

THE EFFECT OF PREPUBERAL AND POSTPUBERAL GONADECTOMY ON RADIAL PHYSEAL CLOSURE IN MALE AND FEMALE DOMESTIC CATS

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Male (n = 6/group) and female (n = 6/group) kittens were gonadectomized at 7 weeks (prepuberally) or 7 months of age (postpuberally), or left intact. Lateral radiographic projections of the right forelimb were made from 4 months of age until the distal radial physis was closed, or 24 months of age. In males, distal radial physeal closure was delayed in both groups of gonadectomized animals, compared to intact males ($p < 0.01$). In females, proximal radial physeal closure was significantly delayed in prepuberally gonadectomized animals ($p = 0.02$), and distal radial physeal closure was significantly delayed in both groups of gonadectomized animals, compared to intact animals ($p < 0.01$). Final radial length (females $p < 0.01$, males $p = 0.01$), and age and radial length at time of the growth plateau ($p < 0.01$) were significantly increased in all gonadectomized animals. Age at gonadectomy had no effect on age and radial length at time of the growth plateau. No puberal growth spurt was observed in any of the cats. *Veterinary Radiology & Ultrasound*, Vol. 38, No. 1, 1997, pp 42–47.

Key words: cat, physeal closure, gonadectomy, growth.

Introduction

LONG BONES OF the limb grow in length by endochondral ossification and in width by intramembranous ossification. In endochondral ossification, a cartilaginous model is formed first, with subsequent replacement of chondrocytes with osteoblasts to form the bone.¹ Longitudinal growth of long bones occurs at the physes. In the physes, calcium is deposited and migrating osteoblasts begin to secrete components of bone matrix. Subsequently, chondrocytes undergo proliferation, hypertrophy and then degenerate as they and the surrounding matrix are invaded by capillaries.^{1,2} When no viable chondrocytes remain at the physeal plate, longitudinal bone growth ceases and the growth plate is defined as closed.¹

Longitudinal bone growth and physeal closure are complex processes, dependent on growth hormone, insulin and insulin-like growth factors, thyroid hormone, and the gonadal steroids, estrogens and androgens.^{2–13} Low doses of

estrogen stimulate growth, and high doses inhibit growth and promote physeal closure.^{3,4,6,8,11,12} The growth effect may be due to estrogens potentiating the release or activity of growth hormone.^{7,9,12} Estrogens, when present at high concentrations, accelerate physeal closure by promoting calcium deposition at the physes.¹¹ Androgens accelerate physeal closure by promoting degeneration of hypertrophied chondrocytes and proliferation of capillaries and perivascular mesenchymal tissue, and promoting calcium deposition at the physes.^{10,11}

Puberty is the period when the adult pattern of gonadotropin release begins, gonadal steroids are released, and the animal attains the ability to reproduce. The pubertal release of estrogen and androgen in humans causes the pubertal growth spurt, with subsequent cessation of growth due to physeal closure.^{12,13} Although a pubertal growth spurt has not been identified in dogs^{14,15} or cats,^{16,17} physeal closure and cessation of longitudinal bone growth occur at about the time of first estrus in females or appearance of sperm in the ejaculate in males in these species.

In the domestic cat, the average age at onset of puberty is 8.5 to 10 months.^{18,19} A wide range of ages for puberty onset occurs, however, as onset of puberty in both males and females is dependent on body weight, with queens reaching body weight of 2.3 to 2.5 kg²⁰ and males 3.5 kg²¹ at this time. Season of the year is also a factor; cats exhibit a prolonged anestrus linked to short daylength.²² Young animals achieving the appropriate age and body weight during seasons with short daylength will not enter puberty.²³ Conversely, puberty and year-round reproductive cycling

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can be stimulated by maintaining cats under artificial lighting to simulate long daylength.^{22,24,25}

Physal closure in normal intact domestic cats begins as early as 4 months of age and is completed by 20 months of age.²⁶ Smith documented physal closure in domestic cats radiographically and histologically,²⁶ and defined three major groups of physes that close at or about the same time. The proximal radial physis is in the group that closes the earliest, at 4 to 7 months, and the distal radial physes falls in the group that closes the latest, at 14 to 20 months of age.²⁶

By comparing times of pubertal onset and physal closure, it becomes clear that some physes close prepuberally while others do not. Gonadectomy before or near the time of puberty may alter the time of closure of those physes that normally close at about this time, with the hypothesized alteration due to withdrawal of gonadal steroids. The relationship between age at gonadectomy and time of physal closure has been studied in male and female dogs.¹⁴ Physal closure in gonadectomized dogs was delayed compared to that in intact dogs, with those animals that were gonadectomized at 7 weeks of age exhibiting the longest delay. The relationship between gonadectomy at the time of puberty and time of physal closure has been studied in male cats,²⁷ and the time of physal closure was compared in neutered and intact male and female cats.²⁸ These studies attempted to determine whether gonadectomy of immature animals predisposed them to delayed physal closure and increased risk of Salter fractures, which are partial or complete disruptions of the cartilaginous physal plate at the ends of long bones. In both studies, physal closure was delayed in castrated male cats.^{27,28} The effect of gonadectomy on physal closure in female cats or of prepuberal gonadectomy on physal closure in male cats has not been reported.

Purposes of this study were to determine time of closure of the proximal and distal radial physes, to determine radial length in adult male and female cats gonadectomized before or after puberty, or left intact, and to determine age and radial length at the time of the growth plateau, which is defined as the time when bone length no longer increases significantly.

Materials and Methods

Experimental Animals

Adult intact female and male domestic shorthair cats were purchased from a commercial facility and bred to produce 15 litters of kittens, as described previously.²⁹ The kittens were weighed daily for the first 3 weeks of life. At 3 weeks of age, they were given moistened commercial kitten food* *ad libitum*. They were weighed every other day

from 3 to 6 weeks of age, and were completely weaned by that time.

At 6 weeks of age, the kittens were housed individually in fiberglass or stainless steel cages. All had continuous access to a resting bench and litter pan. The kittens were fed a commercial dry kitten food* until 1 year of age, at which time they were fed a commercial dry adult food.† All had *ad libitum* access to fresh water. They were maintained under artificial lights with a cycle of 16 hours light and 8 hours darkness.

A complete blood count, serum chemistry profile, and urinalysis were performed when the kittens were 6 weeks of age, and every 6 months thereafter until 24 months of age. At 6.5, 10 and 14 weeks of age, the kittens were vaccinated for feline viral rhinotracheitis, calicivirus, and panleukopenia.‡ Cats were vaccinated for rabies§ at 14 to 16 weeks of age and again one year later. General health was monitored by weighing the animals weekly and performing physical examinations biweekly.

The gender of the kittens was recorded at birth. Twenty male and 20 female kittens were randomly assigned to Group I (gonadectomy at 7 weeks of age; females *n* = 6, males *n* = 6), Group II (gonadectomy at 7 months of age; females *n* = 6, males *n* = 6), or Group III (intact; females *n* = 6, males *n* = 6). Twenty kittens of each gender were assigned in order to allow for juvenile mortality. Kittens not assigned to a group were placed in homes as pets.

Gonadectomy

Group I consisted of 6 female and 6 male kittens that were gonadectomized at 7 weeks of age. Group II consisted of 6 female and 6 male kittens that were gonadectomized at 7 months of age. Group III consisted of 6 female and 6 male kittens that were not gonadectomized during the 24 month project in order to serve as controls.

Group I kittens were fasted for 4 hours, and Group II kittens for 8 to 12 hours before induction of anesthesia prior to gonadectomy. Kittens of both groups received atropine (0.045 mg/kg SQ) as a pre-anesthetic. Group I kittens were masked with isoflurane^{||} and maintained by mask throughout the surgery. One female kitten in Group I required supplemental anesthesia with ketamine hydrochloride[#] (11 mg/kg IM) and midazolam** (0.11 mg/kg IM) intraoperatively. Group II male and female kittens were induced with ketamine hydrochloride (22–33 mg/kg IM) and midazolam (0.06–0.11 mg/kg IM). Group II females were intubated, and maintained on isoflurane if needed.

*ProPlan Adult, Ralston Purina Company, St. Louis, MO 63164.

†Felocell CVR, SmithKline Beecham Animal Health, West Chester, PA 19380.

‡Imrab 3, Rhone Merieux Inc., Athens, GA 30601.

§Aerrane, Anaquest, Madison, WI 53713.

#Ketaset, Fort Dodge Laboratories Inc., Fort Dodge, IA 50501.

**Versed, Hoffmann-La Roche Inc., Nutley, NJ 07110.

*ProPlan Kitten, Ralston Purina Company, St. Louis, MO 63164.

Ovariohysterectomy or castration was performed in the same manner in all animals, regardless of age at time of surgery. Ovariohysterectomy was performed using a routine technique, with use of 2/0 polyglactin suture material^{††} and placement of subcuticular sutures for closure of the skin. Castration was performed using a routine open technique in all males. The spermatic vessels and ductus deferens were ligated with 2/0 polyglactin suture material as far proximally as possible and transected caudally to the ligatures. The bilateral scrotal incisions were not sutured.

Radiography

Radiography of the right forelimb was performed on all 36 kittens at monthly intervals, from 4 months of age until maturity or completion of the project at 24 months of age. Lateral and craniocaudal radiographs were made, using high speed screens and compatible film, with radiographic technique varying as necessary as the kittens grew. Maturation was considered complete when both the proximal and distal radial physes were closed, as defined by lack of a radiolucent line marking the cartilaginous epiphyseal plate, and when bone length, measured on the lateral projection radiographs as the distance from the most caudal aspect of the articular fovea to the most dorsal aspect of the carpal articular surface, did not increase. Age and radial length at the time of acquisition of the growth plateau were also assessed for each animal, with the growth plateau defined as the first point in time when bone length did not increase from one month to the next.

Statistical Analysis

Age at time of closure of the proximal and distal radial physes, radial length, and age and radial length at the time of the growth plateau were compared between groups, separately by gender using analysis of variance (ANOVA). Pairwise comparisons were made using the least significant difference technique. A *p* value of less than 0.05 was considered significant. Linearity of growth rate prior to acquisition of the growth plateau was assessed by the product moment correlation coefficient, *r*, with *r* greater than 0.90 considered significant.

Results

Radial Physeal Closure

Male cats in Groups I, II and III did not differ in time of proximal radial physeal closure ($p > 0.05$) (Fig. 1). Female cats differed by group ($p = 0.02$); those that were prepuberally gonadectomized (Group I) had delayed physeal closure compared to those gonadectomized at 7 months of age (Group II) or left intact (Group III) (Fig. 1).

Both males ($p < 0.01$) and females ($p < 0.01$) differed

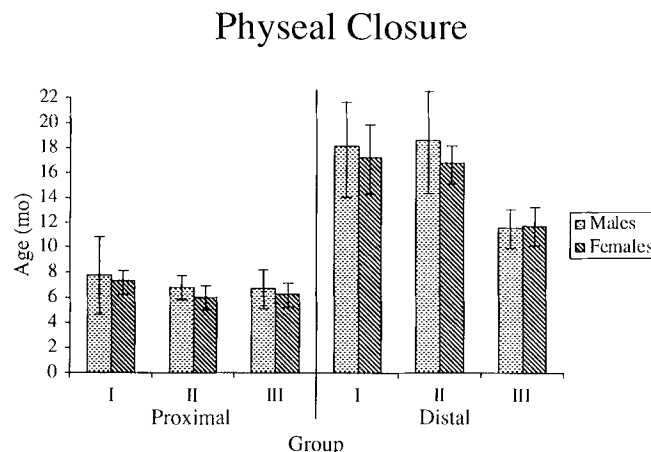


FIG. 1. Mean age at time of proximal and distal radial physeal closure in male and female cats gonadectomized prepuberally (Group I) or postpuberally (Group II) or left intact (Group III).

by group in time of closure of the distal radial physis; both groups of gonadectomized animals (Group I and II) had delayed physeal closure compared to intact animals (Group III). One male cat in Group II died accidentally at 11 months of age, before achieving distal physeal closure. One male each from Groups I and II did not have distal physeal closure at 24 months of age, when they completed the project.

Radiographic physeal closure was easily identified at the proximal radial physes in all animals. At the distal radial physes, apparent islands of cartilage within the physeal plate sometimes prevented complete radiographic physeal



FIG. 2. Radiographic cartilage island within the closing distal radial physeal plate of an 18-month-old male cat.

^{††}Vicryl, Ethicon Inc., Somerville, NJ 08870-0151.



FIG. 3. Lateral radiographic view of the right forelimb of 3 11-month-old full sisters. (A) Group I (gonadectomy at 7 weeks of age) and (B) Group II (gonadectomy at 7 months of age); proximal radial physis closed, distal radial physis open, (C) Group III (intact); both radial physes closed.

closure (Fig. 2). Physeal closure was not considered complete until cartilage islands were no longer visible, and there was complete disappearance of a radiolucent line marking the physeal plate (Fig. 3).

Radial Length

Length of the radius did not differ between groups of cats at 4 months of age for either males ($p > 0.05$) or females ($p > 0.05$) (Fig. 4). Radial length did differ by groups for both males ($p = 0.01$) and females ($p < 0.01$) at 24 months of age, with males gonadectomized at 7 weeks or 7 months of age having final radial length an average of 13% greater than intact males, and females gonadectomized at 7 weeks or 7 months of age having final radial length an average of 9% greater than intact females (Fig. 4). Increase in radial

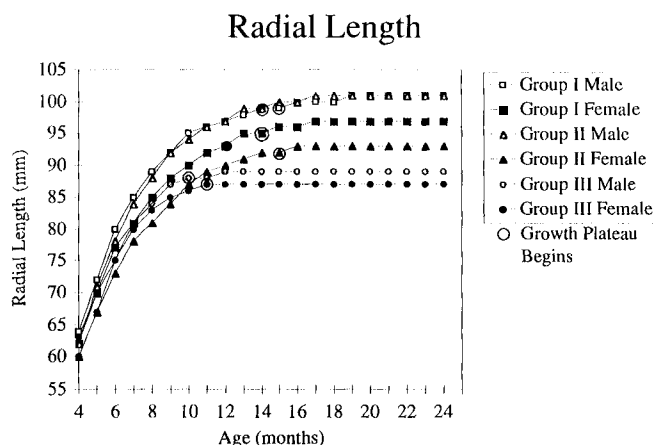


FIG. 4. Mean length of the radius in male and female cats gonadectomized at 7 weeks (Group I) or 7 months of age (Group II), or left intact (Group III), from 4 months of age to maturity.

length was positively correlated with age, and was linear until acquisition of the growth plateau for both males and females in all 3 groups (Males: Group I $r = 0.93$, Group II $r = 0.95$, Group III $r = 0.97$; Females: Group I $r = 0.96$, Group II $r = -0.95$, Group III $r = 0.95$).

Age and Radial Length at Growth Plateau

Age at the growth plateau differed by group for both males ($p < 0.01$) and females ($p < 0.01$), as did radial length at the growth plateau (males $p = 0.02$, females $p < 0.01$). For both age and radial length, both gonadectomized groups differed from the intact group, with gonadectomized animals reaching their growth plateau when older and having a greater radial length compared to intact animals (Fig. 4). Male cats gonadectomized at 7 weeks or 7 months of age reached the growth plateau an average of 35% later, and achieved radial length at the growth plateau an average of 13% greater than intact males. Female cats gonadectomized at 7 weeks or 7 months of age reached the growth plateau an average of 18% later, and achieved radial length at the growth plateau an average of 9% greater than intact female cats.

Discussion

In this study, cats undergoing ovariectomy or castration at 7 weeks of age were defined as having been prepuberally gonadectomized, and assumed to have been exposed to minimal levels of gonadal steroids for the length of the study. Those animals undergoing ovariectomy or castration at 7 months of age were defined as having been gonadectomized postpuberally. Those animals were assumed to have been exposed to gonadal steroids, with duration and magnitude of exposure unknown. All of the animals were fed for optimal growth and housed with lighting simulating long daylength to promote normal puberty onset. No attempt was made to verify estrous activity in females or presence of viable sperm in the ejaculate in males before surgery was performed at 7 months of age.

The presence of apparent islands of cartilage within the distal physis prevented complete radiographic physeal closure, despite disappearance of a radiolucent line in other areas of the plate and static bone length, denoting functional physeal closure. It is possible that these islands are an optical illusion, with the apparent discontinuity caused by viewing the irregular contour of the physis in two dimensions. This phenomenon has also been described in other studies.²⁶⁻²⁸

The proximal radial physis in the intact cat closes at 4 to 7 months of age, before the average time of puberty onset.²⁶ Distal radial physeal closure occurs postpuberally, at 14 to 20 months of age.²⁶ Closure of the distal physis may be dependent on the presence of gonadal steroids. Therefore, a significant delay in physeal closure in gonadectomized an-

imals may be more likely to occur at the distal physis than at the proximal physis. Data from the current study support this hypothesis.

In male cats, there was no effect of sexual status on timing of proximal radial physeal closure. Animals castrated either at 7 weeks or 7 months of age had delayed distal radial physeal closure compared with that of intact animals. Age at time of gonadectomy had no effect, suggesting that the proximal radial physis closes in response to lower levels of gonadal steroids or some other signal, while the distal physis is more dependent on gonadal steroids. In female cats, proximal radial physeal closure was delayed in prepuberally gonadectomized animals, and distal physeal closure was delayed in both prepuberally and postpuberally gonadectomized animals. Age at gonadectomy had no effect on distal radial physeal closure.

The difference in response between males and females is most likely due to a gender-specific reaction of the normal physis to gonadal steroids. Male human beings with familial precocious puberty, a hereditary disorder of accelerated growth, require both anti-estrogen and anti-androgen therapy to control growth.¹² Female human beings with McCune-Albright syndrome, a similar disorder of accelerated growth and precocious puberty, require only anti-estrogen therapy to control growth.¹² This suggests that the mechanisms controlling physeal closure are dependent on the expected hormonal milieu for that individual's gender. In dogs, osteoblasts do not decrease in number, but do decrease in function after ovariectomy.³⁰ Perhaps a decrease in osteoblast function contributes to the delay in physeal closure in gonadectomized cats. Study of the pop-

ulation of receptors for gonadal steroids at the physes, or comparison of physeal closure time to serum concentrations of gonadal steroids would further characterize these relationships.

Radial length at 4 months of age did not differ by group in either males or females, implying a random mix of sizes of male and female kittens in the 3 treatment groups. Final radial length, measured at the time of complete distal physeal closure, was significantly greater for both groups of male and female gonadectomized animals compared to intact animals. Age and radial length at the growth plateau also were significantly greater in both groups of gonadectomized males and females compared to intact animals. It has been demonstrated that increased limb length in gonadectomized dogs compared to intact dogs was not due to increased growth rate but rather to delay in physeal closure.¹⁴ This allowed for a longer period of chondrocyte proliferation and hypertrophy, and subsequent increased longitudinal bone growth. A similar mechanism may be present in cats. As expected, increase in radial length was strongly positively correlated with age. Both males and females in all groups exhibited growth curves that were linear to the growth plateau; no puberal growth spurt was observed, agreeing with previous work in this species^{16,17} (Fig. 4).

The data presented here support the hypothesis that physeal closure is dependent on gonadal steroids in male and female cats, and that gonadectomy prior to 7 months of age will retard physeal closure. Delay in physeal closure and possible subsequent predisposition to Salter fractures were not different in cats neutered at 7 weeks or 7 months of age.

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