Cortisol Profile and Clinical Evaluation of Canine Neonates Exposed Antenatally to Maternal Corticosteroid Treatment

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Cortisol Profile and Clinical Evaluation of Canine Neonates Exposed Antenatally to Maternal Corticosteroid Treatment

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Contents
The effects of glucocorticoids on both foetal canine lung and endogenous serum cortisol concentration have not been clearly delineated. Therefore, we aimed to investigate whether maternal corticosteroid treatment can alter maternal and neonatal cortisol profile and improve neonatal vitality. We allocated six bitches of different breeds and their neonates into two groups: control group (CONT) – maternal administration of saline solution at 55 days post-ovulation (n = 3); and betamethasone group (BETA) – administration of a single dose of 0.5 mg/kg betamethasone (Celestone Soluspan®) at 55 days post-ovulation (n = 3). Caesarean sections were scheduled for day 63 after ovulation. However, BETA group dams showed precocious signs of labour, and c-sections were performed at 58 days post-ovulation. Maternal and neonatal evaluations were performed periodically between betamethasone administration and birth, respectively. Neonates from both groups presented unsatisfactory (<5) Apgar score at birth. However, in spite of an earlier improvement on vitality found on CONT group and the premature delivery on BETA group, both groups showed acceptable Apgar score 120 min after birth. Neonatal cortisol concentrations were higher on CONT group compared to BETA group at birth. In addition, a gradual decrease on maternal cortisol concentrations was observed in the BETA group from treatment until parturition. These findings suggest that despite the down-regulation on the hypothalamic-pituitary-adrenal axis and the induction of premature delivery, betamethasone treatment was able to provide similar vitality when compared to the untreated neonates born at term.

Introduction
The physiological increase on endogenous cortisol concentrations, days before labour, is of utmost importance for overall foetal foetal maturation (Bonacci and Wapner 2009). Previous study suggests the important role of distinct endocrinological factors, including glucocorticoids, on foetal pulmonary development of several species, such as rodents, primates and human (Bolt et al. 2001). On the other hand, during premature labour, various adaptive mechanisms are delayed, and these diminish neonatal survival likelihood. Of the major pathological conditions of foetal immaturity, the Respiratory Distress Syndrome (RDS) is one of the most significant, deriving from an impairment on surfactant synthesis due to pulmonary immaturity. Therefore, antenatal corticosteroid treatment to pregnant women, aiming to artificially induce pulmonary foetal maturation, is a standard treatment in human medicine, initially proposed in 1972 (Peltoniemi et al. 2007). Data in newborn humans suggest that corticotherapy also support the decrease in the incidence and severity of other neonatal pathological conditions, such as necrotizing enterocolitis and periventricular leukomalacia. Betamethasone is the drug of choice to improve neonatal pulmonary function, with a greater bound affinity to the glucocorticoid receptor compared to endogenous cortisol (Ballard and Ballard 1995). According to the trial conducted by Liggins and Howier (1972), a combination protocol with two forms of betamethasone results in a rapid onset with betamethasone phosphate combined with a prolonged exposure due to the slower hydrolysis of betamethasone acetate.

Even though antenatal corticotherapy is constantly applied in human medicine, yet in some veterinarian patients, there is still a need for consistent research to establish the ideal protocol and therapeutic action, allowing for the safe use without empirical basement. In veterinary medicine, especially in dogs, no research has been undertaken in relation to maternal or foetal cortisol profile after glucocorticoid administration, as well as the influence of such drugs on neonatal vitality improvement. Hence, the aims of the present study were to investigate whether maternal corticosteroid treatment can improve neonatal vitality and alter maternal and neonatal endogenous cortisol profile.

Materials and Methods
This study was approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Science – USP (protocol no. 1672/2009). In this study, we used six females of different breeds (body weights from 15 to 20 kg; ages from 2 to 6 years) and 12 neonates (two puppies per bitch), randomly allocated in the following groups: Betamethasone (BETA) – maternal administration of a single dose of 0.5 mg/kg of maternal body weight of betamethasone (Celestone Soluspan®), Merck & Co., Inc., Whitehouse Station, NJ, USA) at 55 days post-ovulation (n = 3 females and six neonates); and Control (CONT) – maternal administration of saline solution (0.9% NaCl) at 55 days post-ovulation (n = 3 females and six neonates). The prediction of parturition date was based upon estimation of the LH peak and ovulation from vaginal cytology, vaginoscopy and progesterone concentration. The ovulation day was considered to occur 48 hours after the LH surge, this later determined through both progesterone and LH assays.

C-sections were performed at 63 days post-ovulation through the following anaesthetic protocol: sedation with acepromazine (0.02 mg/kg, Acepran 0.2%, Vetnil, Louveira, Brazil), tramadol (2 mg/kg, Cloridrato...
de Tramadol®; Hipolabor, Belo Horizonte, Brazil) or morphine (0.3 mg/kg, Sulfato de Morfina®; Hipolabor) injected by the intramuscular route. Anaesthesia was induced with slow intravenous administration of propofol (1 mg/kg, Fresfof®; Fresenius Kabi, Bad Homburg, Germany) and epidural blockage in the lumbosacral intervertebral space using an association of lidocaine chloride (2 mg/kg, Xylestesin®; Cristália, São Paulo, Brazil) and morphine (0.1 mg/kg, Dimorf®; Cristália, São Paulo, Brazil). General inhalational anaesthesia was maintained with isoflurane (Isoflurano; BioChimico®; Rio de Janeiro, Brazil).

Maternal and neonatal analysis
Maternal venous blood samples were collected immediately prior to betamethasone or saline solution administration (55 days post-ovulation); 1 h after betamethasone or saline solution administration (55 days post-ovulation); and daily until parturition (56–63 days post-ovulation), always during the same time of day. After birth and neonatal resuscitation, puppies were maintained in a neonatal incubator (Berço Aquecedor AQS®; Fanem, São Paulo, Brazil), which allowed implementation of the experimental procedures. Neonatal blood samples were collected from the right or left jugular vein. Cortisol concentrations were determined immediately at birth (0 h) and 2 h (2 h) later. The blood samples were allowed to clot at room temperature and centrifuged for 10 min at 1500 g. The serum was drawn off and stored at −20°C until analysis.

The neonatal vitality was verified using the Apgar system adapted for canine neonates (Silva 2008) at the following moments: at birth (0), at 5 min, at 60 min, at 2 h (120 min) and at 4 h (240 min) of life. To establish the Apgar score (0–10), the heart (0–2) and the respiratory (0–2) rates and effort were evaluated. Furthermore, the muscle tone (0–2) of each neonate was appraised mainly by observing the neonate’s ability to maintain strength at dorsal recumbency position; their head movements were also evaluated. An irritability reflex (0–2) was estimated from the neonate’s responsiveness to manipulation during the examination. Finally, the colours of the mucous membranes (0–2) of the gums were inspected to verify the presence of pallor or cyanosis. For each of the referred variables, a score from 0 to 2 was attributed, thus summarizing the Apgar score ranging from zero to 10. The neonates’ rectal temperatures were also recorded at the same moments in which Apgar assessment was performed.

Serum cortisol concentrations were measured by radioimmunoassay using a commercial kit (Cortisol Coat-A-Count; Genese®, São Paulo, Brazil), previously validated for canine serum. Results were expressed as µg/dl after conversion according to a calibration curve simultaneously prepared. The sensitivity of the cortisol assay at 92% binding was 0.11 µg/dl, and the low and high intra-assay coefficients of variation were 1.84% and 1.21%, respectively.

Table 1. Means and standard deviations (mean ± SD) of the neonatal (n = 12) Apgar score (0–10) at 0, 5, 60, 120 and 240 min after birth for the CONT and BETA groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Minutes after birth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>CONT</td>
<td>4 ± 0.77°C</td>
</tr>
<tr>
<td>BETA</td>
<td>3 ± 0.41D</td>
</tr>
</tbody>
</table>

CONT, control group, BETA, betamethasone group.

A,B,D: Different letters within the same row differ significantly (p < 0.05).

Within the same column differ significantly (p < 0.05).

Statistical analysis
Data were evaluated using Statistical Analysis System (SAS®, SAS Institute Inc., Cary, NC, USA). The effects of treatment (CONT and BETA) and the evaluation moment were estimated using PROC GLM. The differences between the treatments were analysed using parametric (the GLM procedure for each factor separately or LSD when combining factors) and non-parametric (Wilcoxon) tests, according to the residual normality (Gaussian distribution) and variance homogeneity. Whenever necessary, the data were transformed to obey these statistical assumptions. The differences between treatment and time were analysed using PROC NPAR1WAY and LSD test for multiple comparisons for non-parametric and parametric variables, respectively. The results were described as untransformed means and standard deviations (mean ± SD). Statistical differences were considered to have occurred if p < 0.05.

Results
Neonates from CONT group were born at 63 days after ovulation, while BETA neonates had to be urgently delivered at 58 days, as the treatment induced precocious labour signs (such as restless, vaginal mucous discharge, fall in body temperature and abdominal contractions), approximately 76 h after administration. Neonates of both groups presented similar unsatisfactory (<5) Apgar score at birth (Table 1). However, newborns from BETA group showed lower Apgar score (9 ± 0.3) when compared to CONT group (10 ± 0) 240 min (4 h) postpartum (Table 1). Furthermore, acceptable Apgar scores (>7) were achieved on the CONT group neonates earlier than the BETA group (60 min: 8.17 ± 0.70 vs 6.25 ± 0.75; and 120 min: 9.17 ± 0.4 vs 8.25 ± 0.63, respectively, Table 1). No differences were found between groups on all evaluation moments when variables used to calculate the Apgar score (heart and respiratory rates, muscle tone,
irritability reflex and mucus colour) were considered separately. Hypothermia was verified at 5 min post-partum in both groups. A gradual increase in body temperature occurred during periods of observation, with differences between groups (Table 2).

Neonatal cortisol concentrations were higher in the CONT group when compared to the BETA group only at the moment of parturition (3.85 ± 0.56 and 0.17 ± 0.06 µg/dl, respectively; p = 0.0012, Table 3). No difference was noticed among periods of analysis within the same group (Table 3). Within groups, maternal cortisol concentration differed only 24 h after betamethasone or saline injection (CONT: 14.1 ± 2.6 µg/dl and BETA: 1.1 ± 0 µg/dl; p = 0.037, Table 4). Moreover, BETA group exhibited a gradual decrease on cortisol concentrations from treatment until parturition (pre-treatment: 10.3 ± 1.7 µg/dl; post-treatment: 5.4 ± 1.3 µg/dl and delivery day: 1.1 ± 0 µg/dl, Table 5).

Discussion

Cortisol, a corticosteroid hormone, is synthesized by the adrenal glands. This hormone is regulated by the anterior pituitary adrenocorticotropic hormone (ACTH) which, in turn, is stimulated by the corticotrophin-releasing hormone (CRH) secreted by the paraventricular nucleus of the hypothalamus (Cunningham 2008). The hypothalamic-pituitary-adrenal (HPA) axis is triggered prior to parturition, but the timing of this event varies by species and is not known in carnivores.

Maternal endogenous cortisol concentration decrease within 2 h after betamethasone treatment followed by a more pronounced drop after 12 h (Ballard et al. 1980). In the present study, we verified that bitches treated with betamethasone showed lower concentrations of cortisol when compared to the control group 24 h after treatment. Despite several beneficial pulmonary effects, prenatal administration of betamethasone is recognized to be transiently suppressive to foetal and maternal adrenal cortex, as it inhibits the release of pituitary ACTH. According to Ballard and Ballard (1995), foetal cortisol concentration measured in the blood collected from the umbilical cord presents a 50% decrease 6 h after maternal corticotherapy. Corroborating such findings, we observed, at birth, higher concentrations of neonatal endogenous cortisol in the CONT group when compared to the animals delivered from bitches treated with betamethasone (CONT: 3.85 ± 0.56 µg/dl and BETA: 0.17 ± 0.06 µg/dl). These results may be associated with the suppressive influence of the corticosteroid treatment on the HPA axis. Furthermore, the CONT group presented the expected response to labour stress with a decrease in cortisol concentration occurring 120 min after birth, while BETA group remained unchanged.

Regarding the Apgar score analysis, neonates of BETA group presented delayed vitality in comparison with CONT group. Indeed, the Apgar score at 240 min of birth was statistically lower in the treated group. In spite of the premature labour, neonates of BETA group accomplished adaptive capability and favourable clinical condition after 2 h of life. Hence, maternal corticotherapy was able to simulate the effect of endogenous cortisol on foetal maturation notwithstanding the adrenal suppression at birth. In fact, Regazzi (2011) observed a structural pulmonary maturation simultaneously to an improvement on neonatal clinic condition in response to canine prenatal betamethasone administration. Therefore, the overall clinical recovery and neonatal favourable outcome at 58 days post-ovulation can be exclusively attributed to maternal betamethasone treatment.

We can conclude that maternal administration of betamethasone at 0.5 mg/kg induced premature labour with no long-term clinical negative effects in canine preterm neonates. Corticosteroid treatment induced
labor onset and foetal maturation, especially in vital organs. Despite the readily apparent foetal adrenal suppression caused by maternal betamethasone, treated neonates are responsive to the exogenous induction of overall maturation.

Acknowledgment

Conflicts of interest
None of the authors have any conflicts of interest to declare.

Author contributions
All authors carried out the experiment. Camila I. Vannucchi, Fernanda M. Regazzi and Marilia M.M. Barbosa analysed the data and drafted the paper.

References