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Tese

Crescimento e desenvolvimento de filhotes de cães da raça Australian Cattle Dog

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Resumo

DODE, Maria Eduarda Bicca. **Crescimento e desenvolvimento de filhotes de cães da raça Australian Cattle Dog.** 2022. 62f. Tese (Doutorado em Ciências) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2022.

O Australian Cattle Dog (ACD) é um boiadeiro robusto e musculoso, classificado no grupo I da Federação Cinológica Internancional. O objetivo dos estudos foi analisar progenitores e filhotes nos primeiros 45 de vida relacionando os dados obtidos com a mortalidade e crescimento de filhotes da raça no período neonatal através de relações entre o crescimento, peso ao nascer, número médio de filhotes da ninhada e ganho de peso de filhotes da raça nos primeiros 45 dias e também descrever através de parâmetros a relação entre peso ao nascer, ganho de peso e o coeficiente de inbreeding (CoI), e a mortalidade observada no período neonatal. Filhotes foram pesados ao nascer e até os 45 dias de vida. Efeitos das ninhadas foram comparados através do teste de Tuckey (p≤ 0,05). Para se estabelecer o comportamento de crescimento de cada um dos grupos, o peso médio dos filhotes em relação aos dias de pesagem foi ajustado através do modelo de regressão e calculada a Correlação de Pearson entre as variáveis. Os grupos também tiveram a heterogeneidade do peso ao nascer, ganho de peso médio diário e CoI de cada ninhada calculados, seguidos a obtenção dos quartis para as variáveis. 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). As matrizes apresentaram idades entre 18 e 70 meses e o peso médio das mães no momento da cobertura foi de 13,5+- 1,4kg (variando entre 10,4 e 16,7Kg) e o peso médio dos pais foi de 16,05+-1,67Kg, (variando de 13,1 a 18,5Kg). Foram incluídas 17 ninhadas nascidas em 2020, totalizando 100 filhotes, 54 machos e 46 fêmeas. O número médio de filhotes por ninhada foi 5,88 +- 1,93 e peso médio ao nascer foi 258,98+-47,19g. O ganho de peso médio diário nos diferentes grupos esteve mais fortemente relacionado ao peso médio ao nascer e moderadamente ao peso da mãe e fracamente relacionado ao peso do pai. O ganho de peso médio diário dos filhotes dos oito grupos analisados durante os primeiros 45 dias de vida variou de 60,12 a 40,16g. Observou-se a validade da relação entre mortalidade no período neonatal e o fato da mãe ser primípara (AUROC≥0,7). Medidas simples como pesagem e acompanhamento de filhotes através de curvas de crescimento devem ser implementadas intensificando atenção em ninhadas numerosas, heterogêneas e de fêmeas de primeira cria.

Palavras-chave: cão; canino; curva de crescimento; ganho de peso; cinotecnia

Abstract

DODE, Maria Eduarda Bicca. **Puppy growth and development of Australian Cattle Dogs**. 2022. 62f. Thesis (Doctor degree in Sciences) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2022.

The Australian Cattle Dog (ACD) is a robust and muscular working dog, classified in group I of the International Cynological Federation. The aim of the studies was to analyze parents and pups in the first 45 years of life, relating the data obtained with the mortality and growth of puppies of the breed in the neonatal period through relationships between growth, birth weight, average number of pups in the litter and weight gain. weight of puppies of the breed in the first 45 days and also to describe through parameters the relationship between birth weight, weight gain and the inbreeding coefficient (CoI), and the mortality observed in the neonatal period. Puppies were weighed at birth and up to 45 days of age. Litter effects were compared using the Tuckey test ($p \leq 0.05$). To establish the growth behavior of each of the groups, the average weight of the pups in relation to the days of weighing were adjusted through the regression model and Pearson's Correlation between the variables was calculated. The groups also had birth weight heterogeneity, average daily weight gain and CoI of each litter calculated, followed by obtaining the quartiles for the variables. Multicollinearity was estimated between the predictors using Cramer's method and the median area under the receiver operating characteristic curve (AUROC). The bitches were aged between 18 and 70 months and the average weight of the mothers at the time of mating was 13.5+- 1.4 kg (ranging from 10.4 to 16.7 kg) and the average weight of the fathers was 16, 05+-1.67Kg, (ranging from 13.1 to 18.5Kg). 17 litters born in 2020 were included, totaling 100 puppies, 54 males and 46 females. The mean number of pups per litter was 5.88 +- 1.93 and mean birth weight was 258.98+-47.19g. The average daily weight gain in the different groups was more strongly related to the average birth weight and moderately to the mother's weight and weakly related to the father's weight. The average daily weight gain of the offspring of the eight groups analyzed during the first 45 days of life ranged from 60.12 to 40.16g. The validity of the relationship between mortality in the neonatal period and the fact of the mother being primiparous (AUROC≥0.7) was observed. Simple measures such as weighing and monitoring pups through growth curves should be implemented, intensifying attention in large litters, heterogeneous and first-birth females.

Keywords: dog; canine; growth curve; weight gain; cynotechnique

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Lista de Abreviaturas e Siglas

ACD	Australian Cattle Dog
AUROC	Curva característica de operação do receptor
CoI	Coeficiente de inbreeding
FCI	Federaçao Cinologica Internacional
g	Gramas
GPD	Ganho de peso diário
Kg	Kilogramas
M	Com perdas
NF	Número de filhotes na ninhada
PM	Peso da mãe
PN	Peso ao nascer
PP	Peso do pai
SM	Sem perdas
TNM	Taxa de morte neonatal

Sumário

1 Introdução

Assim como em outras espécies de mamíferos, a transição fetal/neonatal em caninos requer um processo adaptativo multissistêmico (VERONESI, 2016) que por ser delicado, não raramente exige intervenção do médico veterinário. No período neonatal, os filhotes são frágeis, incapazes de enxergar e ouvir, totalmente dependente de cuidados da mãe (SCOTT, 1957).

Prendergest (2011), infere que passadas 48h do parto, as perdas neonatais estejam fortemente relacionadas a fome sendo que filhotes com baixo peso ao nascer tendem a maiores complicações e menor desempenho o que produz também reflexos na morbidade e mortalidade. Relata ainda que filhotes com 25% a menos de peso da média esperada tem risco aumentado de hipoglicemia, hipotermia e pneumonia corroborando com as indicações de perdas por hipóxia, hipotermia e desnutrição apontadas por Peterson (2011).

Superado o período perinatal, neonatal e de transição, filhotes caninos crescem rapidamente nos primeiros seis meses de vida (FARMINA VET, 2011). Garantir ninhadas com filhotes saudáveis a partir de cruzamentos planejados, contribui para a manutenção de raças puras, preservando suas características e funções, sendo a base da criação responsável de cães.

O Australian Cattle Dog (ACD) é um pastor robusto e musculoso, de porte médio e que tem força e agilidade. O surgimento da raça data do início do século XIX, motivado pela necessidade de um cão para movimentar o gado de corte na Austrália. O resultado foram cães resistentes às altas temperaturas, terrenos acidentados e capazes de acompanhar longas jornadas de pastoreio percorrendo extensas distâncias (AKC, 2020; FCI, 2020; GREEN CROSS VETS, 2020).

Analisar o registro genealógico dos pedigrees facilita estudo da variabilidade genética disponível na população e permite a seleção de progenitores e planejamento dos cruzamentos, monitorando níveis de consanguinidade e seus efeitos. Tais informações passam a ser relevantes complementando escolhas meramente morfológicas e muitas vezes subjetivas.

Avaliação da fertilidade dos progenitores seguida de protocolos sanitários básicos para o início do manejo reprodutivo contribui para prolificidade, saúde e bem estar dos animais. Avançar após estas etapas inclui acompanhar o período pré-natal através, o parto e a vitalidade dos neonatos que em caninos como em outras espécies de mamíferos, durante a transição fetal/neonatal exige um processo adaptativo multissistêmico delicado (VERONESI, 2016).

No período neonatal, os filhotes são frágeis, incapazes de enxergar e ouvir, totalmente dependente de cuidados da mãe (SCOTT, 1957). O peso ao nascer, o número médio de filhotes por ninhada e o registro do ganho de peso diário, fornece informações relevantes para identificação de filhotes em risco (LAWLER, 2008; MILA et al., 2015; MILA et al., 2017).

Estratégias de fácil implementação e que não exijam equipamentos sofisticados podem contribuir para redução de perdas no período neonatal. O objetivo deste trabalho é apresentar resultados da aplicação de avaliações morfométricas dos progenitores e filhotes nos primeiros 45 de vida e a utilização das informações obtidas no registro genealógico para acessar a variabilidade genética da população estudada, buscando relacionar os dados obtidos com a mortalidade neonatal e acompanhamento do crescimento de filhotes da raça ACD do parto ao desmame.

2 Revisão da Literatura

2.1 Fatores de risco e seus impactos no sucesso reprodutivo na criação de cães de raça pura

Mila e colaboradores (2017) enfatizam a necessidade de maiores informações sobre os impactos do período gestacional sobre a vitalidade dos filhotes ao nascer, não limitando a atenção ao monitoramento do parto. Os cães são multíparos e o trabalho de parto pode ser prolongado por inúmeras razões em períodos podem se estender em condições normais até 12h e excepcionalmente 24h em ninhadas muito numerosas (INDREBO et al., 2009). Ao acompanhar o trabalho de parto é necessário observar o comportamento da cadela, o intervalo entre as expulsões e a presença de contrações produtivas, identificando intercorrências e intervindo de forma a garantir o bem estar da mãe e dos filhotes. A decisão sobre intervenções sejam elas físicas, medicamentosas ou cirúrgicas deve ser tomada em tempo reduzir risco e danos a mãe e neonatos (WEIJEN e TAVERNE, 1994; DAVIDSON, 2003; ROMAGNOLI, 2009).

Além de abordar a relevância da mortalidade observada em filhotes caninos, Ogbu e colaboradores (2016) estabelecem definições para classificar as fases iniciais de crescimento e desenvolvimento considerando a subdivisão do período neonatal em neonatal precoce (do nascimento aos 7 dias de vida) e neonatal tardio (dos 8 aos 42 dias de vida).

Fatores que ampliam o risco da mortalidade neonatal foram revisados por Ogbu e colaboradores (2016) que enumeraram raça, idade da mãe, número de crias, duração do trabalho de parto e tamanho da ninhada como relevantes. Os autores sugerem também especial atenção ao peso ao nascer, avaliações da placenta entre outras análises capazes de agregar informações às observações empíricas de criadores, destacando a importância do acompanhamento veterinário obstétrico.

Distocia é a alteração no andamento normal do trabalho de parto e pode ser influenciada por características maternas, do filhote ou de ambos (MUNNICH e KUCHENMEISTER, 2014). A condição aumenta a chance de ocorrência de hipoxemia (síndrome do estresse respiratório) uma vez que a homeostase cardiorrespiratória do neonato é facilmente alterada, resultando também em um desequilíbrio ácido-base.

Após o parto, a ativação da respiração é um momento crítico, porém essencial para a sobrevivência do neonato, tendo impactos no sistema respiratório e cardiovascular. (GRUNDY, 2006; LAWLER, 2008; MUNNICH e KUCHENMEISTER, 2009; VERONESI et al., 2009; MUNNICH e KUCHENMEISTER, 2014; VERONESI et al., 2016). Da Luz e colaboradores (2021) ao relatar a mortalidade de neonatos caninos em um hospital veterinário do Brasil apontou perdas superiores a 50% em filhotes nascidos de cesariana em condições de distocia.

2.2 Avaliação dos filhotes durante o período neonatal precoce

Sabendo que após o parto, o monitoramento da vitalidade dos filhotes pode contribuir para eleição de medidas de apoio ao filhote reduzindo a mortalidade neonatal. O sistema de pontuação através do Score APGAR fornece uma avaliação padronizada que compreende cinco pontos: coloração das mucosas, frequência cardíaca, reflexos, tônus muscular e frequência respiratória. Cada um dos parâmetros recebe uma pontuação quantificando os sinais clínicos observados no neonato e fornecendo um escore individual. A avaliação da vitalidade neonatal através do estabelecimento de escores não é invasiva e permite de forma ágil analisar a necessidade de suporte e acompanhar a evolução dos neonatos.

O teste de APGAR modificado tem sido adotado com sucesso em medicina veterinária como protocolo auxiliar na identificação de neonatos em risco imediatamente após o nascimento e também após adoção de ações de suporte. A obtenção de escores individuais contribui para eleição e adoção de medidas de suporte individualizadas, concentrando atenção e esforços em filhotes mais vulneráveis (VERONESI et al., 2009)

Os escores fornecem um panorama da viabilidade neonatal precoce e pode ser utilizado de forma geral para todos as raças caninas ou adaptado para raças específicas ou filhotes nascidos de cesariana. Neonatos com escores de 0-3 são considerados críticos, enquanto aqueles com escores entre 4-6 em sofrimento moderado enquanto valores de 7-10 indicam neonatos normais. A aplicação do teste permite a identificação das vulnerabilidades dos neonatos, facilitando concentrar atenção imediata nos filhotes sob maior risco (escores de 0-6) reduzindo a mortalidade neonatal precoce (VERONESI et al., 2009; VERONESI, 2016). Em estudo realizado na raça chiuaua, Fusi e colaboradores (2020) ao analisarem os escores obtidos em 176 filhotes nascidos através de cesariana eletiva, confirmaram a utilidade do teste APGAR para predizer a viabilidade dos neonatos nas primeiras 24h após o parto.

Estudo conduzido por Vassalo e colaboradores (2015) observou imediatamente após o parto escores APGAR superiores em filhotes de diferentes raças nascidos de parto normal (49) considerando a depressão observada em filhotes nascidos de cesariana (55) reflexo do sofrimento fetal relacionado a distocia e aos efeitos dos anestésicos. Análises realizadas após 60 min apontaram uniformidade nos escores obtidos em filhotes nascidos através de parto normal e cesariana, sendo útil assim para constatar a efetividade das intervenções para recuperação: desobstrução das vias respiratórias superiores, secagem, ressuscitação cardiopulmonar, oxigenação, fluidoterapia e medicação intravenosa quando necessário.

Os autores além de analisar os escores APGAR procederam também avaliação clínica, aferindo a temperatura retal e reflexos presentes no período neonatal precoce. Tanto escores de APGAR quanto de reflexos foram superiores nos filhotes nascidos através parto normal. Estes autores consideram ambos procedimentos importantes para identificação de filhotes em risco e avaliação da efetividade das ações de reanimação, porém não apontaram utilidade como parâmetros de prognóstico. A terapia intensiva no recém nascido inclui suporte cardiorrespiratório, recuperação da normotermia, cuidados com a imunidade passiva e glicemia contribuindo para o correto estabelecimento das funções vitais do neonato canino, ampliando as chances de sobrevivência pela redução de fatores desencadeadores de afecções neonatais (MUNNICH e KUCHENMEISTER, 2014; VANUCCI e ABREU, 2017).

A avaliação da vitalidade dos filhotes através do teste de APGAR juntamente com análise dos níveis plasmáticos de glicose durante as primeiras 24 horas foram indicados pelos estudos de Mila e colaboradores (2017) como relevantes para monitoramento e identificação de filhotes em risco. Além da condução do teste de APGAR, avaliações morfométricas dos neonatos podem auxiliar médicos veterinários e criadores a identificação de filhotes vulneráveis. Neonatos com baixo peso ao nascer apresentam maior risco de mortalidade e morbidade. Autores relatam o peso ao nascer inversamente relacionado ao tamanho da ninhada e também observam diferenças entre raças. (GROPETTI, 2017). Porém, complexas interações entre fatores relacionados ao feto, a mãe e o ambiente merecem ser consideradas e estudadas mais aprofundadamente em caninos.

Intercorrências durante o parto e período neonatal precisam ser consideradas e identificadas a fim de que medidas corretivas possam ser rapidamente tomadas. Análise dos níveis de lactato do cordão umbilical pode fornecer informações importantes sobre estresse respiratório (GROPETTI et al., 2010). Em estudo que envolveu 68 filhotes saudáveis, Mc Michael e colaboradores (2005) indicaram que até os 28 dias, neonatos apresentam níveis de lactato venoso superiores aos observados em adultos e que em filhotes de quatro dias, estes valores superam de forma significativa os observados em filhotes de 10 a 28 dias.

Os níveis de lactato obtidos a partir da análise de amostras da veia umbilical apontaram menor sofrimento fetal em filhotes nascidos de parto normal quando comparados aos de cesariana não eletiva (KUTTAN et al., 2016). Antoriczyk e colaboradores (2021) reuniram informações sobre resultados de análises de sangue de cordão umbilical de filhotes nascidos por cesariana eletiva e o teste APGAR. Foi observada acidose respiratória leve. Filhotes com escores APGAR baixos apresentaram glicemia elevada e mortalidade superior. Da mesma forma, a bradicardia nos 4 primeiros dias de vida deve ser considerada um sinal de hipoxemia (GRUNDY, 2006).

Baixas reservas de tecido adiposo ao nascer implicam em dificuldades em suprir as demandas metabólicas sem aportes nutricionais. Mesmo neonatos caninos saudáveis apresentam uma menor capacidade de gerar glicose, principalmente devido as baixas reservas de glicogênio e imaturidade do sistema. Durante as primeiras 24h de vida, há intensa depleção das reservas de glicogênio com a produção sistêmica de glicose predominantemente via glicogenólise passando para uma combinação glicogenólise e gliconeogênese após 24h.

O rápido esgotamento dos reservas de glicogênio aponta importância da alimentação regular. Alterações no metabolismo de carboidratos de reserva, endotoxemia, septicemia, presença de shunt portossistêmico também podem contribuir para hipoglicemia. Sinais como letargia, depressão, vocalização ou convulsões devem ser imediatamente considerados principalmente em neonatos de raças pequenas, mais suscetíveis a quadros de hipoglicemia. (KLIEGMAN, 1988;

DAVIDSON, 2003; GRUNDY, 2006; MUNNICH e KUCHENMEISTER 2014). Mila e colaboradores (2017) destacam que a habilidade de transição fetal-neonatal através das adaptações às condições extrauterinas e a ingestão de colostro são fundamentais para a sobrevivência dos filhotes caninos enquanto Munnich e Kuchenmeister (2014) enfatizam a importância do exame físico para identificação de sinais patológicos precocemente.

Os neonatos estão predispostos a desidratação pela elevada relação superfície/volume, características da pele que é mais permeável e imaturidade do funcionamento renal. A disparidade entre as perdas e a ingestão de água em neonatos pode ser acentuada por fatores extrínsecos como o comportamento materno, agalactia e temperatura ambiental elevada. Prematuridade e processos patológicos tais como pneumonia e diarreia que ampliam tal desproporção acabam levando a hipovolemia, hemorragias, choque e perdas neonatais (LAWLER, 2008; MUNNICH e KUCHENMEISTER, 2014).

Especial cuidado deve ser dado ao ambiente onde o neonato será mantido após o parto até o desmame, pois por não apresentar o sistema nervoso autônomo totalmente maduro estará menos apto a responder aos estresses fisiológicos. (GRUNDY, 2006). Davidson (2003) ressalta os necessários cuidados com o conforto térmico destacando que a maturidade do sistema termorregulatório em caninos é atingida apenas em quatro semanas, A temperatura e manutenção do espaço ambiente livre de substâncias potencialmente irritantes também deve ser mantida (GRUNDY, 2006) contribuindo para qualidade ambiental, pois especial atenção deve ser dada a prevenção da síndrome do estresse respiratório que é uma das condições não infecciosas mais preocupantes, pois está relacionada a mais de 60% das perdas neonatais (MUNNICH, 2008).

Após o nascimento, condições amenas de temperatura para mãe e filhotes devem ser garantidas. A hipotermia é uma das respostas fisiológicas de proteção pós-parto capaz de afetar a fisiologia normal de vários sistemas, impactando negativamente na imunidade, digestão e na amamentação. Filhotes caninos saudáveis apresentam temperaturas corporais entre 35 e 36,5°C 24h após o parto. Porém, até seis dias de vida neonatos necessitam ambiente com conforto térmico para manutenção da temperatura independente de serem mantidos ou não com a mãe devendo ser evitada exposição a baixas temperaturas.

A adequada e manutenção da homeostase metabólica exigem a estabilidade da temperatura corporal e temperaturas ambientais muito elevadas reduzem a capacidade respiratória e promovem a desidratação enquanto que em temperaturas baixas, a capacidade de sugar é diminuída, levando a desidratação, falência cardiorrespiratória, alteração na motilidade intestinal predispondo também a infecções. Em caso de intervenção, é importante lembrar que a temperatura corporal deve ser elevada gradualmente e a alimentação restabelecida apenas quando normotermia for alcançada (DAVIDSON, 2003; LAWER, 2008; MUNNICH e KUCHENMEISTER, 2014).

2.3 Acompanhamento do crescimento dos filhotes durante o período neonatal

Superado o delicado processo de adaptação a vida extrauterina, novos desafios devem ser superados, exigindo atenção e cuidados. Muitas vezes, as perdas neonatais após as primeiras 24h de vida ocorrem sem haver um diagnóstico preciso. A síndrome do definhamento canino é um termo coloquial utilizado para descrever a mortalidade de filhotes que nascem aparentemente saudáveis, porém falham em manter crescimento e desenvolvimento nas primeiras duas semanas de vida e perecem. Ainda que ao descrever a síndrome se agrupe um conjunto de sinais patológicos de diferentes origens, suas causas podem ser consideradas de origem ambiental, genética ou infecciosa. Ainda alternativamente, podem ser classificadas em causas de origem materna, de origem neonatal e de manejo. Nestas circunstâncias, sinais clínicos são inespecíficos, mas em geral relacionados ao baixo peso ao nascer, baixo ganho de peso, redução do reflexo de mamada fazendo com que a mãe tenda a segregar tais indivíduos mantendo-os isolados (KHAN et al., 2009). Tanto a avaliação do peso ao nascer quanto do ganho de peso diário a partir da pesagem regular podem oferecer indicativos de filhotes em severo risco (INDREBO et al., 2007) contribuindo para identificação e correção das causas primárias. Hedberg (2015) destaca as principais causas de ganho de peso insuficiente ou perda de peso durante o período neonatal precoce: desidratação, hipoglicemia, manejo inadequado condições ambientais interferindo no equilíbrio térmico dos filhotes, falta de leite ou negligencia materna. A perda de peso no período neonatal precoce devido a causas fisiológicas pode alcançar até 10%. O monitoramento da variação diária de peso dos filhotes pode contribuir para identificação das situações de risco, porém curvas específicas deverão ser elaboradas para atender as peculiaridades do crescimento e desenvolvimento das diferentes raças (BIGLIARDI et al., 2013).

O peso do filhote ao nascer, o peso 12 e 24h após o parto, peso antes e depois da alimentação, ganho de peso diário, peso médio e ganho de peso médio da ninhada são facilmente obtidos e podem ser realizados isoladamente ou em conjunto pelo próprio criador, contribuindo para o monitoramento diário dos neonatos. O registro e acompanhamento dos dados além de fornecer um histórico do crescimento e desenvolvimento do filhote e da ninhada, contribui para a ampliação das informações sobre a ingestão de colostro, habilidade materna, presença de condições adversas, nutrição dos filhotes e patologias (LAWLER, 2008; DODAMANI et al., 2017). Os riscos reduzindo a sobrevivência de filhotes com peso muito baixo ao nascer são reconhecidos tanto por cientistas quanto por criadores contribuindo para adoção de medidas preventivas e corretivas ainda que não haja práticas homogêneas. Mugnier e colaboradores (2020) identificaram as práticas mais usuais no manejo de filhotes caninos e felinos com baixo peso ao nascer e reuniram informações sobre ações implementadas por criadores franceses para reduzir as perdas neonatais. Mila e colaboradores (2015) apontaram o impacto do peso ao nascer e o desenvolvimento neonatal com a mortalidade neonatal precoce (primeiros dois dias) e também aquela observada entre a segunda e terceira semana após o parto.

3 Artigos

3.1 Artigo 1

Neonatal care and the reproductive success of purebred dogs: challenges and goals

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Neonatal care and the reproductive success of purebred dogs: challenges and goals

Cuidados neonatais e o sucesso reprodutivo de cães de raça pura: desafios e objetivos

ABSTRACT
There are over 400 dog breeds that differ in size, morphology, and fitness. The correct management of pure breed kennels is essential for reproductive success. This requires multiple approaches that help control risk factors both intrinsic and extrinsic to progenitors and pups. Essential stages precede conception. Additionally, the fetal-neonatal transition is a complex period requiring greater attention to the pups. Once these challenges have been overcome, the breeder and veterinarian must apply tools to reduce the morbidity and mortality of pups. These rates remain high, impacting animal welfare and breed conservation while also resulting in notable economic damage. In this review, we present risk factors and their impacts on the success of breeding and preserving purebred dogs. Moreover, we highlight the importance of monitoring pup health and growth throughout the neonatal period.

Keywords: newborn, welfare, growth, kennel, Apgar, pup.

INTRODUCTION

Responsible dog breeding requires overcoming the reproductive challenges of the species aided by canine obstetrics and neonatology. Advances in theriogenology have stimulated the research and development of methods to reduce mortality and morbidity during the prenatal, perinatal, and neonatal periods in canines. The mortality of pups remains elevated, causing important impacts for breeders, not only economically but also for animal welfare. Nonetheless, there are new techniques to support birth, comprehend the physiology and evaluate the vitality of neonates, as well as tools for early diagnostics. Reproductive management requires multiple strategies since it is influenced by complex factors both extrinsic and intrinsic to the pup. Appropriate monitoring of pups requires knowledge and specific skills that enable disease prevention, as well as identification of practices to preserve the life and welfare of the neonates. In this study, we reviewed the data pertaining to noninfectious causes of pup mortality and the monitoring processes that reduce mortality in neonatal canines.

Dogs originated from the divergence of the domestic canine from its ancestor. This resulted from an unintentional domestication process that possibly began over 36 thousand years ago, although the precise period remains unclear. Abundant genetic and archeological research has been focused on explaining the origin of the species. The process was likely accelerated by empirical artificial selection of canines for different morphological and behavioral characteristics that originated the species *Canis lupus familiaris* (domestic dog). This species has an important social role, performing multiple functions that require extraordinary variability in morphology and behavior. Recently, reproductive isolation and selection have resulted in over 400 dog breeds with distinct appearance and behavioral traits. The term breed in this review refers to genetically isolated canine populations displaying characteristic sets of physical and behavioral features maintained under artificial selective pressure. Responsible dog breeding promotes pure breed preservation, contributing to the conservation of populations and their diversity. These breeds are registered in their origin throughout generations, forming closed populations (DIAS, 2019; SERPELL and DUFFY 2014).

The success in breeding purebred dogs is based on pillars of breeding excellence that guarantee adequate kennel management. These consider physiological, genetic, sanitary, nutritional, and environmental aspects integrating scientific and technical approaches to dog breeding. Implementing rigorous processes contributes to the control of risk factors, reducing mortality and assuring the welfare of mothers and pups during the pregnancy and neonatal periods (DAVIDSON, 2003; MUNNICH, 2008; ROMAGNOLI, 2009; BARTGES et al., 2012; MUNNICH and KUCHENMEISTER, 2014; DODAMANI et al., 2017; LUZ and FREITAS, 2019).

Dog breeding includes prenatal, perinatal, and neonatal losses for varying reasons related to breed, the progenitors, the environment, or intrinsic to the neonate. Intrinsic factors affect fetal viability and can result from gestational issues, litter size, gestational duration, fetal malformation, and maternal age, among others. Close monitoring during the prenatal, perinatal, and neonatal periods is necessary to control and mitigate these factors (TONESSEM et al., 2012; MUNNICH and KUCHENMEISTER, 2014; GROPPETTI et al., 2015; LUZ and FREITAS, 2019; SCHRANK et al., 2020; TESI et al., 2020). The impact of pup mortality and morbidity becomes greater when considering other aspects, including emotional distress, animal welfare, and breed conservation (VERONESI et al., 2009; DODAMANI et al., 2017; GROPPETTI et al., 2017; MUGNIER et al., 2020).

An appropriate breeding plan must consider consanguinity levels, hereditary conditions, and pathological and sanitary conditions of the progenitors. In particular, factors associated with the reproductive history of females dictate the amount of care required during the prenatal period. These factors include morphology, age, number of litters and interventions in previous gestations, frequency of stillborn pups and dystocia. Optimal nutritional and sanitary conditions as well as vaccination programs should be rigorously maintained during gestation to ensure the welfare and health of dams. Moreover, pharmacological interventions should be carefully evaluated to reduce teratogenic or other risks to the fetuses (SOMMERFELD-STUR, 2006; ROMAGNOLI, 2009).

Knowledge of the reproductive physiology of canines and prenatal exams facilitate the monitoring of fetal development and a more accurate prediction of the delivery date. Obstetric evaluations include examining the birth canal and nipples, imaging (X-rays and pelvic ultrasound), and biochemical analysis (red blood cell count, hormonal levels). Knowledge of the breed in question also allows the implementation of appropriate obstetric conduct and interventions that will reduce perinatal and neonatal suffering. (WEIDJEN and TAVERNE, 1994; DAVIDSON, 2003; ROMAGNOLI, 2009; FUSI et al., 2020).

The term neonate is used to describe canine pups during the first weeks of life. Canine neonates are totally dependent on care, sensitive to environmental changes and susceptible to microbial infections. The fetal-neonatal transition is an important period requiring coordinated activation of multisystem adaptive processes. Prevention and control of physical, sanitary, and environmental risks reduce chance of suffering and the high rate of neonatal mortality and morbidity reported in purebred kennels. The morphological and physiological characteristics of the canine neonate hinder diagnostics and treatment. To overcome these challenges, the veterinarian requires skills and tools to implement the necessary care, which guarantees the survival of the pups (GRUNDY 2006; MUNNICH, 2008; VERONESI, 2016; MUGNIER et al., 2020;)

RISK FACTORS AND THEIR IMPACT ON REPRODUCTIVE SUCCESS IN PUREBRED CANINES

Mila *et al.* (2017) emphasized the need to further understand the impacts of the gestational period on pup vitality at birth, instead of only monitoring birth. Dogs are multiparous, and labor can be prolonged for several reasons; periods can extend under normal conditions up to 12 h or 24 h in very large litters (INDREBO et al., 2009). When monitoring labor, the dam's behavior should be observed as well as the interval between expulsions and the presence of productive contractions to identify complications and intervene to guarantee the well-being of the dam and pups. Physical, medical, or surgical interventions should occur in time to reduce risk and harm to the dam and neonates (WEIDJEN and TAVERNE, 1994; DAVIDSON, 2003; ROMAGNOLI, 2009).

Factors that increase the risk of neonatal mortality were reviewed by Ogbu *et al.* (2016), who emphasized race, the dam's age, number of pups, duration of labor, and litter size. The authors highlighted the importance of obstetric veterinary monitoring by recommending birth weight, placental assessments and other analyses capable of adding information to the empirical observations of breeders.

Dystocia is a change in the normal course of labor influenced by characteristics of the dam and of the pups (MUNNICH and KUCHENMEISTER, 2009). The condition increases the chance of hypoxemia (respiratory stress syndrome) as the neonate's cardiorespiratory homeostasis is easily altered, also resulting in an acid-base imbalance. After delivery, the activation of breathing is a critical moment for the survival of the neonate, affecting the respiratory and cardiovascular systems. (GRUNDY, 2006; MUNNICH and KUCHENMEISTER, 2009; VERONESI et al., 2009; MUNNICH and KUCHENMEISTER, 2014; VERONESI et al., 2016). Brum *et al.* (2021) reported mortality rates greater than 50% in pups born by cesarean section under dystocia conditions in a veterinary hospital in Brazil.

ASSESSMENT OF PUPPIES DURING THE EARLY NEONATAL PERIOD

After birth, monitoring the vitality of pups can contribute to the selection of measures to reduce neonatal mortality. Therefore, the modified Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) test has been successfully adopted in veterinary medicine as an auxiliary protocol to identify neonates at immediate risk. Obtaining individual scores enables individualized support measures, focusing on the most vulnerable pups (VERONESI et al., 2009). The use of a score to assess neonatal vitality is noninvasive and allows rapid analysis of heart rate, respiratory rate, motility, irritability reflex and mucosal color. The scores provide an overview of the main vulnerabilities in the early neonatal period and can be used for all dog breeds or adapted for specific breeds or pups delivered via cesarean section. Neonates with scores between 0 and 3 are considered critical, those with scores between 4 and 6 are in moderate distress, and scores of 7 to 10 indicate normal neonates. The test identifies vulnerabilities in neonates, prompting immediate attention to the pups at greatest risk (scores of 0-6), thereby reducing early neonatal mortality (VERONESI et al., 2009; VERONESI, 2016). In a study carried out with the Chihuahua breed, Fusi *et al.* (2020) analyzed the scores obtained for 176 puppies born through elective cesarean section. The authors confirmed the utility of the APGAR test to predict the viability of neonates in the first 24 h after delivery. A study conducted by Vassalo *et al.* (2015) observed higher APGAR scores immediately after delivery in pups of different breeds born vaginally (49) than pups born by cesarean section (55), which were prostrated. This effect reflected fetal distress related to dystocia and anesthetic effects. After 60 min, the scores were similar between vaginally and cesarian section-delivered pups. Therefore, the study verified the effectiveness of the interventions used for pup recovery, including clearing the upper airways, drying, cardiopulmonary resuscitation, oxygenation, fluid therapy, and intravenous medication when required. In addition to analyzing the APGAR scores, the authors also performed a clinical evaluation, measuring rectal temperature and reflexes in the early neonatal period. Both APGAR and reflex scores were higher in pups delivered vaginally. The authors consider both important parameters for identifying at-risk pups and evaluating the effectiveness of resuscitation actions. Nonetheless, APGAR and reflex scores were not considered useful as prognostic parameters. Intensive care for the neonate includes cardiorespiratory support, recovery from normothermia, care with passive immunity and blood glucose, which contribute to healthy neonatal vital functions, increasing the chances of survival by reducing the factors triggering neonatal diseases (MUNICH and KUCHENMEISTER, 2014; VANNUCCI and ABREU, 2017).

Mila et al. (2017) recommend the assessment of pup vitality through the APGAR test combined with analysis of plasma glucose levels during the first 24 h to monitor and identify at-risk pups. In addition to conducting the APGAR test, morphometric assessments of neonates can help veterinarians and breeders identify vulnerable pups. Low birth weight neonates are at increased risk of mortality and morbidity. The authors reported that birth weight is inversely related to litter size and observed differences between breeds. (GROPETTI, 2017). However, the complex interactions between factors related to the fetus, the dam, and the environment should be further investigated in canines.

The identification of issues during delivery and in the neonatal period allows the quick establishment of corrective measures. Analysis of umbilical cord lactate levels can provide important information about respiratory stress (GROPETTI et al., 2010). In a study involving 68 healthy pups, McMichael *et al.* (2005) indicated that venous lactate levels in neonates up to 28 days are higher than those in adults. Additionally, in four-day-old pups, these values significantly exceeded those observed in 10- to 28-day-old pups. The lactate levels in umbilical vein blood samples showed less fetal distress in vaginally delivered pups born than in those delivered through nonelective cesarean section (KUTTAN et al., 2016). Antoriczyk *et al.* (2021) evaluated umbilical cord blood samples and APGAR scores from pups delivered by elective cesarean sections and reported mild respiratory acidosis. Puppies with low APGAR scores had high blood glucose and higher mortality than those with high scores. Considering this, bradycardia in the first 4 days of life should be considered a sign of hypoxemia (GRUNDY, 2006).

Low adipose tissue reserves at birth imply difficulties in meeting metabolic demands without nutritional inputs. Healthy canine neonates have a lower ability to generate glucose, mainly due to low glycogen stores and system immaturity. During the first 24 h of life, glycogen is stored and severely depleted, with systemic glucose production predominantly via glycogenolysis, followed by a combination of glycogenolysis and gluconeogenesis after 24 h. The rapid depletion of glycogen stores highlights the importance of regular eating. Changes in reserve carbohydrate metabolism, endotoxemia, septicemia, and the portosystemic shunt may also contribute to hypoglycemia. Signs such as lethargy, weakness, vocalization, or convulsions should be immediately considered, especially in small-breed neonates, which are more susceptible to hypoglycemia. (DAVIDSON, 2003; GRUNDY, 2006; MUNNICH and KUCHENMEISTER 2014). Mila *et al.* (2017) emphasized that the ability to transition to extrauterine conditions and colostrum ingestion is fundamental for pup survival, while Munnich and Kuchenmeister (2014) highlighted the importance of physical examination to identify early pathological signs.

Neonates are predisposed to dehydration due to the high surface/volume ratio, which occurs because the skin is more permeable and due to renal function immaturity. The disparity between water losses and intake in neonates can be accentuated by extrinsic factors such as maternal behavior, agalactia and high environmental temperature. Prematurity and pathological processes such as pneumonia and diarrhea that magnify this discrepancy result in hypovolemia, hemorrhages, shock, and neonatal losses (MUNNICH and KUCHENMEISTER, 2014).

The environment where neonates are kept after delivery until weaning is highly important since pups do not have a fully mature autonomic nervous system and are less able to respond to physiological stresses (GRUNDY, 2006). Davidson (2003) emphasized the importance of thermal comfort, highlighting that maturity in the thermoregulatory system of canines is only reached in four weeks. The environment should be at an appropriate temperature and free of irritant substances to prevent respiratory stress syndrome. This is one of the most worrisome noninfectious conditions in pups and is related to more than 60% of neonatal losses (MUNNICH, 2008). After birth, the dam and pups should remain under mild temperature conditions. Hypothermia is a postpartum protective physiological response affecting the normal physiology of several systems, negatively impacting immunity, digestion, and breastfeeding. Healthy pups had body temperatures between 35 and 36.5 °C 24 h after delivery. However, in the first six days after birth, neonates require thermal comfort interventions to maintain body temperature regardless of whether they are kept with the mother, and exposure to low temperatures should be avoided. Adequate maintenance of metabolic homeostasis requires a stable body temperature, and very high ambient temperatures reduce respiratory capacity and promote dehydration. Furthermore, low ambient temperatures hinder suckling, leading to dehydration, cardiorespiratory failure, alteration in intestinal motility, and predisposing pups to infections. Body temperature should be raised gradually, and food should only be reestablished when normothermia is reached (DAVIDSON, 2003; LAWER, 2008; MUNNICH and KUCHENMEISTER, 2014).

MONITORING THE GROWTH OF PUPS DURING THE NEONATAL PERIOD

After adaptation to extra uterine life, pups face new challenges demanding attention and care. Often, neonatal losses after the first 24 h of life occur without an accurate diagnosis. Canine wasting syndrome is a colloquial term used to describe apparently healthy pups that perish after failing to maintain growth and development in the first two weeks of life. The causes of this syndrome can be environmental, genetic, or infectious, although descriptions included a grouped set of pathological signs of different origins. Alternatively, they can be classified into maternal, neonatal and management causes. In these circumstances, clinical signs are nonspecific but generally related to low birth weight, low weight gain, and reduced suckling reflex leading to the segregation of the pup by the dam (KAHN et al., 2009). Both the assessment of birth weight and daily weight gain from regular weighing can reveal the pups at severe risk (INDREBO et al., 2007), contributing to the identification and correction of primary causes. Hedberg (2015) highlighted the main causes of insufficient weight gain or weight loss during the early neonatal period, including dehydration, hypoglycemia, inadequate management, environmental conditions interfering with the thermal balance of the pups, lack of milk, or maternal neglect. Weight loss in the early neonatal period due to physiological causes can reach 10%. Monitoring the daily variations in pup weight can contribute to the identification of risk situations. However, specific curves should be prepared to meet the peculiarities of the growth and development of each breed (BIGLIARDI et al., 2013).

Pup weight at birth, weight at 12 and 24 h after delivery, weight before and after feeding, daily weight gain, average weight and average litter weight gain are easily obtained, can be performed alone or with the breeder, and contribute to the daily monitoring of newborns. Recording data and providing the developmental history of the pup and litter expand the information on colostrum intake, maternal ability, adverse conditions, pup nutrition, and presence of pathologies (DODAMAI *et al*., 2017). The risks of reducing pup survival with very low birth weight are recognized by both scientists and breeders, leading to the adoption of preventive and corrective measures, although interventions vary between kennels. Mugnier *et al.* (2020) identified the most common practices to manage low-birth-weight canine and feline pups by reviewing interventions by French breeders to reduce neonatal losses. Mila *et al.* (2015) highlighted the impact of birth weight and neonatal development on early neonatal mortality (first two days) and on that observed between the second and third weeks after delivery.

FINAL CONSIDERATIONS

Understanding the fragility of the neonate and the complexity of the measures adopted to manage the kennel, the progenitors, and the neonate are essential to reduce and control risks in the first days of life. These constitute an essential step to reduce neonatal mortality of purebred dogs. Sanitary, nutritional, and reproductive planning and management in kennel can reduce pup mortality. Additionally, prenatal care, birth supervision, and neonatal care through biochemical analyses, vitality tests, and pup growth monitoring reduce losses by increasing reproductive success with important economic and animal welfare impacts.

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3.2 Artigo 2

Growth of Australian Cattle Dog puppies in the neonatal period and its relation to birth weight, parent weight and little size

Maria Eduarda Bicca Dode; Luciana Bicca Dode; Carine Dahl Corcini

Submetido à revista Research, Society and Development

Growth of Australian Cattle Dog puppies in the neonatal period and its relation to birth weight, parent weight and little size

Crescimento de filhotes de Australian Cattle Dog no período neonatal e sua relação com o peso ao nascer, peso dos pais e pequeno tamanho

Crecimiento de cachorros de pastor ganadero australiano en el período neonatal y su relación con el peso al nacer, el peso de los padres y tamaño de la camada

Resumo

Monitorar crescimento e desenvolvimento dos filhotes durante o período neontal contribui para adoção de medidas preventivas e curativas. O objetivo deste estudo foi relacionar o peso ao nascer, número médio de filhotes da ninhada e ganho de peso nos primeiros 45 dias de vida com peso dos progenitores. Estudo prospectivo, longitudinal incluiu 17 ninhadas de cães da raça Australian Cattle Dog, Medidas morfométricas dos progenitores foram coletadas. Peso diário foi obtido até os 45 dias e as ninhadas agrupadas conforme o número de filhotes e aplicados os testes de Shapiro Wilk, teste de Hartley e à independência dos resíduos através da análise gráfica e Analise de variância através do teste F ($p \leq 0{,}05$). Os efeitos das ninhadas foram comparados através do teste de Tuckey ($p \leq 0{,}05$). o peso médio dos filhotes em relação aos dias de pesagem foi ajustado através do modelo de regressão ($p \leq 0{,}05$): $y=y0ax+bx2$ e calculada a Correlação de Pearson entre as variáveis ganho de peso médio diário (GPMD), número de filhotes (NF), peso ao nascer (PN), peso da mãe (PM), peso do pai (PP). O número médio de filhotes por ninhada foi 5,88 +- 1,93 e peso médio ao nascer foi 258,98+-47, 19g. O ganho de peso médio diário nos diferentes grupos esteve mais fortemente relacionado ao peso médio ao nascer e moderadamente ao peso da mãe e fracamente relacionado ao peso do pai.

Palavras-chave: Cinofilia; kennel; Filhote; Curva de crescimento; Criação de preservação.

Abstract

Monitoring the growth and development of puppies during the neonatal period contributes to the adoption of preventive and curative measures. The aim of this study was to relate birth weight, mean number of pups in the litter and weight gain in the first 45 days of life with the weight of the parents. Prospective, longitudinal study included 17 litters of Australian Cattle Dogs. Morphometric measurements of the parents were collected. Daily weight was obtained up to 45 days and the litters were grouped according to the number of pups and applied the Shapiro Wilk test, Hartley test and the independence of residues through graphic analysis and analysis of variance through the F test ($p \leq 0, 05$). Litter effects were compared using the Tuckey test ($p \leq 0.05$). the mean weight of the pups in relation to the days of weighing was adjusted through the regression model ($p \leq 0.05$): $y=y0ax+bx2$ and Pearson's Correlation was calculated between the variables mean daily weight gain (GPMD), number of pups (NF), birth weight (PN), mother's weight (PM), father's weight (PP). The mean number of pups per litter was 5.88 +- 1.93 and the mean birth weight was 258.98+-47.19g. The mean daily weight gain in the different groups was more strongly related to the mean birth weight and moderately to the mother's weight and weakly related to the father's weight.

Keywords: Canine; kennel; Puppy; Growth curve; Preservation breeding.

RESUMEN

El seguimiento del crecimiento y desarrollo de las crías durante el período neonatal contribuye a la adopción de medidas preventivas y curativas. El objetivo de este estudio fue relacionar el peso al nacer, el número medio de crías por camada y la ganancia de peso en los primeros 45 días de vida con el peso de los padres. Estudio longitudinal prospectivo en el que se incluyeron 17 camadas de perros boyeros australianos, se recogieron medidas morfométricas de los progenitores. Se obtuvo peso diario hasta los 45 días y las camadas se agruparon de acuerdo al número de crías y se aplicó la prueba de Shapiro Wilk, prueba de Hartley y la independencia de residuos mediante análisis gráfico y análisis de varianza mediante la prueba F (p≤ 0,05). Los efectos de la camada se compararon mediante la prueba de Tuckey (p≤ 0.05). el peso medio de las crías en relación a los días de pesaje se ajustó mediante el modelo de regresión (p≤ 0.05): y=y0ax+bx2 y se calculó la Correlación de Pearson entre las variables ganancia media diaria de peso (GPMD), número de crías (NF), peso al nacer (PN), peso de la madre (PM), peso del padre (PP). El número medio de crías por camada fue de 5,88 ± 1,93 y el peso medio al nacer de 258,98 ± 47,19 g La ganancia media diaria de peso en los diferentes grupos estuvo más fuertemente relacionada con el peso medio al nacer y moderadamente con el peso de la madre y débilmente relacionado con el peso del padre.

Palabras clave: Canino, Criadero, Cachorro, Curva de crecimiento, Crianza de preservación.

1. Introduction

The Australian Cattle Dog (ACD) is a Cattle Dog classified in Group 1 section 2 (Serpell and Duffy, 2014; Dias, 2019; FCI, 2021). Selected based on the need for a resistant dog to drive cattle in adverse conditions, the breed has fixed characteristics of medium size, robust and agile (FCI, 2012; AKC, 2020; Ciccarelli et al., 2021) having the size defined in the standard of 43 cm. to 51 cm measured at the withers. Smooth, double-coated dog with a short, dense undercoat (FCI, 2012).

Obtaining specific growth rates for each breed throughout life is particularly relevant in the early stages, including the neonatal period. Characteristics such as birth weight, average number of pups per litter and average and daily weight gain patterns, contribute to a personalized zootechnical management and to reduce the high losses observed in the species, helping owners, breeders, management routines and veterinarians in clinical practice (Lawler, 2008; Mila et al., 2015; Mila et al., 2017).

Specific information on ACD is scarce and it is known that dogs with different morphological characteristics have developmental patterns influenced not only by size in adulthood, but also by temperament and coat type, resulting in different growth curves (Peterson, 2011). Analysis of the growth and development of pups in the neonatal period will contribute to the reproductive success and conservation of the breed.

Growth curves available in the canine species are related to breed, age, sex and food, and it is important to know the expected patterns not only in the neonatal period, but also in the other phases of the animal's life. Such conservation are able to provide relevant studies both for nutritional studies and development in genetic improvement and pure breeds (Ardelean, 1999; Hoskins, 1999; Trangerud et al., 2006). The aim of this study was to relate weight to weight, mean number of puppies in the litter and weight gain of Australian Cattle Dog puppies in the first 45 days of life with parent weight.

2. Material and Methods

The study was conducted in a prospective, longitudinal manner, including 17 litters of Australian Cattle Dogs born in 2020, in a kennel located in the city of Pelotas, registered in the CBKC/FCI system. The parents were weighed using a digital scale and measured by measuring the height at the withers (cm) using a rigid meter. Such data were obtained prior to coverage. The animals received uniform handling, super premium feed and periodic control of endo and ectoparasites 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 delivery was monitored and the pups were weighed at birth using a portable digital scale with a precision of 3 houses. The vitality of the pups was analyzed using the modified APGAR test (Vassalo et al., 2015; Veronesi, 2016) at 1 and 60 minutes after birth. Litters whose delivery was normal, without interventions and whose pups presented APGAR scores between 7 and 10 were included in the study. The weighings were repeated after 12 and 24 hours of birth using a precision digital scale. A daily monitoring routine was established, keeping the weighing time uniform until the 45th day of life.

In order to analyze the data obtained, the litters were grouped according to the number of pups and the results obtained were tested for normality using the Shapiro Wilk test; to homoscedasticity by the Hartley test and to the independence of the residuals by means of graphical analysis. Then, they were submitted to analysis of variance using the F test (p≤ 0.05). With statistical significance, the effects of litters were compared using the Tuckey test (p≤ 0.05).

To establish the growth behavior of each of the groups, the average weight of the pups in relation to the days of weighing were adjusted through the regression model ($p \leq 0.05$):y=y0ax+bx2, where y=response variable; yo= corresponding response variable at the minimum or maximum point of the curve; a= maximum estimated value of the response variable; b=slope of the curve; x=day after birth.

Pearson's correlation was performed between the variables mean daily weight gain (GPMD), number of pups (NF), birth weight (PN), mother's weight (PM), father's weight (PP).

3. Results and Discussion

All parents showed measurements within the stipulated breed standard (FCI). Dams aged between 18 and 70 months, of which eight primiparous, seven mothers of second birth and two of third birth were evaluated. The average weight of mothers at the time of mating was 13.5+-1.4kg (ranging from 10.4 to 16.7kg) and the average weight of fathers was 16.05+-1.67kg, (ranging from 13.1 to 18.5 kg).

In all, 17 litters born in 2020 were followed, totaling 100 puppies, 54 males and 46 females. In our study a 1.17:1 male/female ratio was observed. The ratio of 1:1.25. (male/female) showed a higher proportion of females in the local Nigerian population studied by Ajala et al. (2012), and Gravrilovik et al. (2008) observed values close to 50% of males in the Drever breed. Tedor and Reif (1978) suggested that the ratio between males and females may have a genetic origin, being influenced by breed.

The mean number of pups per litter was 5.88 +- 1.93, with litters ranging from 2 to 10 pups. Gavrilovic et al. (2008) point out the influence of the number of litters and the mother's age on litter size. The mean weight of the pups at birth was 258.98+-47.19g). In his study, Alves (2020) when evaluating different pure breeds, obtained results in pure breeds of Group I (FCI) in the Shetland Shepherd breed with 4 puppies and weight between 200 and 280g; Belgian Shepherd Laekenois with litters between 6 and 8 puppies and average weight ranging from 240 to 410 g and the Old English sheep dog breed with litters ranging from 2 to 10 puppies and weight between 265 and 500g. Mutembei et al. (2000) reported in their studies litters with an average number of 6.34+-0.4, ranging from 1 to 14 pups, while Chatdarong et al. (2007) observed litters with an average of 6.6+-2.8 pups with mean weight of 505.9+-77.3g also in the German Shepherd breed, emphasizing in their observations the effect of the breed on the number and mean weight of puppies. Although dogs can also be grouped by size, it is possible to observe significant differences in puppies’ birth weights (Mugnier et al, 2019)

The registration, identification and control of risk factors are essential for the full reproductive success of purebred dogs. Low birth weight is recognized as a risk factor in the neonatal period (Indrebo et al., 2007; Gropetti et al., 2017; Mugnier et al., 2021) with an inverse relationship between litter size and average pup weight in animals politocic (Schellin et al., 2019). Alves (2020) points out that even in the same litter, puppies with heterogenous weights can be observed. In our study, no significant differences were identified in relation to average birth weight in most groups established by the number of puppies in the litter, which is in agreement with what was observed by Gropetti et al. (2015) litter and birth weight, while Ajala et al. (2012) observed an inverse relationship between the number of pups and average birth weight in their studies (Table 1).

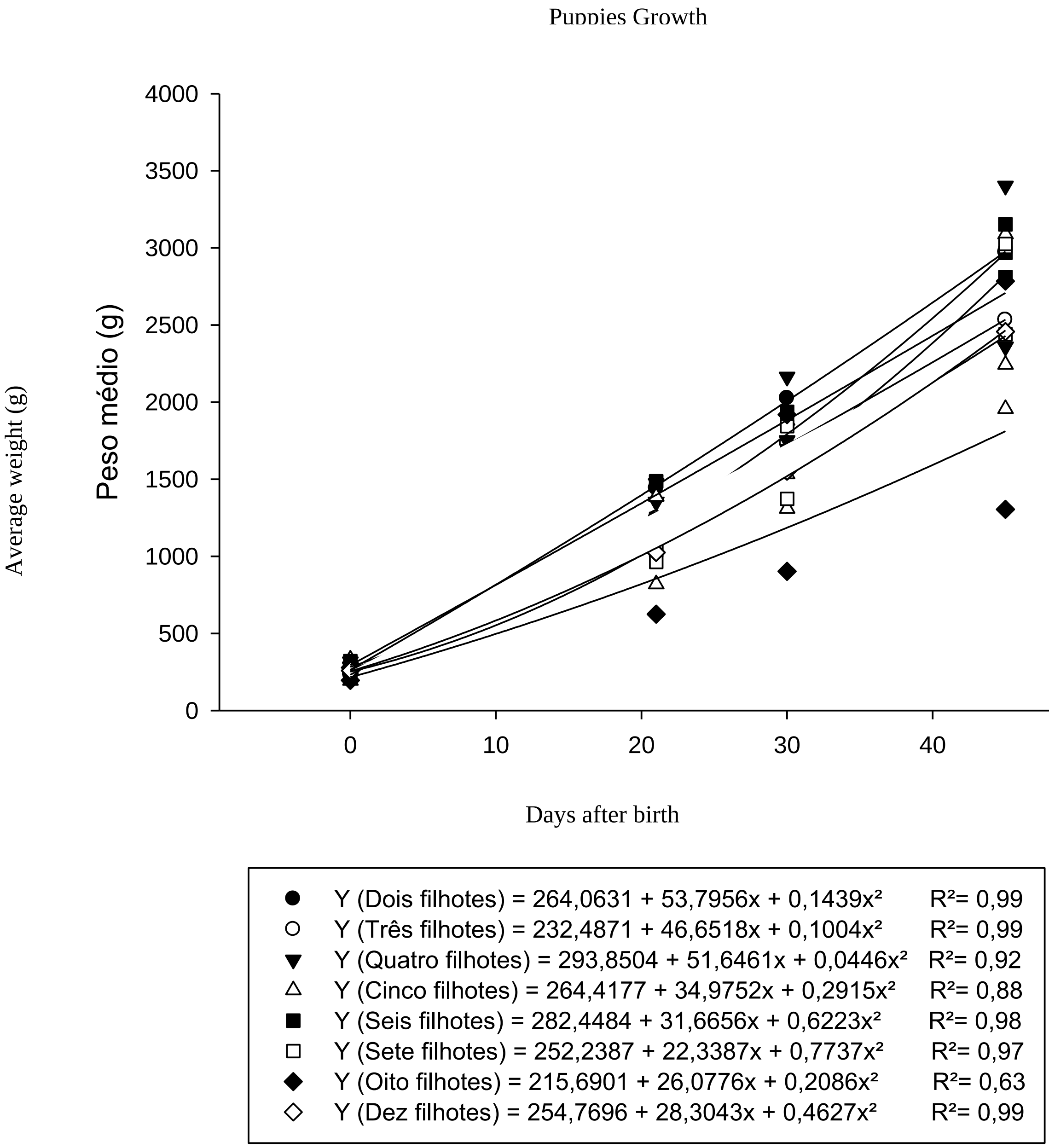
A pattern similar to that obtained with birth weight was also observed in the periods evaluated at 21, 30 and 45 days, where it is possible to observe that in the group of 8 pups, with lower mean birth weight, it remained lower (Table 1). Alves (2020), when evaluating 345 puppies from 60 litters of 19 breeds, observed that 45% of the followed puppies were able to gain weight from birth, while a third did not show gains or losses greater than 20% in the first 48 hours of life. In our study, 19% of the puppies showed some weight loss in the first 48 hours of life. Although the growth observed in the analyzed population has pointed to differences in relation to the mean values, the observed linear model explains the weight gain of the different groups (Figure 1).

Table 1- Average weight (g) of pups from the eight litter groups, on days (0, 21, 30 and 45 days after birth).

N Pups	N Litters	Days after birth			
		0	21	30	45
Two pups	1	ab 266,50±40,3	a 1.442,0±8,49	a 2.024,5±112,43	a 2.972,0±236,17
Three pups	1	b 234,00 ±8,19	ab 1.247,0±19,80	ab 1.753,6 ±22,63	ab 2.532,5 ±40,31
Four pups	1	a 294,63 ±13,57	a 1.393,2±90,83	ab 1.888,9±108,90	a 2.706,9 ±98,70
Five pups	4	ab 264,07±63,32	ab 1.129,6±289,09	ab 1.573,6±399,42	ab 2.429.2 ±714,14
Six pups	4	a 276,89±39,56	ab 1.256,6 ±257,39	ab 1.753,6±340,57	a 2.977,3 ±565,00
Seven pups	3	ab 252,76±33,88	ab 1.059,3 ±135,80	ab 1.622,4 ±253,99	a 2.823,4±351.02
Eigth pups	2	b 223,63±52,50	b 805,7 ±372,46	b 1.241,3±531,77	b 1,797,8 ±936,25
Ten pups	1	ab 259,33±45,13	ab 1.024,7 ±199,59	ab 1.552,3 ±325,43	ab 2.457,5±544,87
	CV (%)	12,78	14,88	15,88	17,18

Means followed by the same letter in each column did not differ, comparing the mean weight of litters on the days of weighing, by Tukey's test (p≤0.05).

Figure 1- Linear regression analysis of weight in g of different groups of Australian Cattle Dog puppies from birth to 45 days.



Source: Authors.

Although birth weight is a characteristic influenced by numerous factors (Gropetti, et al., 2017; Schellin et al., 2019), weight gain during the neonatal period can be considered an important parameter for identifying puppies at risk, contributing to adopt support measures and reduce neonatal mortality (Kham et al., 2009; Gropetti et al., 2015; Mugnier et al., 2019).

The average daily weight gain of the pups in the eight groups analyzed during the first 45 days of life ranged from 60.12 to 40.16g. Puppies with lower birth weight and lower weight gain during the period did not achieve the same performance as those whose growth was maximal (Table 2) (Figure 1).

The demand for energy and maternal skill in litters with a greater number of pups are higher than those with a smaller number of neonates, favoring more patient and experienced mothers in the management of larger litters.

Table 2- Average daily weight gain (g) of the eight litter groups of Australian Cattle Dog puppies during the first 45 days of life.

Litter	Daily weight gain (g)
Two pups	a 60,12±2,08
Three pups	ab 51,08 ±1,40
Four pups	cd 46,12 ±2,12
Five pups	bc 53,23 ±7,12
Six pups	ab 57,13 ±5,63
Seven pups	ab 57,12 ±3,58
Eigth pups	d 40,16 ±8,85
Ten pups	bc 48,85±4,99
CV (%)	18,14

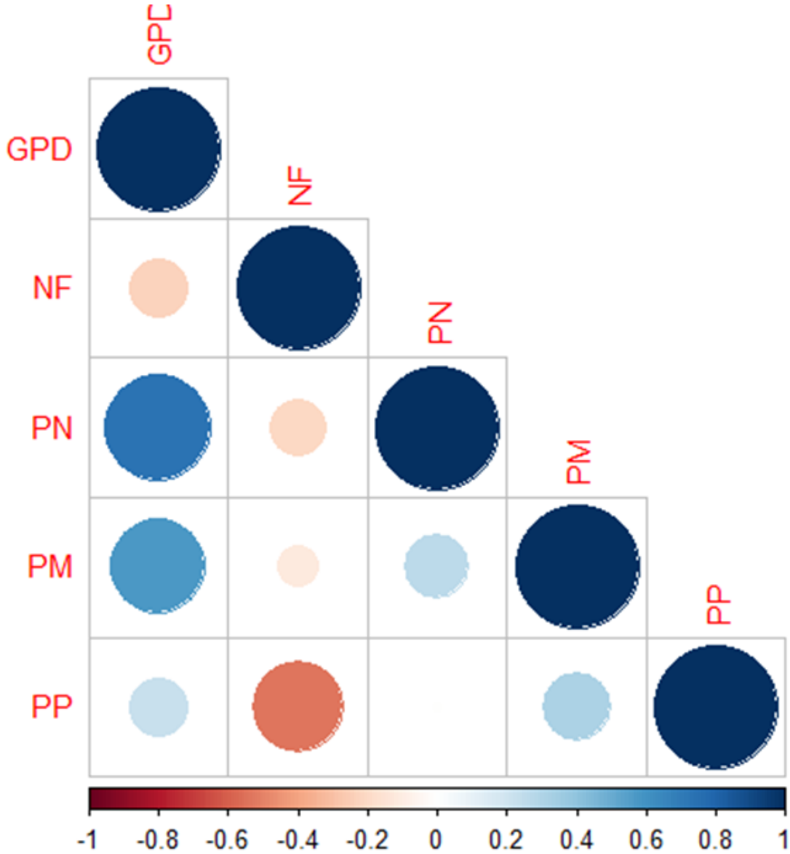
Means followed by the same letter in each column did not differ, comparing the mean weight of litters on the days of weighing, by Tukey's test ($p \leq 0.05$).

The group of 8 pups, with the lowest average birth weight, maintained a significantly lower weight throughout the evaluated period. A lower average daily weight gain was also observed (Table 2) (Figure 1) corroborating information from Alves (2020) who infers that pups keep their growth within limits, and thus, changes in this pattern are not recovered in the first 21 days of life, a fact that was also observed during the period of our study that followed the groups up to 45 days of life. Litters with larger numbers of pups require higher milk production in order to meet the demand of all offspring. Considerations about individual maternal behavior including during the breastfeeding period were also highlighted by Alves (2020) and Boutigny et al. (2016).

The average daily weight gain in the different groups was more strongly related to the average birth weight and moderately to the mother's weight and weakly related to the father's weight. (Figure 2). In their studies Gropetti et al. (2017) observed a relationship between the mother's weight and size and birth weight in puppies of different pure breeds (Figure 2).

Willham (1972) highlights that growth from birth to weaning is related to factors intrinsic to the litter and extrinsic in which the mother is included. Gowane et al. (2014) in their review on sheep highlight the importance of maternal care after delivery. Obtaining an adequate growth curve for the breed can contribute to breeding programs as well as adaptations of zootechnical and nutritional management of the litters, contributing to the preservation of pure breeds.

Figure 2- Pearson's correlation matrix between daily weight gain (GPD) and the variables number of pups in the litter (NF); birth weight (PN), father's weight (PP) and mother's weight (PM).



Source: Authors.

4. Conclusions

Growth followed by daily weight gain was related to mean birth weight, mother's weight and more weakly related to father's weight. Although the population effectively analyzed in the present study is still small when compared to large population studies developed by different authors, the importance of integral monitoring of the reproductive process of purebred dogs, since reproductive success is not limited to fertilization or the number of puppies born in a litter. Numerous steps must be taken until the puppy overcomes the main risk phases. Thus, the registration and extensive monitoring will provide veterinary and breeder auxiliary tools and achieve reproductive success in the breed.

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3.2 Artigo 3

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

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Submetido à revista Research, Society and Development

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

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 binaria 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±1,93, PN médio foi de 258,98±47,19g. 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≥0,7) foi validada na população estudada.

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

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±47.19g. 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≥0.7) was validated in the population studied.

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

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±1,93, el peso corporal medio fue de 258,98±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≥0.7) en la población estudiada.
Palabras clave: Cachorros; Curva de crecimiento; Camada; Canino; Cuidado del recién nacido.

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 (Munnich, 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 (Munnich and 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. Material and Methods

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, 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 et al., 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±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 (Ciccarelli et al., 2021).

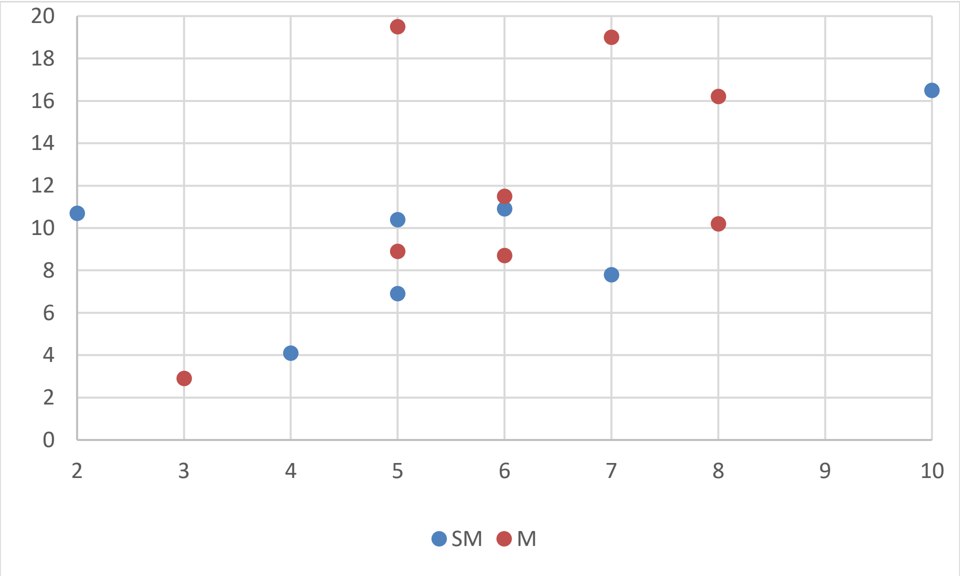
Table 1- Quartile values of groups classified by number of pups born, used to classify 100 pups from 17 litters for three parameters: birth weight, daily weight gain in the first 45 days and inbreeding coefficient.

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.

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, Wooton 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.

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 (Mila et al., 2015; Veronesi et al., 2020).

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 et al (1999) developed a growth modeling proposal. in the German Shepherd and Labrador Retriver 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, ≤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.

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 Moutain 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 and 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 et al., 2018) while in the Tatra Shepherd breed the inbreeding of the population ranged from 6.68 to 6.85%. (Sweklej et al., 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 (Leroy et al., 2015; MarellI et al., 2020; Sweklej et al., 2020; Sargolzaei et al., 2021).

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.

Tonessem 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 and 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-birth 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≥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.

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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-birth females.

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4 Considerações Finais

Ainda que a população efetivamente analisada no presente estudo ainda seja pequena quando comparada a estudos populacionais amplos desenvolvidos por diferentes autores, os dados analisados refletem uma população conduzida sob condições uniformes e controladas, trazendo informações específicas para a raça Australiam Cattle Dog de forma prospectiva e longitudinal. A relevância do acompanhamento integral do processo reprodutivo de cães de raça pura evidenciada, reforça que o sucesso na criação de conservação não se resume a fertilização ou ao número de filhotes nascidos em uma ninhada. Reflexos do sucesso reprodutivo serão observados na raça através de novas gerações de animais saudáveis dentro dos padrões almejados, capazes de assumir diferentes funções. A atividade de criação de preservação deve superar as barreiras impostas pela cegueira de criador passando a ser exercida de forma técnica e cientificamente embasadas com apoio de médicos veterinários capazes de assessorar a criação responsável.

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