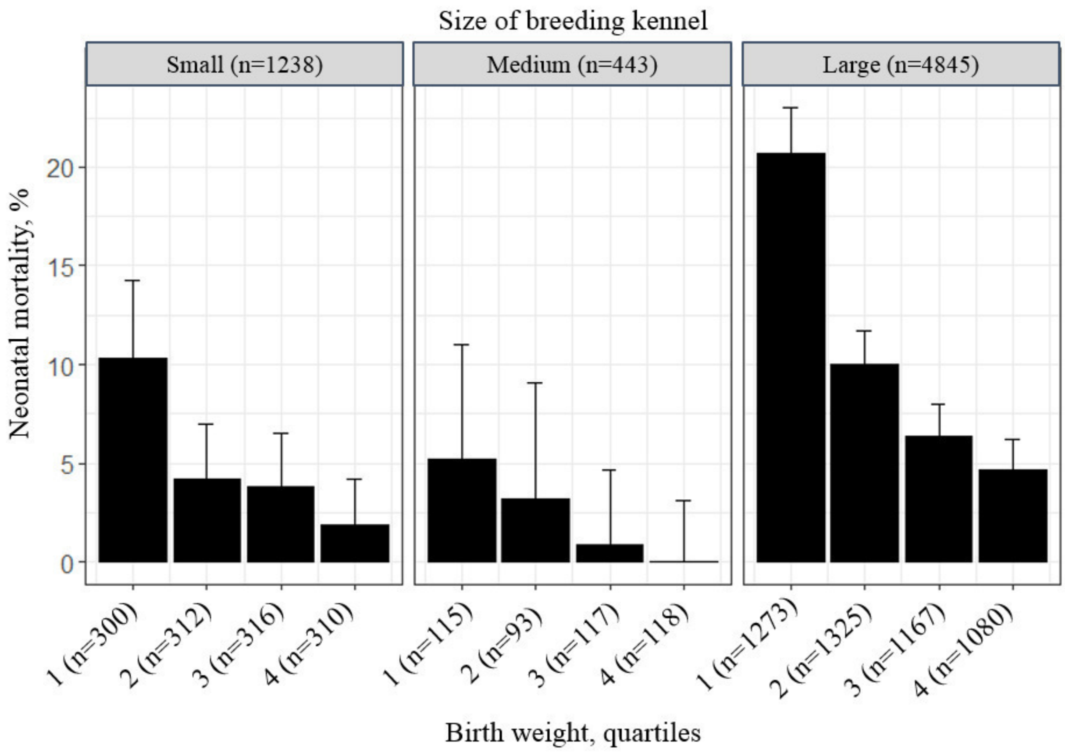

Fig. 1 Neonatal mortality rate according to birth weight and size of breeding kennel (proportion and exact binomial confidence interval)

Birth weight cut-off value determination

Birth weight cut-off values regarding neonatal mortality have been identified for 12 breeds with AUROC greater than or equal to 0.70 (Table 3). For all the breeds, the birth weight cut-off value defining the at-risk puppies was greater than the first quartile range.

Table 3. Breed-specific cut-off values for birth weight

Breed	Number of puppies included	AUROC[a] (95%CI)	BW[b] cut-off value, grams (Se[c];Sp[d])	BW first quartile value, grams
Australian Shepherd Dog	420	0.72 (0.58–0.85)	375 (75;45)	315
Berner Sennenhund	265	0.75 (0.65–0.84)	480 (100;50)	445
Cocker Spaniel	477	0.73 (0.65–0.81)	280 (84;45)	223
French Bulldog	111	0.71 (0.57–0.85)	230 (82;60)	210
German Shepherd Dog	197	0.75 (0.62–0.88)	480 (75;59)	436
Jack Russell Terrier	122	0.71 (0.54–0.88)	202 (83;59)	185
Labrador Retriever	1,846	0.75 (0.70–0.79)	406 (77;56)	365
Leonberger	216	0.75 (0.65–0.85)	480 (77;68)	450
Lhasa Apso	153	0.81 (0.70–0.91)	184 (84;60)	165
Maltese	178	0.76 (0.64–0.88)	162 (75;57)	146
Rottweiler	111	0.70 (0.58–0.83)	410 (81;52)	370
West Highland White Terrier	164	0.70 (0.58–0.82)	209 (85;41)	177

[a]AUROC = Area Under the ROC Curve, [b]BW = birth weight, [c]Se = sensitivity, [d]Sp = specificity

DISCUSSION

In the present study, including 6,694 puppies from 27 breeds represented by at least 100 puppies, the neonatal mortality rate (mortality within the first three weeks of life) was 9%. In a large French canine purebred population including 248 breeds with 204,537 puppies, postnatal mortality rate (mortality within the first two months of life) was 6.5% (Chastant-Maillard et al., 2017) and was very similar to the rate observed in Norway over the same period of age by Indrebø et al. (2007; n=744). In a cohort study including 58,439 puppies, first-week mortality rate of born alive puppies was 3.7% (Tønnessen et al., 2012). In the study of Gill (2001), 13.2% of the puppies that were born alive, died during the first six weeks (n=2,574). Thus, the current results are in the range of postnatal mortalities previously reported.

A huge variation in birth weight was observed between breeds with values ranging from 119.6 in the Chihuahua to 630.3 grams in the Newfoundland. Indeed, an influence of breed, in terms of adult body weight, on birth weight has already been demonstrated in canine species (Čechová, 2006; Chatdarong et al., 2007; Fiszdon & Kowalczyk, 2009; Groppetti et al., 2017). Our study highlights not only the importance of breed size-specific study, but also breed-specific, as birth weight was demonstrated significantly different within the same breed-size puppies. For example, adult body weights of the German Shepherd Dog, Boxer and Golden Retriever are similar, ranging from about 25 to 30 kg (Helmink et al., 2000; Hawthorne et al., 2004; Trangerud et al., 2007; Posada et al., 2014), whereas their average birth weights are quite different (506 g, 464 g and 395 g, respectively).

In this study, birth weight was strongly associated with the risk of neonatal mortality. Puppies with birth weight at or below the first quartile value, considered as low birth weight puppies, presented three times higher odds of death during the neonatal period compared to normal birth weight puppies. Various mechanisms, like an increased risk of hypoglycaemia and hypothermia, could explain the higher losses in low birth weight puppies (Mila et al., 2017): such newborns will thus require specific attention, appropriate nursing and medical care to improve their chances of survival (Mila et al., 2015). Although the impact of birth weight was already described as a major determinant for puppy survival (Lawler, 2008; Mila et al., 2015), it was arbitrarily considered as low when belonging to the 25% lighter weights. In the present study, the critical threshold was defined objectively based on an increased risk of neonatal death through ROC analysis. Specific thresholds were possible to determine for 12 breeds and 48% of the puppies were to be considered at risk for neonatal mortality (2,026 puppies of 4,260 had birth weights lower than the cut-off). These results suggest that puppies susceptible to neonatal death are not only the ones which belong to the 25% lighter birth weights as previously reported (Münnich & Küchenmeister, 2014; Mila et al., 2015). Nevertheless, specific care of almost half of the puppies (48%) would be time-consuming for breeders. Thus, it could be interesting to refine the classification of newborn puppies into puppies with moderate or critical risk of neonatal death.

Neonatal mortality was also significantly different depending on the size of the breeding kennel. Interestingly, it was increased on one hand in high-producing breeding kennels (selling 10 to 50 puppies per year) and, on the other hand, in those with a low annual number of births (less than 10 puppies). The higher probability of neonatal loss in large breeding kennels could be explained by increased puppies' susceptibility to infectious diseases. Indeed, a significantly higher prevalence of enteropathogens was described in large breeding kennels (i.e. selling more than 30 puppies per year) compared with smaller facilities (Grellet et al., 2014). Conversely, higher mortality rate in breeding kennels with less than 10 puppies sold per year could be due to a low technical level of the breeder, usually with a non-professional approach.

To conclude, evaluation of weight at birth is an easy-to-use tool for the early identification of canine newborns at higher risk of neonatal mortality. Critical thresholds presented in this study would allow the identification of puppies with higher risk of neonatal death and the provision of appropriate nursing and medical care. Further investigations are needed to define birth weight thresholds for the numerous remaining canine breeds. Moreover, due to a possible 'bloodline effect', birth weight thresholds within a given breed across different countries remain to be investigated.

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REFERENCES

Bigliardi, E., Ianni, F.D., Parmigiani, E., Morini, G. and Bresciani, C. (2013). Physiological Weight Loss in Newborn Puppies of Boxer Breed. Ital. J. Anim. Sci. 12, e77

Boyko, A.R., Quignon, P., Li, L., Schoenebeck, J.J., Degenhardt, J.D., Lohmueller, K.E., Zhao, K., Brisbin, A., Parker, H.G., vonHoldt, B.M. et al. (2010). A simple genetic architecture underlies morphological variation in dogs. PLoS Biol. 8, e1000451

Čechová, M. (2006). Analysis of some factors influencing the birth weight of piglets. J. Anim. Sci. 3, 139–144

Chan, P.K. and Stolfo, S.J. (1998). Toward scalable learning with non-uniform class and cost distributions: a case study in credit card fraud detection. In, Proceedings of the 4th International Conference on Knowledge Discovery and Data Mining, New York, US, 164–168

Chastant-Maillard, S., Guillemot, C., Feugier, A., Mariani, C., Grellet, A. and Mila, H. (2017). Reproductive performance and pre-weaning mortality: preliminary analysis of 27,221 purebred female dogs and 204,537 puppies in France. Reprod. Domest. Anim. 52, 158–162

Chatdarong, K., Tummaruk, P., Sirivaidyapong, S. and Raksil, S. (2007). Seasonal and breed effects on reproductive parameters in bitches in the tropics: a retrospective study. J. Small Anim. Pract. 48, 444–448

Fédération Cynologique Internationale. (2018). Nomenclature des races de la FCI. Available at: http://www.fci.be/fr/Nomenclature/. Accessed 26/11/2018

Fiszdon, K. and Kowalczyk, I. (2009). Litter size, puppy weight at birth and growth rates in different breeds of dogs. Ann. Warsaw Univ. of Life Sci. 46, 161–168

Fix, J.S. (2010). Relationship of piglet birth weight with growth, efficiency, composition, and mortality. PhD thesis, North Carolina State University, Raleigh

Gill, M.A. (2001). Perinatal and late neonatal mortality in the dog. PhD thesis, University of Sydney, Sydney

Greiner, M., Pfeiffer, D. and Smith, R.D. (2000). Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev. Vet. Med. 45, 23–41

Grellet, A., Chastant-Maillard, S., Robin, C., Feugier, A., Boogaerts, C., Boucraut-Baralon, C., Grandjean, D. and Polack, B. (2014). Risk factors of weaning diarrhea in puppies housed in breeding kennels. Prev. Vet. Med. 117, 260–265

Groppetti, D., Ravasio, G., Bronzo, V. and Pecile, A. (2015). The role of birth weight on litter size and mortality within 24h of life in purebred dogs: what aspects are involved? Anim. Reprod. Sci. 163, 112–119

Groppetti, D., Pecile, A., Palestrini, C., Marelli, S. and Boracchi, P. (2017). A national census of birth weight in purebred dogs in Italy. Animals, 7:pii: E43

Hajian-Tilaki, K. (2013). Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. Casp. J. Intern. Med. 4, 627–635

Hawthorne, A.J., Booles, D., Nugent, P.A., Gettinby, G. and Wilkinson, J. (2004). Body-weight changes during growth in puppies of different breeds. J. Nutr. 134, 2027S–2030S

Helmink, S.K., Shanks, R.D. and Leighton, E.A. (2000). Breed and sex differences in growth curves for two breeds of dog guides. J. Anim. Sci. 78, 27–32

Indrebø, A., Trangerud, C. and Moe, L. (2007). Canine neonatal mortality in four large dog breeds. Acta Vet Scand. 49, S1–S2

Lawler, D.F. (2008). Neonatal and pediatric care of the puppy and kitten. Theriogenology 70, 384–392

Mila, H., Grellet, A., Feugier, A. and Chastant-Maillard, S. (2015). Differential impact of birth weight and early growth on neonatal mortality in puppies. J. Anim. Sci. 93, 4436–4442

Münnich, A. and Küchenmeister, U. (2014). Causes, diagnosis and therapy of common diseases in neonatal puppies in the first days of life: cornerstones of practical approach. Reprod. Domest. Anim. 49, 64–74

Nielen, A.L., Janss, L.L. and Knol, B.W. (2001). Heritability estimations of diseases, coat color, body weight and height in a birth cohort of Boxer dogs. Am. J. Vet. Res. 62, 1198–1206

Posada, O., Gomez, O. and Rosero, N. (2014). Aplicación del modelo logístico para describir la curva de crecimiento en perros de diferentes razas. Rev. MVZ Córdoba 19, 4015–4022

R Core Team (2016). R: A language and environment for statistical computing. The R Foundation, Vienna, Austria. Available at: https://www.r-project.org/

Société Centrale Canine. (2018). Les statistiques du LOF depuis 1969. Available at: https://www.centrale-canine.fr/articles/les-statistiques-du-lof-depuis-1969. Accessed 26/11/2018

Trangerud, C., Grøndalen, J., Indrebø, A., Tverdal, A., Ropstad, E. and Moe, L. (2007). A longitudinal study on growth and growth variables in dogs of four large breeds raised in domestic environments. J. Anim. Sci. 85, 76–83

Weiss, G.M. (2004). Mining with rarity: a unifying framework. SIGKDD Explor. 6, 7–19

Wu, G., Bazer, F.W., Wallace, J.M. and Spencer, T.E. (2006). Intrauterine growth retardation: implications for the animal sciences. J. Anim. Sci. 84, 2316–2337

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