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Original article

Oral folic acid supplementation decreases palate and/or lip cleft occurrence in Pug and Chihuahua puppies and elevates folic acid blood levels in pregnant bitches

A. Domośławska, A. Jurczak, T. Janowski

Department of Animal Reproduction with Clinic, Faculty of Veterinary Medicine,
University of Warmia and Mazury, Oczapowskiego 14, 10-719 Olsztyn, Poland

Abstract

The aim of this study was to compare the frequency of the occurrence of lip and/or palate cleft (CL/CP) in new-borns of two breeds, Pugs and Chihuahuas, and to measure the folic acid blood levels in bitches during gestations both with and without folic acid oral supplementation. Bitches of 13 Pugs and 17 Chihuahuas with CL/CP cases were used in the study. In trial 1, the animals of the experimental group (n=25) were given additional folic acid from the onset of heat till the 40th day of gestation. The females of the control group (n=12) were fed a traditional diet. From all the animals blood was collected at the onset of heat, 14 days later and on the 30th day of the gestation to estimate folic acid concentration. In trial 2, the prevalence of CP/CL cases in litters from pregnancies before and after supplementation was compared. The percentage of puppies with CL/CP after supplementation decreased in both Pugs and Chihuahua puppies (10.86% and 15.78% vs. 4.76% and 4.8% respectively). On Day 0, the concentrations of folic acid were at a low physiological level (around 8 ng/ml) in all the animals. In bitches of the experimental group the blood level of folic acid on day 14th and 30th of the treatment showed an increase in both breeds (13.65 ± 4.27 ng/ml in Pugs, 10.79 ± 2.84 ng/ml in Chihuahuas, and 14.94 ± 3.22 ng/ml in Pugs, 12.95 ± 3.58 in Chihuahuas, respectively) while in the control group, this level decreased with time of gestation both in Pugs and in Chihuahuas (around 6 ng/ml). Folic acid supplementation seems to be a simple, effective preventive method to reduce the risk of CL/CP, especially in the predisposed breeds.

Key words: folic acid supplementation, dogs, palate/lip clefting

Introduction

The relationship between nutritional and genetic factors causing congenital malformations during embryonic development has already been documented, mainly in humans (Mills et al. 1995, Finelli et al. 2004, Friso et al. 2006, Kim et al. 2009). Foliates are essen-

tial factors for the activity of enzymes modulating genomic DNA synthesis and methylation of homocysteine into the methionine. Disorders or deficiency in folate metabolism can cause craniofacial anomalies such as lip and/or palate cleft (CL, CP), neural tube defects (NTD's) and conotruncal heart defects (Friso et al. 2006, Kim et al. 2009) and also have a negative

effect on general fetal development and increased numbers of dystocia (caesarean sections) (Mills et al. 1995, Elwood et al 1997, Murray 2002, Finelli et al. 2004, Friso et al. 2006, Greco 2008, Kim et al. 2009). On the other hand, the counteraction of CL/CP may be folic acid supplementation as a daily nutritional addition (Elwood et al. 1997, Murray 2002, Guilloteau et al. 2006, Greco 2008). CL or CP is the most often appearing malformation, in contrast to other disorders that occur rarely or are not detected (Mills et al. 1995, Finelli et al. 2004). Pathogenesis of the lip and/or palate cleft is connected with gene mutations of the 5.10-methylenetetrahydrofolate reductase (MTHFR) which play a central role in the folate cycle (Mills et al. 1995, Finelli et al. 2004, Kim et al 2009). In dogs, transformations controlled by this enzyme occur during organogenesis in the first half of gestation. In this period, the embryo develops in the cephalocaudal direction. On day 23 of the gestation, the upper and lower jaw are developed. On day 33, the ossification of the nasal, incisive, zygomatic, palatine and parietal bones occurs. In this pregnancy phase, the fusion of palatal shelves also takes place (Pretzer 2008). Till day 35 of the gestation, the above described organogenesis is completed and the eventual disturbance in this process can result in many disorders seen after parturition as craniofacial anomalies. These can be lethal or cause many difficulties in the further development of puppies (Harvey 1987, Finelli et al. 2004, Pretzer 2008, Kim et al. 2009). In dogs, craniofacial anomalies can appear in every breed, but the brachycephalic breeds and dogs with wide skulls seem to be the most predisposed (Richtsmeier et al. 1993, Guilloteau et al. 2006). Studies of particular breeds (Boston terriers, French bulldogs and Brittany spaniels) also showed that the appearance of palate clefting can be expected as an autosomal recessive Mendelian inheritance (Richtsmeier et al. 1993, Elwood et al. 1997, Guilloteau et al. 2006). Information about the occurrence of the above-mentioned disorders in other breeds is still lacking as well as the concentration of folic acid in pregnant bitches.

The aim of this study was to compare the frequency of lip and/or palate cleft in new-borns of two breeds (Pugs and Chihuahuas) and to measure the folic acid blood levels in pregnant bitches during gestations both with and without oral supplementation of folic acid.

Materials and Methods

Bitches of 17 Pugs and 20 Chihuahuas aged from 2-6 years from different kennels with lip and/or palate

cleft cases in puppies were used in the study. In the past, all the animals were fed with the kibble containing usual dose of folic acid (0.6 mg/kg). On the basis of a questionnaire filled out by the dog's owners, data were collected regarding the number of puppies with CL/CP in previous litters and the number of caesarean sections.

The present study included two trials.

In trial 1, the folic acid blood levels during gestation were measured both with and without supplementation. The study group included 11 Pugs and 14 Chihuahuas which were given additional folic acid (Acidum folicum, Hasco, 5 mg tablets) at a dose of 5.0 mg per Pug and 2.5 mg per Chihuahua daily p.o. from the onset of heat till the 40th day of gestation. Six Pugs and 6 Chihuahuas females fed a traditional diet without any folic acid supplementation during pregnancy were used as a control group.

Blood was collected from the cephalic vein from the animals of both the experimental and control groups at the onset of heat (day 0), 14 days later and on the 30th day of the gestation. Folic acid level (standard 7-17 ng/ml) was assayed by the chemiluminescence technique (Elecsys Folate III, Cobas e411, Roche Diagnostic 2011).

In trial 2, the prevalence of CP/CL cases in litters delivered after pregnancies before and after supplementation was compared. This comparison only involved puppies of the experimental group of females from the first trial (11 Pugs and 14 Chihuahuas). Only one litter per bitch, a total of 84 puppies (46 Pugs and 38 Chihuahuas) before supplementation and 83 puppies (42 Pugs and 41 Chihuahuas) after the treatment were evaluated. Detection of CL/CP was performed by both the dogs' owners and veterinarians. In these bitches, the occurrence of the caesarean sections was also evaluated and compared in parturitions before and after supplementation.

A statistical analysis by ANOVA was performed for folic acid blood concentration between the experimental and control groups. Fischer's exact test was used to assess the statistical significance of the influence of supplementation on CL/CP appearance. The data were expressed as an odds ratio (OR) with a confidence interval as well. The data analyses were conducted with Statistica 9.1 (StatSoft, Inc. (2010). STATISTICA (data analysis software system), version 9.1. www.statsoft.com.)

Results

In trial 1 the concentrations of folic acid on Day 0 were at a low physiological level in both breeds and did not differ between the experimental and control

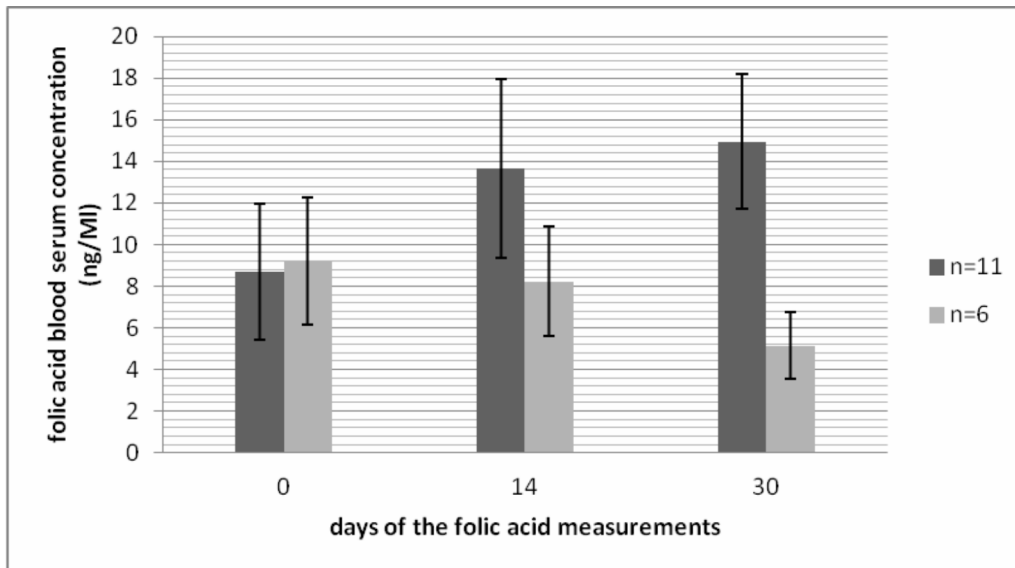


Fig. 1. Folic acid blood serum concentrations in experimental ■ and control group ■ of trial 1 in Pugs.

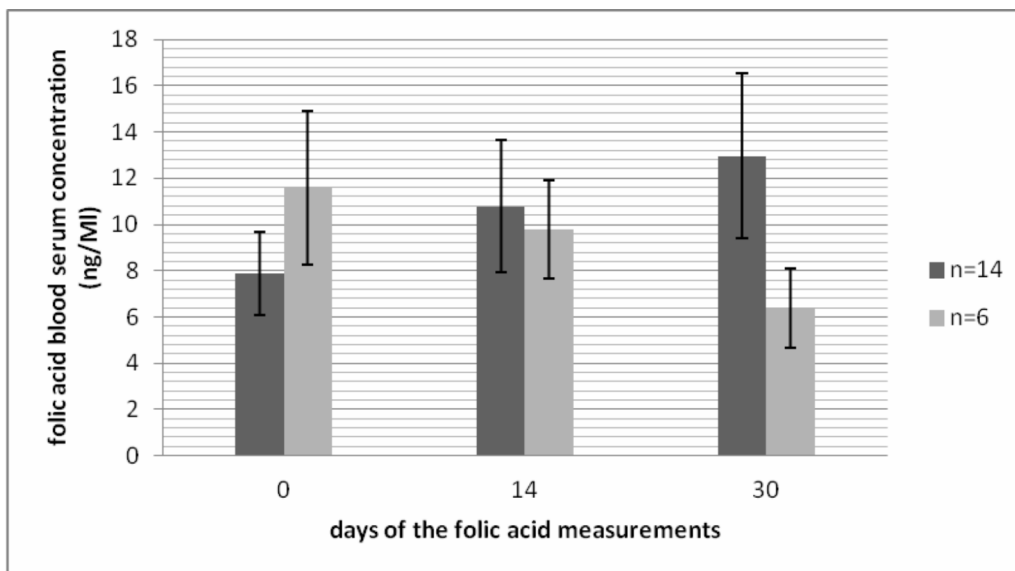


Fig. 2. Folic acid blood serum concentrations in experimental ■ and control group ■ of trial 1 in Chihuahuas.

groups ($P=1.00$). In all litters under supplementation, the blood level of folic acid on day 14 and 30 of the treatment showed an increase (Fig. 1, 2). In contrast, in the control group of both breeds this level decreased with the time of gestation (Fig. 1, 2). However, a statistically significant difference between the experimental and control groups was found only on day 30 ($P=0.001$) in both Pugs and Chihuahuas. The odds ratio (OR) computed for CL/CP incidence in supplemented vs control puppies in the study was 0.56 for Pugs and 0.52 for Chihuahuas.

In trial 2, the prevalence of puppies with CL/CP before and after supplementation with folic acid is presented in Table 1. The appearance of clefts de-

creased under supplementation from 15.78% to 4.87% ($P=0.1449$ OR=0.23) in Chihuahua puppies and from 10.86% to 4.76% ($P=0.4373$ OR=0.41) in Pug puppies. These differences using Fisher's test were not statistically significant. However, when the odds ratio was calculated, which is a statistical method independent from the number of animals used in the experiment, it revealed that in groups after supplementation, the probability of CL/CP was 3.66 times lower for Chihuahuas and 2.44 times for Pugs (Fig. 3).

Moreover, a decrease in caesarean section number in litters after supplementation was observed. In pregnancies before folic acid supplementation,

Table 1. Prevalance of clefts before and after supplementation with folic acid in Pug and Chihuahua puppies (trial 2).

Breed	Folic acid	Number of puppies	Number of puppies with CP/CL	Percentage of CP/CL
Chihuahua (n=14)	before supplementation	38	6	15.78%
	after supplementation	41	2	4.87%
Pug (n=11)	before supplementation	46	5	10.86%
	after supplementation	42	2	4.76%



Fig. 3. Cleft of lips and palate in littermate Chihuahua puppies.

caesarean sections were performed 8 times (4 times in Pugs and 4 times in Chihuahuas), whereas, after supplementation it was performed 3 times (2 caesarean sections in Chihuahuas and 1 in Pug).

Discussion

A novel aspect of our trial was the breeds of experimental animals. To date, Pugs and Chihuahuas had not been used in such studies. Although, there are no differences in folate metabolism between breeds, such differences can occur in the development of anatomical structures of skulls and, consequently, can cause disturbances in the fusion of the palatal shelves (Richtsmeier et al. 1993). In our research, the appearance of clefting and other malformations decreased in Pug and Chihuahua new-born under supplementation with folic acid. This reduction of cleftings incidence in new-borns has also been recorded by other authors. In French bulldogs, such a reduction of CL/CP was documented by Guilloteau et al. (2006). In their

comprehensive study on 140 puppies, supplementation of folic acid lasted from 15 days before mating till the end of gestation. The appearance of palate and lip clefts decreased from 8% to 4.4%. Similar observations were perceived by Elwood and Colquhoun (1997), who observed that in Boston terriers, the frequency of cleft palate decreased 4 times from 17.6% to 4.2%. It should be stressed that, in this study, folic acid at a dose of 5 mg was supplemented as a regular diet component throughout the entire reproductive life of the bitches. As a result, after 5 years of supplementation only 2 puppies appeared with clefts of the palate. Moreover, these authors observed that more vital puppies were born, probably due to the shorter time of expulsion phase, which was reduced by about 30-50%. In this study, caesarean sections were not fully eliminated, although, their number also decreased. In our trial, we observed a similar tendency. Under supplementation with folic acid only 3 caesarean section were performed compared to 8 before the treatment. In general, our results also showed a significant diminishing of CL/CP after

supplementation of folic acid in most predisposed breeds.

An interesting aspect of our study was also the evaluation of folic acid levels, because information about the dynamics of these levels during pregnancy in dogs is still limited. It has been suggested that, in humans, the physiological decline of folic acid concentration measured during early and mid-pregnancy is associated with the development of the fetuses and their demand for this compound (Finelli et al. 2004, Kim et al. 2009). Such a fall during canine pregnancy was described by Kalender (2006), who found higher concentrations of folic acid in early pregnancy (days 15-25) than in its later stages (days 46-63). The same tendency was observed in the control group of our study, while in the experimental group the level of folic acid remained consistently higher. To our knowledge, to date there have been no reports on the levels of folic acid in pregnant bitches after supplementation.

Our results revealed that cleftings can still appear after supplementation of folic acid, although, we noted a lower occurrence of this disorder. However, it has been shown that the appearance of clefting depends on both nutritional and genetic factors. Richtsmeier (1993) suggested, on the basis of pedigree analysis, that cleftings were inherited as an autosomal recessive. This disorder was recognized in 14 from 52 Brittany spaniel puppies (26.9%). In the opinion of these authors, the failure to fuse the palatal shelves can originate from developmental disturbances caused by different factors. It can be induced by inadequate growth of palatine shelves and an incorrect time of their elevation, extremely wide heads and failure of the shelves to fuse or secondary ruptures after fusion (Richtsmeier et al. 1993). Such hereditary clefting was also reported by Weber (1995) who diagnosed nasal fissures, cleft of lips and palates in 11 of 26 puppies sired by one male affected by a nasal fissure. This last report is in agreement with our clinical observations, because in this study, the same couple of Pugs gave two of five puppies with palate clefting in two consecutive litters. This disorder disappeared after the bitch was mated with another male. Such observations suggest the possibility of male carriers, which is in line with general opinion (Richtsmeier et al. 1993, Weber 1995). However, to our knowledge, the exact genetic background (gene or group of genes) responsible for this disorder in dogs has not yet been identified. The relationship between genetic and

nutritional factors, as well as their impact on early embryogenesis disorders in dogs, is still unclear and may reflect the epigenetic mechanism of action on embryo-fetal development (Kim et al. 2009).

In general, it seems that there are two ways to diminish the occurrence of cleftings. Folic acid oral supplementation seems to be a good and simple preventive method to reduce the risk of these disorders, especially in the predisposed breeds. Moreover, the hereditary aspects of reproduction related to carriers should also be taken into consideration.

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