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SHORT-TERM EFFECTS OF PREPUBERTAL OVARIOHYSTERECTOMY ON SKELETAL, PHYSICAL AND BEHAVIOURAL DEVELOPMENT OF DOGS UP TO 24 WEEKS OF AGE

B. H. SONTAS* and H. EKICI

Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Istanbul University, Avcılar Campus, 34850 Avcılar, Istanbul, Turkey

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This study investigated the effects of ovariohysterectomy performed at 10 weeks of age on body weight, behaviour, bone length, serum calcium and phosphorus concentrations and gonadal hormone (progesterone and oestradiol) levels until 24 weeks of age in 31 clinically healthy mixed-breed female puppies. The littermates were randomly assigned to three groups. Group I (n = 11) was completely ovariohysterectomised, Group II (n = 10) was incompletely ovariohysterectomised with one ovary left intact, and Group III (n = 10) was sham operated. No significant differences were found between the groups for any of the parameters evaluated. It was determined that, except for two measurements of two animals in Group II at 20 and 24 weeks of age, all animals had undetectable concentrations of oestradiol 17-β. These findings suggest that an ovariohysterectomy performed on a mixed-breed dog at 10 weeks of age does not affect her skeletal, behavioural or physical development in the period studied, i.e. until 24 weeks of age.

Key words: Dog, early-age spaying, bone, behaviour, growth

According to cultural, ethical, economic and legislative differences among countries, different methods are used to control pet overpopulation in the world. Early-age spaying/neutering, often referred to as prepubertal gonadectomy, is the surgical sterilisation of sexually immature companion animals aged 6 to 14

*Corresponding author: Besim Hasan Sontas, Lamartin Cad. 49/7 Sontas Ap., Taksim, 80090, Istanbul, Turkey; E-mail: bhsontas@gmail.com; Phone: 0090 (532) 324-3713; Fax: 0090 (212) 473-7241

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weeks, which has been suggested as a tool to combat pet overpopulation in the United States. No dog or cat adopted or taken from a shelter would have a chance to reproduce as a result of this procedure. Several important organisations including the American Veterinary Medical Association (AVMA), the American Humane Association (AHA) and the American Animal Hospital Association (AAHA) endorse the practice of prepubertal gonadectomy because of the benefits of the surgery for combating the problem of pet overpopulation (Lieberman, 1987; Kahler, 1993; Kustritz, 1999).

In our previous study (Ekici et al., 2005) we investigated the changes in bone mineral density and bone mineral content of the calcaneus and the accessory carpal bone after prepubertal ovariohysterectomy using dual energy X-ray absorptiometry. No differences were found between puppies that underwent prepubertal ovariohysterectomy or sham surgery. We suggested that bone mineral density and bone mineral content of the calcaneus and the accessory carpal bone were not affected by prepubertal ovariohysterectomy until six months of age.

In the present study, we attempted to demonstrate the effects of prepubertal ovariohysterectomy on body weight, behaviour, bone length, serum calcium and phosphorus concentrations and gonadal hormone levels until 24 weeks of age in mixed-breed dogs.

Materials and methods

Animals and study design

In this study, a total of 31, six- to eight-week-old, mixed-breed female puppies with a mean weight of 2.97 kg were used. All procedures that were performed in this study were approved by the Ethics Committee of the Faculty of Veterinary Medicine, Istanbul University (69/09.07.2003). The puppies were taken from an animal shelter and housed with their littermates in an indoor facility. They were individually fed dry dog food containing 1.43% calcium and 1.04% phosphorus (ANF Advantage Puppy, ANF Specialities, Inc., USA) twice daily according to the recommendations of the company. Water was provided *ad libitum*. The animals were taken outside in groups once daily for at least 4 h for exercise and play. Vaccination and deworming protocols used in this study were the same as previously reported (Ekici et al., 2005).

Surgeries

Littermates were randomly placed into three groups. The surgeries were performed at 10 weeks of age under isoflurane (Isoflurane, Rhodia Organique Fine Ltd., UK) anaesthesia following premedication with atropine (0.04 mg/kg SQ, Atropin, Vetaş, Turkey) and carprofen (4 mg/kg SQ, Rimadyl, Pfizer, Belgium) in the same manner as previously described (Ekici et al., 2005). Group I

consisted of 11 animals that were completely ovariohysterectomised using the technique described by Howe and Olson (2000), Group II consisted of 10 animals that were incompletely ovariohysterectomised, leaving the left ovary intact, and Group III consisted of 10 animals that had a sham surgery. At the end of the study, the remaining reproductive organs of Group II and Group III puppies were surgically removed and the animals were returned to animal shelters where they had been taken from.

Radiography of the left and right antebrachium was performed to monitor the growth of the bone on each animal at 12, 16, 20 and 24 weeks of age. Ulnar and radial lengths were measured in millimetres on mediolateral views. The measurement was performed from the most proximal to the most distal extent of both ulna and radius. To determine the effect of the surgery on body weight, each animal was weighed weekly on the same day beginning at 12 weeks of age until the end of the study.

All animals were observed during the entire period of the experiment and six behavioural characteristics were assessed by two researchers and two students who did not have knowledge of which treatment group each animal was in. These characteristics which included general activity, barking, playfulness, aggression and affection towards people and intraspecies aggression were graded on a scale from 0 (absent) to 4 (highest).

Blood samples were collected by cephalic or jugular venipuncture at 12, 16, 20 and 24 weeks of age and analysed for calcium (Ca), phosphorus (P), oestradiol 17-β (E2) and progesterone (P4). Sera were obtained immediately following venipuncture and stored at –20 °C until assayed. Serum Ca and P concentrations were measured with an auto-analyser (Vitalab Flexor, The Netherlands) using commercial kits (Kalsiyum Arsenazo DDS and Fosfor UV DDS, Diasis Diagnostik Sistemler Tic. Ve San. A.S., Turkey).

Serum concentrations of E2 and P4 were determined by radioimmunoassay using commercial kits (Ultra-Sensitive Estradiol RIA DSL-4800 and Active Progesterone DSL-3900; Diagnostic Systems Laboratories, Inc., USA) according to the manufacturer's instructions. The sensitivity of the assay was 2.2 pg/ml, and the intra- and interassay coefficients of variation (CV) were 6.5% and 8.6%, respectively. The minimal assay sensitivity for P4 was 0.1 ng/ml, and the intra- and interassay CV were 4.8% and 12.2%, respectively.

Statistical methods

Statistical analysis was performed with statistical packages for social sciences for Windows 11.5 package program (SPSS, 2004). The differences between the groups were analysed by one-way ANOVA and a value of $P < 0.05$ was considered to be statistically significant.

Results

Thirty out of the 31 animals completed the study. One animal from Group II died six weeks after the surgery of a severe respiratory infection. The animals tolerated the surgeries well and they were alert and active within an hour after the surgery.

Bone length

Monthly measurements taken from the lateral radiographs of the antebrachium showed that ulnar and radial lengths were greater in Group I animals than in animals of Groups II and III (Fig. 1). However, the difference between the groups was statistically insignificant ($P > 0.05$). The opposite radius and ulna did not display any difference. No orthopaedic problems were noted.

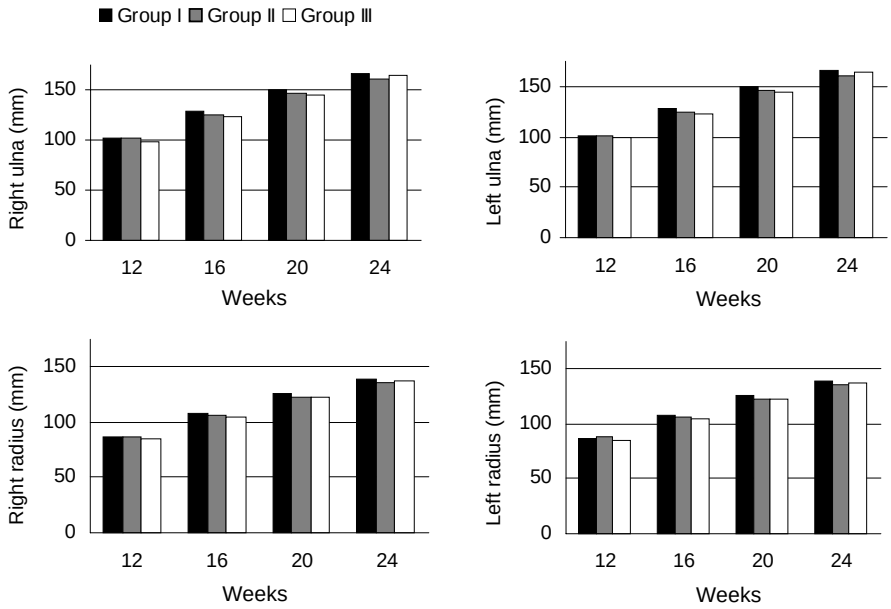

Fig. 1. Comparison of groups for radial and ulnar bone lengths

Body weight (BW)

Group I animals were heavier than Group II and Group III animals, although the difference between the groups was not statistically significant ($P > 0.05$) (Fig. 2). Subjectively, none of the animals were obese or overweight throughout the experiment.

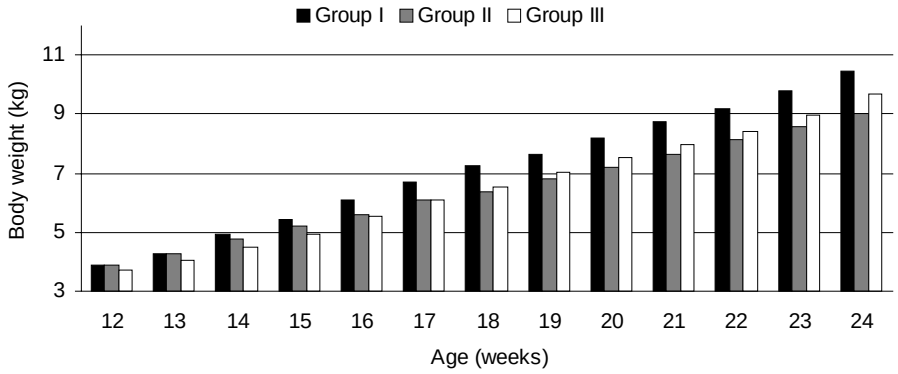

Fig. 2. Body weight data of Groups I, II and III

Behaviour

During the experimental period, no differences were observed between the three groups in terms of general activity, barking, playfulness and affection towards people. Lethargy or unresponsiveness to external stimuli was not observed in any animal throughout the study. None of the animals displayed aggressive behaviour towards their own species or towards people.

Biochemical and hormonal analysis

The serum concentrations of Ca and P of each group was in the normal range and no significant changes were seen throughout the experiment (Fig. 3a, b). However, at week 24 P was significantly lower in Group II animals compared to Group I and Group III animals ($P < 0.01$).

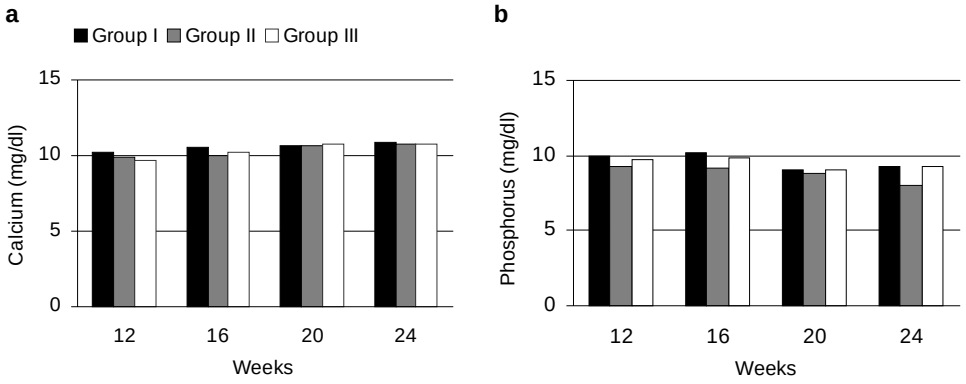

Fig. 3a, b. Comparison of the mean serum calcium and phosphorus concentrations of dogs in Groups I, II and III

No significant difference was observed in serum P4 levels between the groups (Fig. 4). Serum E2 levels of the groups were not statistically analysed because of undetectable concentrations. Serum E2 levels could be measured only in two animals of Group II in the 20th (2.9 pg/ml) and 24th (1.3 pg/ml) weeks.

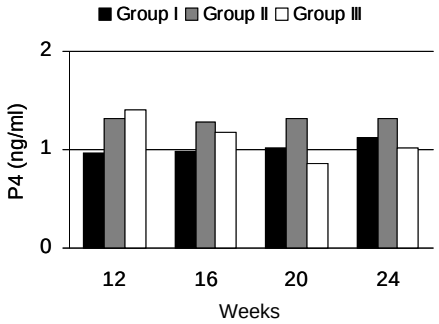

Fig. 4. Comparison of the mean serum progesterone concentration of dogs in Groups I, II and III

Discussion

In the present study, the final radial and ulnar lengths of Group I puppies were found to be greater than those of Group II and Group III puppies, although the difference was not statistically significant. The results of studies conducted by Salmeri et al. (1991*a*), Stubbs et al. (1996) and Root et al. (1997) showed that the final radial or ulnar lengths of prepubertally gonadectomised animals were found to be greater than those of sexually intact animals, which is consistent with our findings. The final radial or ulnar lengths of prepubertally gonadectomised animals are greater than those of sexually intact animals because the closure of the radial or ulnar growth plates is delayed due to the biphasic effects of oestrogen on physeal maturation (Salmeri et al., 1991*b*).

Several researchers (May, 1998; McNicholas et al., 2002; Spain et al., 2004) reported that the incidence of hip dysplasia or growth plate (Salter-Harris) fractures increased among prepubertally gonadectomised dogs. An increase in the risk of growth plate fractures after prepubertal gonadectomy was not observed either in our study or in the studies by Salmeri et al. (1991*a*), Howe et al. (2001) and Spain et al. (2004). Moreover, Howe et al. (2001) did not find any correlation between prepubertal gonadectomy and the frequency of musculoskeletal disorders or hip dysplasia.

The reason why we did not observe any orthopaedic problem in our study might be (a) that the puppies were fed with a fixed amount of balanced dog food, (b) that middle-breed dogs were used, which are not predisposed to skeletal diseases, such as the Boxer (LaFond et al., 2002) or the Bernese Mountain dog (Hedhammar, 2003), and (c) that the study was ended in an early stage following

the surgery, although developmental skeletal diseases are common in growing dogs (Hedhammar, 2003).

Obesity is the most common nutritional disorder affecting 10 to 40% of dogs in developed countries that shortens the life-span by increasing the development of diabetes mellitus, cardiovascular and musculoskeletal diseases, skin problems, heat intolerance, reproductive disorders, as well as the risk of surgery and anaesthesia (Robertson, 2003). This occurs as a result of increased food intake, reduced physical activity, decreased metabolic rate or more efficient utilisation of nutrients (Root and Johnston, 1995; Root et al., 1996; Fettman et al., 1997; Robertson, 2003). Our findings are in agreement with those reported by Salmeri et al. (1991*a*), Howe et al. (2001) and Spain et al. (2004) who found that there was no association between obesity and prepubertal ovariohysterectomy.

This may be due to feeding the animals with a regulated amount of dog food according to the manufacturer's recommendation instead of *ad libitum* feeding, since a*d libitum* feeding after ovariectomy was found to result in significant weight gain whereas feeding with a fixed amount of commercial dog food did not result in obesity (Houpt et al., 1979; Le Roux, 1983). Furthermore, the animals were allowed to exercise in groups for at least four hours which prevented weight gain. The importance of regular exercise in the prevention of obesity in ovariectomised bitches was demonstrated by Le Roux (1983).

No difference between the three groups in terms of general activity, barking, playfulness and affection toward people was observed during the experimental period. These findings were in accordance with those obtained by Salmeri et al. (1991*a*). The dogs gonadectomised at either seven weeks or seven months of age were found to be more active than sexually intact dogs. Results of two other studies (Howe et al., 2001; Spain et al., 2004) also indicated that prepubertal gonadectomy was not associated with increased behavioural problems.

Although the hormonal changes of the prepubertal, pubertal and postpubertal periods were studied in the male dog on 13 male Fox terriers (Mialot et al., 1988), the reproductive endocrinology during the first year of life was not well documented in the female dog. Salmeri et al. (1991*a*) attempted to determine the changes in serum concentrations of E2 in dogs; however, because of inadequate serum samples, the data were only presented graphically and were not analysed statistically. Oestradiol was detected only in one early-age gonadectomised animal at 10 and 30 weeks of age, whereas in sexually intact bitches the E2 concentrations were determined to range from 0 to 3.7 pg/ml prior to 30 weeks of age. In our study, we also failed to demonstrate the effects of prepubertal ovariohysterectomy on E2 concentrations because of insufficient data. The duration of the experiment may be a major factor that prevents us from making a clear statement since we were able to monitor the animals for only 14 weeks after the surgery. None of the animals displayed oestrous behaviour throughout the experimental period and, as a result of this, serum P4 concentrations were determined to be at basal levels.

Except for the P level at 24 weeks of age, no significant difference in Ca or P existed between the groups at the end of the study. Ca and P levels of all animals were determined to be within the normal limits accepted for paediatric dogs (Ca: 8.8–15.9 mg/dl; P: 4.1–10.8 mg/dl) (Turgut, 2000). We had difficulty in comparing our results with those of other studies (Salmeri et al., 1991*a*; Stubbs et al., 1996; Root et al., 1997) that investigated the effect of prepubertal gonadectomy on bone development, since serum Ca or P levels were not measured. However, in previous studies no significant change had been determined in serum Ca or P levels after ovariectomy in adult beagle bitches (Dannucci et al., 1987; Martin et al., 1987).

From the findings obtained in this study in mixed-breed puppies we can conclude that calcium and phosphorus concentrations, skeletal development, body weight and behaviour are not affected by prepubertal ovariohysterectomy until six months of age. However, further studies conducted at least up to one year of age on a larger number of animals are needed to make a firmer statement.

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