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ORIGINAL ARTICLE

# The effect of doxapram on survival and APGAR score in newborn puppies delivered by elective caesarean: A randomized controlled trial

Timothy H. Hyndman[1,2] | Shelby Fretwell[1,3] | Ross S. Bowden[4] | Flaminia Coaicetto[1] | Peter C. Irons[1] | Joshua W. Aleri[1,2] | Nino Kordzakhia[5] | Stephen W. Page[6] | Gabrielle C. Musk[1] | S. Jonathan Tuke[7] | Martina Mosing[1] | Steven S. Metcalfe[3]

[1]School of Veterinary Medicine, Murdoch University, Murdoch, Western Australia, Australia

[2]Harry Butler Institute, Murdoch University, Murdoch, Western Australia, Australia

[3]Applecross Veterinary Hospital, Applecross, Western Australia, Australia

[4]Mathematics and Statistics, Murdoch University, Murdoch, Western Australia, Australia

[5]School of Mathematical and Physical Sciences, Macquarie University, Macquarie Park, New South Wales, Australia

[6]Advanced Veterinary Therapeutics, Newtown, New South Wales, Australia

[7]Mathematics and Statistics, The University of Adelaide, Adelaide, South Australia, Australia

**Correspondence**
Timothy H. Hyndman, School of Veterinary Medicine, Murdoch University, Murdoch, Western Australia, Australia.
Email: t.hyndman@murdoch.edu.au

## Abstract

Doxapram is marketed as a respiratory stimulant and is used by some veterinarians to help with neonatal apnoea, especially in puppies delivered by caesarean. There is a lack of consensus as to whether the drug is effective and data on its safety are limited. Doxapram was compared to placebo (saline) in newborn puppies in a randomized, double-blinded clinical trial using two outcome measures: 7-day mortality rate and repeated APGAR score measurements. Higher APGAR scores have been positively correlated with survival and other health outcomes in newborns. Puppies were delivered by caesarean and a baseline APGAR score was measured. This was immediately followed by a randomly allocated intralingual injection of either doxapram or isotonic saline (of the same volume). Injection volumes were determined by the weight of the puppy and each injection was administered within a minute of birth. The mean dose of doxapram administered was 10.65 mg/kg. APGAR scores were measured again at 2, 5, 10 and 20 min. One hundred and seventy-one puppies from 45 elective caesareans were recruited into this study. Five out of 85 puppies died after receiving saline and 7 out of 86 died after receiving doxapram. Adjusting for the baseline APGAR score, the age of the mother and whether the puppy was a brachycephalic breed, there was insufficient evidence to conclude a difference in the odds of 7-day survival for puppies that received doxapram compared to those that received saline ($p = .634$). Adjusting for the baseline APGAR score, the weight of the mother, the litter size, the mother's parity number, the weight of the puppy and whether the puppy was a brachycephalic breed, there was insufficient evidence to conclude a difference in the probability of a puppy having an APGAR score of ten (the maximum APGAR score) between those that received doxapram compared to those that received saline ($p = .631$). Being a brachycephalic breed was not associated with an increased odds of 7-day mortality ($p = .156$) but the effect of the baseline APGAR score on the probability of having an APGAR score of ten was higher for brachycephalic than non-brachycephalic breeds

($p = .01$). There was insufficient evidence that intralingual doxapram provided an advantage (or disadvantage) compared to intralingual saline when used routinely in puppies delivered by elective caesarean and that were not apnoeic.

**KEYWORDS**
bitch, CONSORT, dog, Dopram, GEE, time to event

## 1 | INTRODUCTION

Doxapram HCl (hereafter doxapram) is used in veterinary and human medicine as a respiratory stimulant for newborn animals and human infants that are not breathing (Papich, 2021; Vliegenthart et al., 2017). In veterinary medicine, doxapram is most commonly used in newborn puppies and can be administered through a number of routes. Venous access in newborn puppies is technically difficult and so most often doxapram is administered as a few drops placed below the tongue. Despite it being first synthesized in the early 1960s (Lunsford et al., 1964), and despite how commonly it is used, the mechanism of action, safety and efficacy of doxapram remains unclear. The mechanism of action appears to be dependent on species (Cunningham et al., 2020) but ventilation seems to be stimulated through excitation of peripheral chemoreceptors (Nishino et al., 1982), which is most likely mediated through inhibition of TASK potassium channels and increased calcium signalling in type-I carotid body cells (O'Donohoe et al., 2018). There are concerns over the safety of doxapram as it is known to cause increases in heart rate and blood pressure (Bamford et al., 1986). These changes have been associated with decreases in cerebral oxygen delivery in preterm human infants (Dani et al., 2006).

In one study assessing its safety and efficacy, 22 puppies from six caesareans were administered doxapram (Holladay, 1971). A total dose that was proportional to the size of the breed was diluted with distilled water to 0.5 mL. This was then administered into the umbilical vein. Two puppies were given a second dose, 15 or 20 min after the first, respectively. All 22 puppies survived to the time they were discharged from hospital. The study was not blinded, no control group was included and survival was the only measure of drug efficacy and safety. In a much larger study, data from 3908 newborn puppies from 807 caesareans were retrospectively analysed, with doxapram use associated with a decreased survival rate at 2 h after birth (Moon et al., 2000). However, it was not recorded whether doxapram was preferentially administered to puppies that were not doing well. Survival rates at other time points were not mentioned.

Doxapram has been investigated for use in human infants more intensively than it has in neonatal animals. Despite this focus on humans, there is no consensus view on its safety and efficacy. In 2004, a Cochrane review (Henderson-Smart & Steer, 2004) listed only one study (Peliowski & Finer, 1990) that met the inclusion criteria for study rigour. In this small study of 21 infants (11 assigned to intravenous doxapram and 10 to placebo), the results were equivocal although the study was likely underpowered due to its size. Subsequent studies and reviews on the use of doxapram in human infants, usually for apnoea of prematurity, have provided a variety of viewpoints including a recommendation that it appears to be an effective second-line drug (behind methylxanthines such as caffeine) with uncertain side effects (Natarajan et al., 2010); that it not be used routinely due to its side effects and unclear long term benefits (Zhao et al., 2011); that it is used cautiously until its safety profile is better understood (Prins et al., 2013); that it may be used effectively but placebo-controlled studies are needed to assess its safety and efficacy (Flint et al., 2017); that routine use cannot be recommended and that large multicentre randomized trials are urgently needed (Vliegenthart et al., 2017); and, most recently, that doxapram is known to be effective but may be associated with adverse side effects (Pergolizzi et al., 2022).

Some of the aforementioned studies have used mortality as a measure of doxapram toxicity while others have used surrogate measures such as blood flow through the brain. An alternative measure of overall neonatal health was conceived by the paediatrician Virginia Apgar (Apgar, 1953). The Apgar scoring system awards scores to various categories of health and then aggregates them to create an overall score. APGAR is now a backronym of Appearance, Pulse, Grimace, Activity and Respiration. An APGAR scoring system was adapted for use in newborn puppies and was validated by comparing it to 2-h survival rate, mammary gland seeking behaviour and suckling reflexes (Veronesi et al., 2009). This scoring system was subsequently found to be associated with the 1-day (Mila et al., 2017; Titkova et al., 2017) and 3-week (Mila et al., 2017) survival rates of neonatal puppies. An almost identical seven-category neonatal APGAR scoring system was developed by Groppetti et al. (2010) where puppies with lower APGAR scores were associated with higher umbilical vein lactate concentrations and emergency caesarean delivery, but not 2-day survival rate. Using a five-category APGAR scoring system, Silva et al. (2009) associated lower scores with puppies delivered by caesarean.

The present study describes a randomized, double-blinded, placebo-controlled clinical study in newborn puppies that assessed the efficacy and safety of doxapram using two outcome measures: 7-day mortality rate and time to reach a maximum APGAR score of ten. Reporting of this study follows the Consolidated Standards of Reporting Trials (CONSORT statement) (Schulz et al., 2010).

Primary (null) hypotheses were formulated for the data analyses:

1. For puppies delivered by elective caesarean, there is no difference in the 7-day survival rate between those puppies that received doxapram compared to those that received saline.

2. For puppies delivered by elective caesarean, there is no difference in the time to reach the maximum APGAR score of ten between puppies that received doxapram compared to those that received saline.

As brachycephalic breeds appear to be predisposed to higher rates of complications during whelping (the birthing process of dogs) (Moon et al., 2000), a secondary set of null hypotheses was also formulated:

1. For puppies delivered by elective caesarean, there is no difference in the 7-day survival rate between brachycephalic and non-brachycephalic breeds.
2. For puppies delivered by elective caesarean, there is no difference in the time to reach the maximum APGAR score of ten between brachycephalic and non-brachycephalic breeds.

## 2 | METHODS

### 2.1 | Study design

Newborn puppies delivered by elective caesarean were randomly allocated to receive, in parallel, either an intralingual injection of doxapram (Dopram®, 20 mg/mL, Elanco Australasia, Macquarie Park, Australia) or isotonic saline (0.9% Sodium Chloride Intravenous Infusion BP, 0.9 g/100 mL, Baxter) in a 1:1 allocation ratio (Figure 1). Three categories of puppy weights were created: <150 grams (g), 150–450 g and >450 g. The smallest puppies received 0.08 mL of either doxapram (20 mg/mL, equating to 1.6 mg) or saline; the medium-sized puppies received 0.16 mL of doxapram (3.2 mg) or saline; and the largest puppies received 0.24 mL of doxapram (4.8 mg) or saline. These doses were chosen so that on average, puppies would receive approximately 10 mg/kg, noting the on-label dose of Dopram® is 1–5 mg per puppy (APVMA PubCRIS Database, 2022), which for a 300 g puppy equates to a dose range of 3.3–16.7 mg/kg. The mean dose of doxapram administered was 10.65 mg/kg.

For the caesarean, anaesthesia was induced with ~2 mg/kg of intravenous alfaxalone (Alfaxan®, Jurox, Rutherford, Australia), followed by endotracheal intubation and maintained with isoflurane in oxygen. The vaporizer was out-of-circuit (VOC) and was calibrated every 2 years. Following intubation, the vaporizer was set to deliver 3% isoflurane in oxygen until surgical preparation was completed, after which, it was reduced to 1%–1.5%. Oxygen flow rates were 1 L/min/20 kg and were delivered by a circle circuit to the lungs of the bitch. No pre-anaesthetic medication was administered and the oxygen saturations were not measured for the bitches or puppies. For all caesareans, a midline incision was used to access the uterine horns. Puppies were extracted and handed to one of the investigators (SF) for APGAR measurements and intralingual injection of doxapram or saline through a 29-gauge needle (BD Ultra-Fine 0.5 mL insulin syringe, Becton Dickinson, Sydney, Australia). Injection was into the ventral (sublingual) aspect of the tongue approximately 0.5–1 cm from the distal tip and was administered within a minute of birth. Degree of placental separation for each puppy was not recorded as the focus was expedient delivery. The number of puppies that were randomly chosen from each litter to be included in this study was based on whether the investigator could confidently assess that number of puppies at all the sampling time points. Bitches received 0.02 mg/kg of intravenous buprenorphine (Temvet, Troy Laboratories, Glendenning, Australia) and 0.2 mg/kg of intravenous meloxicam (Meloxicam 20, Troy Laboratories, Glendenning, Australia) immediately after delivery of the last puppy (before suturing the uterus).

APGAR scores were measured using the scoring system described in Table 1 (Veronesi et al., 2009). The assessment of respiratory effort involved the respiratory rate and the intensity of the puppy's vocalizations. Where there was disagreement between these two measures, vocalization determined the value for respiratory effort. For reflex irritability, leg retraction took priority over vocalization.

Survival was measured for the 7 days following caesarean. This was assessed by hospital staff for approximately the first day and then by the puppies' owners once the bitches left hospital. Owners were informed at the time of patient discharge which puppies were included in the study.

There were two phases of this study (Figure 1). The first phase represented the accumulation of pilot data to inform the second phase of the study. Preliminary assessments of the pilot data using contingency tables to compare the survival rates and using graphs to visualize differences in APGAR scores between puppies that received doxapram to those that received saline, suggested that if doxapram had any effect (either beneficial or harmful) it was subtle. For example, one out of 35 puppies died from the placebo group compared to two out of 35 from the doxapram group. The onset of action for doxapram was expected to be rapid. In foetal lambs, breathing was stimulated within a few seconds of intra-arterial injection and lasted approximately

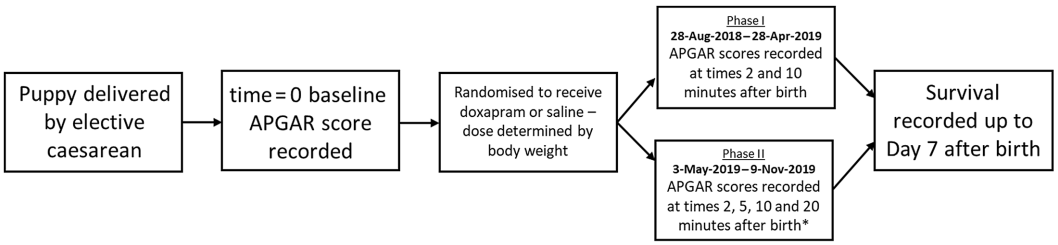


FIGURE 1 Flow diagram of the study. *Three puppies, from the same litter, had APGAR scores measured at times 5 and 20 min after birth.

TABLE 1 APGAR scoring system adapted to newborn puppies.

| Health category | Score = 0 | Score = 1 | Score = 2 |
|---|---|---|---|
| Heart rate (beats per minute) | <180 | 180–220 | >220 |
| Respiratory effort (breaths per minute) – crying is highest priority | <6 no crying | 6–15 mild crying | >15 clear crying |
| Reflex irritability – compression of paw tip | No leg retraction, no vocalization | Weak leg retraction, no/weak vocalization | Crying, quick leg retraction |
| Motility – spontaneous movement | Weak or absent movement | Some flexions, mild movement | Active motion, strong movement |
| Mucous membrane colour | Cyanotic | Pale | Pink |

5 min (Bamford et al., 1986) and the product insert for Dopram® states the onset of action is usually within seconds (APVMA PubCRIS Database, 2022). In case the ostensibly null effect of doxapram from the first phase of the study could be explained by assessments occurring at times when the doxapram had not yet reached its average peak effect, the second phase of the study included an extension of the final sampling time point from 10 to 20 min.

No changes were made to the study outcome measures between the two phases of the study.

## 2.2 | Study population

To be included in this study, a puppy must have been delivered by caesarean and a baseline (time = 0) APGAR score must have been recorded. Puppy that met the inclusion criteria were only excluded from the study if there was insufficient time to collect their APGAR scores at the sampling time points. Following collection of data, only data from elective caesareans were analysed as only four puppies were delivered by emergency caesarean.

All puppies were purebred, client-owned and had been presented to a local veterinary practice with a high dog caesarean caseload.

## 2.3 | Study randomization and blinding

### 2.3.1 | Randomization

One of the investigators (THH) used Microsoft Excel to create the allocation sequence for each weight category. A block size of two was used: for each weight category, the first, third, fifth, seventh (etc.) puppies were each randomly allocated to receive either doxapram or saline. The second, fourth, sixth, eighth (etc.) puppies in that weight category were to receive the opposite treatment to what the previous puppy received. For example, if the first, third and fifth puppies were randomly allocated to receive doxapram, saline and saline, respectively, then the second, fourth and sixth puppies would receive saline, doxapram and doxapram. Syringes containing either doxapram or saline were then labelled sequentially for each weight category according to the allocation sequence: S1, S2, S3 (etc.) for small-sized puppies (<150 g), M1, M2, M3 (etc.) for medium-sized puppies (150–450 g) and L1, L2, L3 (etc.) for large-sized puppies (>450 g) (Figure S1).

### 2.3.2 | Blinding

The same investigator (SF) administered the doxapram or saline to each puppy and was blinded to the treatment that each puppy received. This investigator recorded a baseline (time = 0) APGAR score, weighed the puppy and based on that weight, chose the next syringe that was prefilled with either doxapram or saline. Both doxapram and saline are clear and colourless solutions that cannot be easily distinguished by visual examination.

## 2.4 | Study outcome, sample size and statistical analysis

### 2.4.1 | Study outcome

There were two primary outcomes. The first outcome was puppy survival for the 7 days following caesarean and was recorded as a binary variable. Mortalities included puppies that died naturally and those that were euthanased. The time to reach the maximum APGAR score of ten was the second primary outcome. This measure was chosen due to the high proportion of puppies that reached an APGAR score of ten (143 out of 178 = 80.3%) and because the repeated measures of the APGAR scores collapsed into a single statistic for each puppy.

### 2.4.2 | Sample size

The recruitment of puppies into this study was conditional on the caseload of the participating veterinary practice and also the availability of the investigator who quantified all APGAR scores. It was decided to recruit as many puppies as possible while this investigator was available. Formal sample size calculations were not completed.

### 2.4.3 | Statistical analysis

Data were analysed according to intention to treat. That is, data were analysed according to treatment allocation, irrespective of what happened to each puppy following this allocation. The association between treatment allocation and the two study outcomes (survival and APGAR scores) was the focus of this study. Treatment allocation

was treated as a continuous variable of the dose of doxapram, that is, a dose of zero was used for puppies allocated to saline and the milligram per kilogram dose of doxapram was used for the puppies allocated to doxapram. For five puppies, birthweight was recorded only as >450 g (the exact weight was not recorded) and for these puppies, a birthweight of 450 g was used in all analyses.

Several other covariates were also analysed: brachycephaly, baseline APGAR score (prior to randomized allocation to a treatment group), age of bitch, pre-caesarean weight of bitch, parity number for bitch, the number of puppies in the litter and the birthweight of the puppy. All continuous covariates, except for parity, were centred around their mean. For parity, each value was centred around the median value. Centring was to facilitate the interpretation of interaction terms. Three interaction terms were included in the analyses: dose of doxapram × baseline APGAR score, dose of doxapram × brachycephaly and brachycephaly × baseline APGAR score. For all three interaction terms, it was considered to be biologically plausible that one of the covariates in the interaction term may have a relationship with the outcome variable that was dependent on the other covariate in the interaction term. For example, the effect of doxapram on survival may be different for brachycephalics compared to non-brachycephalics.

As some puppies were from the same litter, the caesarean (litter) that a puppy was from was used as a random effect. This was to account for any within-litter correlation (clustering) of the results.

*Outcome 1: 7-day mortality*

Generalized estimating equations (GEE) were used to compare the 7-day mortality rate in the population of puppies that received doxapram to those that received saline. GEE are an alternative method of fitting random-effects generalized linear models using a form of method-of-moments but which have less stringent modelling assumptions than the maximum likelihood approach (Gardiner et al., 2009). The trade-off is that the parameter estimates are not as precise as when the maximum likelihood assumptions are met. For the GEE model of 7-day mortality rate, the binomial distributional family was used with a logit link function. It was assumed that the correlation structure within a litter of puppies was exchangeable. That is, the correlation between the probability of a puppy dying and the probability of another puppy dying is the same for all puppies that belong to the same litter. Robust estimates of the standard errors of the regression coefficients were calculated. Models were compared using quasi-Akaike Information Criteria (qAIC) (Pan, 2001).

*Outcome 2: Time for APGAR score to reach its maximum value of ten*

Time-to-event analysis was used by considering the APGAR score reaching ten as the event. The outcome variable was therefore the length of time until this event occurred. Puppies that did not reach an APGAR score of ten were considered to be right-censored at their final sampling time point. As there was only a maximum of four post-randomization sampling time points (2, 5, 10 and 20 min), there was considerable interval-censoring resulting in a large number of puppies reaching the maximum APGAR score at the same time points. As a result of these many ties, a mixed-effects regression analysis using a complementary log–log (cloglog) link function was used to accommodate the discrete sampling time points (Rabe-Hesketh & Skrondal, 2022).

The alternative hypotheses to the null hypotheses were two-tailed. That is, where a null hypothesis referred to no difference, the alternative hypothesis was that there *was* a difference. This permitted the effect of doxapram to be positive or negative. All statistical analyses were performed using Stata, version 16.1 (StataCorp., 2019).

## 3 | RESULTS

### 3.1 | Study population

Between 28 August 2018 and 9 November 2019, 179 newborn puppies were recruited into this study (Figure S2; Table S1; Data S1). Four puppies were excluded from analysis as they were from emergency (not elective) caesareans, and the paper-based data recording sheets from four other puppies were lost before they could be stored electronically. The final sample size, from both phases of the study (Figure 1), was 171 puppies: 70 had APGAR scores recorded at times 0, 2 and 10 min after birth; three at times 0, 5 and 20 min; and the remaining 98 at times 0, 2, 5, 10 and 20 min.

Due to the use of a treatment allocation of block size two, the dataset was strongly balanced. For example, of the 83 brachycephalic puppies, 41 received doxapram and 42 received saline; and of the 88 non-brachycephalic puppies, 45 received doxapram and 43 received saline (Figure 2).

A summary of the study population according to breed, brachycephaly, treatment group and mortality outcome is provided in Figure 2. In total, there were 45 caesareans from 43 bitches (two bitches contributed two caesareans each to the dataset).

Summary statistics for the 45 caesareans are presented in Table 2. More than half of the puppies in each litter were recruited into the study. On average, in each litter, almost two puppies received doxapram and almost two received saline.

### 3.2 | Effect of doxapram on 7-day mortality

In the 7 days following caesarean, five out of 85 puppies died after receiving saline and seven out of 86 died after receiving doxapram and most (ten out of 12) of the deaths were in brachycephalics (Table 3).

Using GEE, there was insufficient evidence that the dose of doxapram was associated with the 7-day mortality rate ($p = .634$) (Table 4). The dose of doxapram for the puppies that received saline was 0 mg/kg and so there was insufficient evidence that the effect of doxapram on mortality was different to saline.

The odds of 7-day mortality for puppies born of older mothers was lower than for puppies born of younger mothers ($p = .039$).

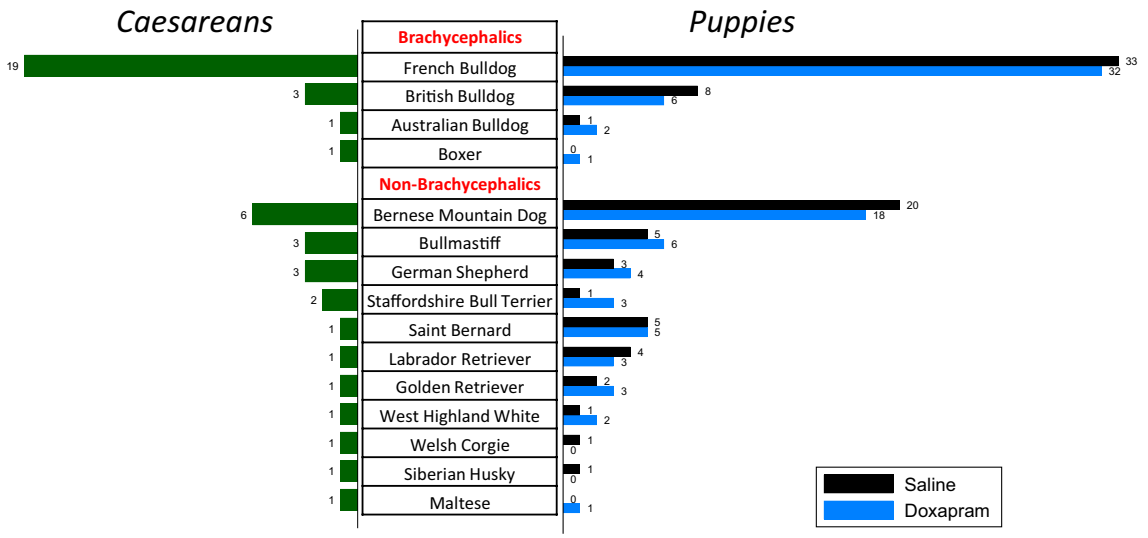


**FIGURE 2** Left: the number of caesareans sorted by brachycephaly and then breed. Right: the number of puppies sorted by brachycephaly and breed that received doxapram or saline.

**TABLE 2** Summary statistics for the 45 caesareans included in this study.

| | Mean | SD | Min. | Max. |
|---|---|---|---|---|
| Age of bitch (yrs) | 3.12 | 1.41 | 1.5 | 6.8 |
| Parity | 1.82 | 0.91 | 1.0 | 4.0 |
| # pups in litter | 5.78 | 2.78 | 1.0 | 12.0 |
| # pups assessed in litter | 3.80 | 2.36 | 1.0 | 12.0 |
| # pups in litter given saline | 1.89 | 1.30 | 0.0 | 6.0 |
| # pups in litter given doxapram | 1.91 | 1.26 | 0.0 | 6.0 |

Abbreviations: #, number of; max., maximum; min., minimum; pups, puppies; SD, standard deviation; yrs, years.

Specifically, the odds of a puppy dying, after adjustment for the other covariates in the model, decreased by 46% (95% CI: 3%, 70%) for each year that the mother was older.

Higher baseline APGAR scores were associated with lower odds of puppy mortality ($p = .026$). After adjustment for covariates, the odds of a puppy dying decreased by 51% (95% CI: 8%, 74%) for each single-unit increase in the baseline APGAR score.

### 3.2.1 | Causes of death

Of the seven puppies that died in the 7 days following doxapram dose, three were euthanased due to anasarca and one was euthanased due to the presence of a cleft palate. The remaining three puppies died naturally from apnoea (on day one, the same day as the caesarean), seizures (day two) and with marked amounts of yellow-tinged oral and nasal serous discharge (day four), respectively.

**TABLE 3** Contingency tables of the 7-day mortality rates for brachycephalic breeds (top), non-brachycephalic breeds (middle) and all puppies (bottom) by treatment group.

| | Saline | Doxapram | Total |
|---|---|---|---|
| *Brachycephalic puppies* | | | |
| Died | 4 | 6 | 10 |
| Survived | 38 | 35 | 73 |
| Total | 42 | 41 | 83 |
| *Non-brachycephalic puppies* | | | |
| Died | 1 | 1 | 2 |
| Survived | 42 | 44 | 86 |
| Total | 43 | 45 | 88 |
| *All puppies* | | | |
| Died | 5 | 7 | 12 |
| Survived | 80 | 79 | 159 |
| Total | 85 | 86 | 171 |

Of the five puppies that died in the 7 days following saline dose, three were euthanased due to anasarca and one was euthanased due to the presence of a cleft palate. The remaining puppy died naturally of suspected respiratory failure (day two).

## 3.3 | Effect of doxapram on APGAR scores

The distribution of the APGAR scores for each of the five sampling time points was highly asymmetrical with a large proportion of the APGAR scores at the maximum value of ten, especially at sampling time points 5, 10 and 20 min (Figure 3). Mean APGAR scores, along with 95% confidence intervals are presented in Figure 4 for all puppies and separately for brachycephalic and non-brachycephalic breeds.

**TABLE 4** Regression analysis results for the two primary outcomes: 7-day survival (represented here as mortality) and the time to reach the maximum APGAR score of ten. The 7-day mortality rate is represented as the odds ratio of a puppy dying (mortality), relative to not dying, within the 7 days following caesarean. For the time to reach the maximum APGAR score of ten, hazard ratios can be viewed as conditional risks and when above one, are analogous to faster times to reach the maximum APGAR score. Doxapram dose and brachycephaly were retained in the final models regardless of *p*-value. n.s. = not significant ($p > .05$) and so not included in the final model.

| | Mortality | | Time to reach APGAR = 10 | |
|---|---|---|---|---|
| **Covariate** | **Odds ratio (95% CI)** | ***p*-value** | **Hazard ratio (95% CI)** | ***p*-value** |
| Per mg/kg increase in doxapram dose | 1.02 (0.95, 1.10) | .634 | 1.01 (0.97, 1.04) | .631 |
| Being a brachycephalic | 3.20 (0.64, 15.94) | .156 | 0.70 (0.24, 2.04) | .516 |
| Per unit increase in baseline APGAR score | 0.49 (0.26, 0.92) | .026* | 1.79 (1.15, 2.78) | .009* |
| *Baseline APGAR score × brachycephaly* | | n.s. | 2.22 (1.21, 4.08) | .010* |
| Per year increase in bitch age | 0.54 (0.30, 0.97) | .039* | | n.s. |
| Per kg increase in bitch weight | | n.s. | 0.95 (0.91, 0.99) | .018* |
| Per unit increase in parity number | | n.s. | 1.66 (1.09, 2.53) | .018* |
| Per puppy increase in litter size | | n.s. | 1.31 (1.10, 1.57) | .003* |
| Per 100 g increase in puppy weight | | n.s. | 1.64 (1.08, 2.48) | .020* |

*Note*: Interaction term is italicized.

*$p \leq .05$.

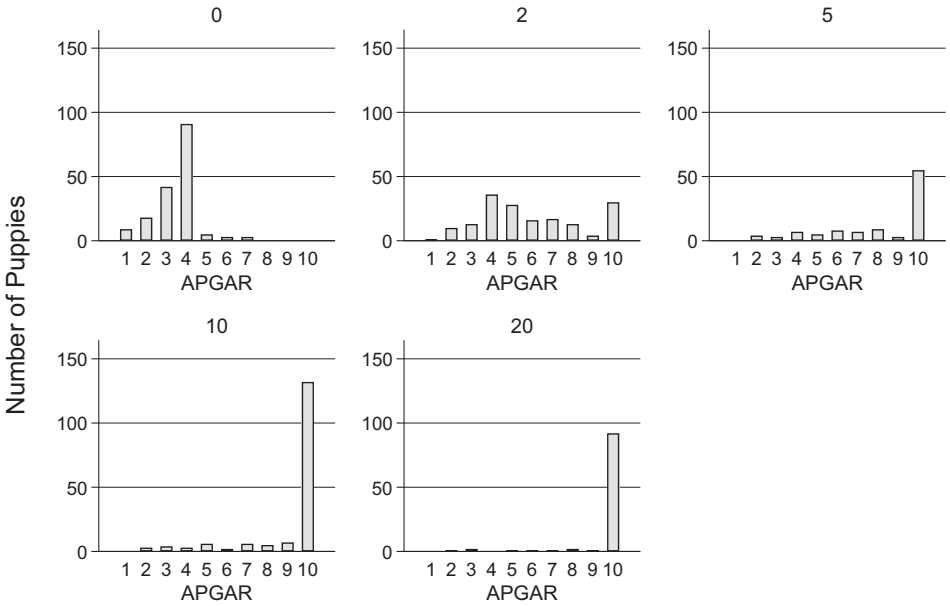


**FIGURE 3** The number of puppies at each APGAR score for the sampling time points of 0 (171 puppies), 2 (168), 5 (101), 10 (168) and 20 (101) minutes.

All but four of the 171 puppies had APGAR scores that monotonically increased. The majority of puppies reached the full APGAR score of ten during the sampling time (Table S2) and of these, the most common time to reach an APGAR score of ten was 10 min after birth (Table S3). For the 28 puppies that did not reach an APGAR score of ten, 14 were from the doxapram group and 14 were received placebo. There were no obvious trends between the APGAR score and the non-zero dose of doxapram at each sampling time point (Figure S3).

Using the final mixed-effect discrete time-to-event (cloglog) regression model, the hazard ratios in Table 4 can be viewed as conditional risks (hereafter referred to only as risks) of having an APGAR score of ten. If greater than one, these risks are analogous to faster times to reach the maximum APGAR score of ten at any time point. For example, after adjusting for covariates, the risk of having an APGAR score of ten was, on average, 5% lower (95% CI: 1% lower, 9% lower; $p = .018$) for puppies for each one-kilogramme increase in body weight of their mother. Using this model, there was insufficient evidence that the dose of doxapram was associated with the time taken for the APGAR score to reach its maximum value of ten ($p = .634$) (Table 4). Hence there is insufficient evidence that the administration of doxapram altered the time to reach the maximum APGAR score.

The risk of having an APGAR score of ten was significantly increased for: each additional litter their mother had had; for each

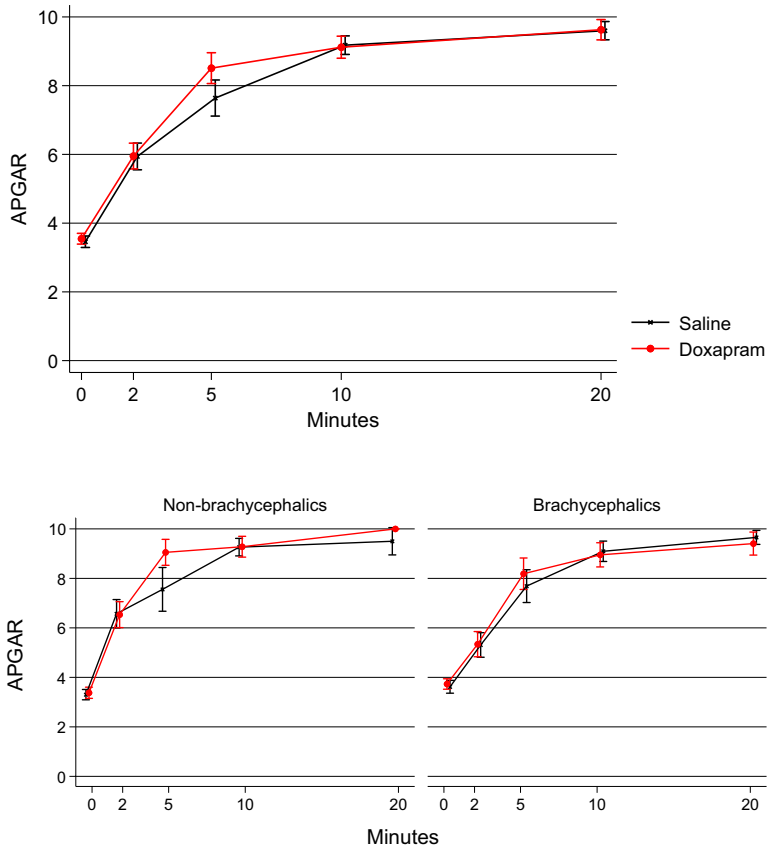


**FIGURE 4** Mean APGAR scores for all puppies (top) or for non-brachycephalic and brachycephalic breeds (bottom) at baseline (time = 0 min) and then at times 2, 5, 10 and 20 minutes following caesarean. The 95% confidence intervals (CIs) have been shortened by a factor of $\sqrt{2}$. This means that overlapping CIs suggest that the associated population means are *not* different at an alpha of 5%, and CIs that do not overlap *are* different at an alpha of 5% (Goldstein & Healy, 1995). Minor horizontal displacement was used to reduce the overlap of plots at each time point.

additional puppy in the litter they were from; and for every 100 grams of additional puppy body weight. To use simpler language, the maximum APGAR score of ten was, on average, reached more quickly in heavier puppies from larger litters from lighter bitches who had had more litters.

The effect of the baseline APGAR score was dependent upon whether the puppy was brachycephalic, that is, there was an interaction between the variables of baseline APGAR score and brachycephaly. To use simpler language, the maximum APGAR score of ten was, on average, reached more quickly when the baseline APGAR score was higher, and this effect was more pronounced in brachycephalics than non-brachycephalics.

## 4 | DISCUSSION

The primary hypotheses of this study related to the effect that doxapram had on two outcome measures: 7-day survival and the time to reach the maximum APGAR score of ten. The results of this study provided insufficient evidence that puppies given ~10 mg/kg of intralingual doxapram had a 7-day survival advantage compared to puppies that received a volume-matched intralingual injection of saline. And using an APGAR scoring system as a surrogate marker of neonatal puppy health, there was insufficient evidence that doxapram offered an advantage (or disadvantage) compared to saline.

There were a number of covariates that could have confounded the relationship between doxapram and the survival and APGAR outcome measures. The secondary hypothesis in this study related to the effect that one of these covariates, brachycephaly, had on the outcome measures. Brachycephalics have been shown to be at a higher risk of whelping complications (Moon et al., 2000), which is in part due to size of the puppy's head relative to the size of the bitch's pelvis (Runcan & da Silva, 2018). The anatomy of the skull also results in alterations in respiratory function resulting in a higher incidence of upper respiratory tract pathology and reduced life expectancy (Ekenstedt et al., 2020; O'Neill et al., 2015).

In the present study, being a brachycephalic did not significantly reduce the odds of 7-day survival relative to non-brachycephalics ($p = .156$). However, when analysing the risk of having an APGAR score of ten (a higher risk is associated with this favourable outcome being more likely), the baseline APGAR score better predicts whether a brachycephalic puppy will reach an APGAR score of ten than it does for non-brachycephalic puppies.

APGAR scores have been positively associated with markers of puppy health and survival (Mila et al., 2017; Veronesi et al., 2009), although the association was weaker in the study by Groppetti et al. (2010). The present study adds to the support for the use of APGAR scores as an indicator of neonatal puppy health as higher baseline APGAR scores were associated with lower probabilities of puppy mortality ($p = .026$). Furthermore, when measured longitudinally, puppy APGAR scores tend to increase in the first hour after delivery (Doebeli et al., 2013; Silva et al., 2009; Titkova et al., 2017). In the present study, all but four of the 171 puppies had APGAR scores that monotonically increased (i.e. did not

decrease). These results are consistent with the conclusion that repeated measures of the APGAR score are unnecessary for investigations that do not involve post-delivery interventions. That is, a baseline APGAR score provides a strong prediction of subsequent APGAR scores.

In the present study, each additional year of age for the bitch was associated with a lower puppy mortality rate ($p = .039$). Also, puppies from bitches with higher parity numbers ($p = .018$) and puppies from larger litter sizes ($p = .003$) had higher probabilities of obtaining the maximum APGAR score of ten. These two results may simply be a form of "survivor bias". For example, bitches that give birth to healthy litters, where a high number of puppies survive, are more likely to be bred from again.

Larger puppies were associated with a higher chance of reaching an APGAR score of ten ($p = .020$). The size of a puppy was defined by its weight and so larger puppies were more likely to be from larger breeds of dog and so it is possible that this result reflects a breed effect. Breed effect could not be analysed as there were too few puppies from most of the breeds: from the 45 caesareans included in this study, there were 15 breeds of dog, nine of which were represented by a single bitch each. The only categorization of breeds in this study was according to brachycephaly. Regardless, Groppetti et al. (2010) demonstrated that for medium-sized puppies (average weight = 396.2 g, standard deviation = 66.8 g), heavier puppies had higher APGAR scores. However, in that study, heavier medium-sized puppies had higher 2-day survival rates, whereas puppy weight was not associated with 7-day survival rates in the present study.

An unexpected result was that heavier bitches were more likely to have puppies with a *lower* chance of obtaining a maximum APGAR score of ten ($p = .018$). It is not known what might have caused this effect. Future studies should consider investigating the association between the body condition of the bitch and the puppy APGAR scores as bitch weight is dependent on both breed and body condition.

In Australia, doxapram is registered to be given sublingually, or into the umbilical vein, to neonatal apnoeic puppies (APVMA PubCRIS Database, 2022). Injection into the umbilical vein of the puppies was considered but it is technically difficult if the umbilical vein is relatively undamaged and is impossible if the umbilical vein is avulsed during caesarean delivery, and so this route of administration was not chosen. The decision was made to administer the drug intralingually, rather than sublingually, to improve the reliability of doxapram absorption. This approach was especially important considering this study was designed to identify a dose-dependent effect of doxapram. The administration was simple to perform and no adverse effects such as haematoma formation or swelling of the tongue were observed in any of the puppies during the 7-day observation time.

It has been shown that blood flow is maintained in the canine tongue even at low perfusion pressures (Koehler et al., 1983) and a number of studies have shown that intralingual drug administration compares favourably with intravenous injection. Intralingual flumazenil has been reported as being a viable alternative to intravenous administration in dogs (Unkel et al., 2006). Furthermore, in a study of 10 beagles, seizures induced by intravenous infusions of lidocaine were stopped by intravenous, intralingual and intramuscular (deltoid) administration of the same bolus dose of pentobarbitone in 27–39, 50–72 and 194–220 s respectively (Cutright & Nichols Jr, 1972). Naloxone has also been shown to be absorbed well from the tongue in dogs (Maio et al., 1984). In contrast, larger doses were required to normalize histamine-induced hypotension in dogs when adrenaline was given intralingually compared to intravenously (Halpern et al., 1978); however, localized pressor effects of the adrenaline may have reduced the systemic absorption of itself (Yagiela et al., 1982). In case the systemic absorption of doxapram was low in the present study, it was decided that a high average dose of doxapram would be given (~10 mg/kg).

## 4.1 | Randomization

A large number of covariates needed to be considered in this study (e.g. litter size, age of bitch, brachycephaly, puppy weight etc.). Naturally, many of these covariates were very similar for puppies born from the same litter. Given the number of covariates and the uncertainty around the number of puppies that would be recruited into this project, the block size for randomization was two. Having such a small block size ensured that if the trial was to stop prematurely, then the covariate values would be split reasonably evenly between the doxapram and saline allocation groups. This appeared to work well as the division of covariates between the two experimental groups was highly balanced.

## 4.2 | Limitations

An important limitation of this study is that the saline (placebo)-control group of puppies received an intralingual injection. Our study does not provide any data as to whether an intralingual injection of saline is different to no intervention. If injecting the tongue of a neonatal puppy improves survival and health measure outcomes, then differences between the doxapram and saline intralingual injections would be reduced due to the underlying effect of the injection itself.

Although painful stimuli can cause respiratory stimulation (Jafari et al., 2017), there are very few precedents in the literature for the effect that injections have on respiratory stimulation. In a study on doxapram in southern elephant seals (*Mirounga leonina*), a species known to breath-hold, two out of four individuals spontaneously ventilated after an intralingual injection (Woods et al., 1996). For one of these animals, spontaneous ventilation occurred when the needle was inserted into its tongue, but before doxapram was administered, and for the other individual, it was after saline was injected. The other two southern elephant seals did not accelerate their respiratory rate when doxapram was injected into their tongues.

The present study was blinded and it was the same investigator that administered the doxapram or saline and measured the APGAR

scores. This study design required the saline to be administered to the puppies in the same way as the doxapram and so there was no opportunity to include a no-intervention group as this would have partially unmasked the blinding. Future studies should consider separating the roles of treatment allocation and APGAR measurements so that treatments do not need to be indistinguishable.

### 4.3 | Generalizability

This study was analysed according to the "intention to treat". This means that puppies were included in the analyses even if there was a cause of death that was clearly unrelated to the treatment allocation, for example, euthanasia of a puppy with a cleft palate. To exclude such a puppy would then set the precedent to exclude all puppies where the cause of death could be confidently attributed to some other event besides the treatment. Making these decisions about which animals to include and exclude from the dataset can introduce a bias.

Whether the results of this study are generalizable to the global population of neonatal puppies should be carefully considered. The present study used a caesarean caseload from a single practice with extensive experience and expertise in canine reproductive medicine and surgery, and has a client base of experienced dog breeders. Furthermore, puppies were from elective caesareans, not emergency caesareans. Finally, this study did not focus exclusively on puppies that were apnoeic or in respiratory distress, which would have aligned more closely with the on-label indications for Dopram® (APVMA PubCRIS Database, 2022).

These factors may explain the relatively high 7-day survival rate seen in the present study (93%, $n=171$). However, survival rates this high are not unprecedented. An observational study of 663 Scandinavian large-breed live-born puppies, most of which were spontaneously delivered, reported a 3-week survival rate of 93% (Indrebø et al., 2007); in the study by Veronesi et al. (2009), mortality rates were similar between spontaneous, assisted and caesarean deliveries and the overall 24-h survival rate for 166 puppies was 95%; and a 94% 24-h survival rate for 32 live-born puppies delivered by caesarean was reported by Titkova et al. (2017). In a larger study ($n=3410$), the 7-day "all-alive" survival rate of puppies in a litter was 75% (Moon et al., 2000). In the present study, the 12 (out of 171) puppies that died were from eight (out of 45) caesareans, but given survival was only recorded for those puppies in each litter that were part of this study, the 7-day "all-alive" survival rate for puppies was, *at most*, 82% (=37/45). Furthermore, the study by Moon et al. (2000), using data from 109 veterinary practices, included puppies delivered by emergency caesarean (58%) and those where the anaesthetic regimen sometimes involved methoxyflurane or xylazine, which were significantly associated with poorer survival outcomes. The data from the Moon study (2000) therefore represented a diverse variety of clinical standards and in that study, doxapram was associated with poorer 2-h survival rates. However, analyses did not adjust for any potentially confounding variables and it was not recorded whether doxapram was selectively applied to puppies that were not doing well. These underlying neonatal puppy mortality and morbidity rates are relevant because studies are more likely to detect differences between doxapram and saline if greater improvements in these outcomes can be achieved.

### 4.4 | Statistical analyses of the repeated APGAR scores

Perhaps the most impactful way to assess the efficacy and safety of doxapram is by the survival outcome but this lacks precision as survival is a binary measurement. The APGAR scoring system provides a method of assessing newborn "health" which is not as direct as a mortality rate but provides a more sensitive measure.

The statistical methods used in this study to analyse the repeated APGAR scores were necessarily complex. The correlation of the repeated measures for puppies born of the same litter needed to be included in the analytical methodology. The APGAR scores presented an additional challenge as 86% of puppies in this study (=148/171) reached the maximum score of ten. In a study by Mila et al. (2017), 51% of puppies (=177/346) reached the maximum score of ten but 75% reached an APGAR score of at least nine. In a smaller study of 48 neonatal puppies, Silva et al. (2009) showed that all puppies reached an APGAR score of at least eight within their first hour of life. These skewed distributions meant that assuming a normal (Gaussian) distribution of APGAR scores was inappropriate.

The decision was made to utilize a time-to-event approach as it would collapse the repeated measures of APGAR score into a single statistic; the time taken to reach the maximum score. Furthermore, time-to-event analyses can accommodate censored values, which in this case refer to the 23 puppies that did not reach the maximum APGAR score of ten. An additional advantage of a time-to-event approach is that quantification of the APGAR score is only needed until the puppy reaches the maximum score. Sampling beyond this point is unnecessary if it can be assumed that APGAR scores monotonically increase, which was the case in this study for all but four puppies. By focusing on the time to reach the maximum APGAR score, the requirement to record the APGAR scores at predefined periods of time is relaxed. The outcome measurement becomes the time that the APGAR score reached ten, or the last sampling time point if the APGAR score never reached ten (which would then be the time point that the measurement became censored).

Time-to-event analyses are best suited to situations where there are a large number of "events" (relative to the sample size) and so is uniquely suitable for APGAR scores in puppies where most puppies reach the maximum value (i.e. the event is reaching the maximum score). A strong contrast between most time-to-event analyses (survival analyses) and the analyses performed in this study is that the event is a positive outcome (having an APGAR score of ten), whereas in survival analyses the event is a negative outcome (death occurring).

Alternative approaches to assess the effect of doxapram on repeated measures of APGAR score (and where the APGAR score

is skewed toward the maximum values) involve transforming the APGAR scores into a binary variable where the APGAR score is counted as being maximum or not, or an ordinal variable (Groppetti et al., 2010; Titkova et al., 2017; Veronesi et al., 2009) where the APGAR scores are divided into ordered categories. If analysed using regression models, an interaction term between time and the treatment variable is needed to define the effect of treatment across multiple sampling time points. This will require the APGAR score to be measured at as many sampling time points as possible.

## 4.5 | Conclusion

In conclusion, this study has not been able to provide support for the use of intralingual doxapram in neonatal puppies above and beyond intralingual saline. Other routes of doxapram were not investigated in this study. The results were derived from data obtained from elective caesareans performed at a single veterinary practice that has extensive experience and a high level of expertise in canine reproductive medicine and surgery. Results may have been different if a wide range of veterinary hospitals were included in this study. Furthermore, a beneficial effect of doxapram may be limited to puppies that are apnoeic. By including several covariates in the statistical analysis, it is unlikely that conclusions were influenced by confounding variables such as brachycephaly, baseline APGAR score and puppy and maternal factors.

### AUTHOR CONTRIBUTIONS

TH Hyndman – study conception and design; analysis and interpretation of data; drafting and editing of manuscript; project supervision. S Fretwell – acquisition of data; editing of manuscript. RS Bowden, N Kordzakhia and SJ Tuke – analysis and interpretation of data; editing of manuscript. F Coaicetto – acquisition of data. PC Irons, JW Aleri, GC Musk and M Mosing – study design; editing of manuscript. SW Page – editing of manuscript. SS Metcalfe – study design; acquisition of data; editing of manuscript; project supervision.

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### CONFLICT OF INTEREST STATEMENT

Doxapram was purchased by the investigators and no drug company contributed funds to this research. The authors, therefore, declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in Appendix S1 of this article.

### ETHICS STATEMENT

The study was performed in accordance with the Code of Practice for the Care and use of Animals for Scientific Purposes (Australian Government, 2013) and approved by the institutional Animal Ethics Committee (AEC) (Permit #: R3063/18). Client-owned dogs were enrolled in the study. Owners voluntarily provided written consent for their dogs to be included in this study after being provided with an information sheet explaining the purpose of the study and how their animals would be used in the study.

### ORCID

*Timothy H. Hyndman* https://orcid.org/0000-0003-4083-562X

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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