

Short Communication

Prepubertal and Pubertal Canine Reproductive Studies: Conflicting Aspects

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Contents

Insufficient knowledge has been acquired regarding the prepubertal and pubertal periods of domestic canids. Until further information becomes available, a better standardization of the definitions, a careful and complete description of experimental variables and end points is necessary to diminish experimental bias in published trials. The aim of this report is therefore to discuss the definition of puberty and some of the most conflicting conditions influencing the pubertal transition (e.g. age and body weight and condition score among others) that, in turn, will be useful for the future design of reproductive studies on the domestic dog. Only trials that could be easily processed by a meta-analysis will contribute to an improvement of our general knowledge on the reproductive physiology of this particular species.

Introduction

Puberty – the process of physical changes by which an animal matures into an adult capable of sexual reproduction – is still a mysterious aspect of mammalian reproductive physiology involving a considerable number of intricately interlinked factors. Particularly, when a Medline search was performed for ‘puberty and dog’, only 62 results appeared, of which listing the majority of the most recent articles were on the effects of prepubertal gonadectomy and/or puberty prevention or postponement. In this respect, the proportion of books vs original articles that had to be cited to accomplish the objective of the present article is also noteworthy. Only few fundamental studies in the literature have evaluated the factors that influence puberty attainment in domestic canids. The insufficient knowledge pertaining specifically to this species’ pubertal process may lead to a conceptual oversimplification of the dog’s reproductive physiology. For example, the use of solely the chronological age, without further anamnesis as well as clinical findings, is one of the most frequent mistakes found in trials. Furthermore, as canine reproduction is one of the most dissimilar among the domestic animals, a simple extrapolation of principles from one species to another could result in mistakes.

The investigator, in planning and conducting a research project, has the responsibility of anticipating and minimizing potential difficulties, which may bias the results and give invalid conclusions (Ginther 2013). The aim of this article is therefore to discuss the definition of puberty and some of the ‘known’ conditions influencing the pubertal transition that, in turn, will be useful for the future design of reproductive studies on the domestic dog.

Definition

An absent, incomplete, or inadequate definition of end points is one of the most frequent shortcomings in reproductive studies (Ginther 2013). According to *Webster’s Dictionary*, puberty is ‘the period of becoming first capable of reproducing sexually, marked by maturation of the genital organs and development of secondary sex characteristics’. Nevertheless, that puberty is a gradual, quantitative process rather than an acute and qualitative transition should be born in mind (Pelletier et al. 1981). The duration of this developmental phase is normally inversely related to the complete lifespan of a particular species. In men, for example, there are five recognized developmental stages within the whole pubertal process – that is starting from the prepubertal Stage I and extending through adult development in Stage V (Tanner 1975). As a similar description of staging is not yet available for dogs, a clear definition that would provide the inclusive and exclusive end points – with respect to the physical, behavioural, seminal or endocrinal milestones involved – is essential for each gender to gain a complete appreciation and in-depth understanding of what is going to be defined as puberty in a particular study. The selection of these end points – although somewhat arbitrary – normally depends on the feasibility of obtaining this information in a given species.

Although, in bitches, because of their important physical manifestations, the onset of the first proestrus is generally considered puberty (Johnston et al. 2001); the actual situation is not so simple: as pubertal bitches are likely to manifest split or silent heats (Wildt et al. 1981; Johnston et al. 2001), what should exactly be considered puberty – for example, the first follicular phase, as shown by physical signs or vaginal cytology; the first time she permits mating; the first time she has a biphasic oestrous cycle, as evidenced by ovulation; the first time she has a normal luteal phase; or, finally, the extreme situation of the first time she becomes pregnant – all of these milestones must be clearly stated.

As in the female, the onset of puberty in the male dog has been defined in several ways in the literature. Although the age at which a dog can successfully be induced to ejaculate is generally considered puberty (Johnston et al. 2001), this event is highly variable. The first canine ejaculations could physiologically be incomplete or accompanied by functional abnormalities such as aspermia or oligospermia (Corrada et al. 2006). Furthermore, young dogs that have not yet reached complete sexual maturity may not ejaculate, even if capable of erection (Johnston et al. 2001).

However, male puberty is also said to occur with the following manifestations: sexual behavioural traits (e.g. mounting), spermatozoa in the ejaculate and/or in urine, or sperm parameters that are compatible with fertilization (Senger 2012); most of these possibilities in practice are limited with respect to species. The initial ejaculates from a dog after he reaches puberty often contain many abnormal and dead spermatozoa (Taha et al. 1981; Mialot et al. 1985.).

Thus, in both genders, a great effort will be required to define what exactly are going to be the characteristics considered to determine puberty. In this regard, the selection of objective criteria over subjective impressions is always preferable, and even the use of *ad hoc* scoring tables would be useful.

Conditions That Influence the Pubertal Transition

The developmental process that leads to puberty and the next step – that is sexual maturity – is based on an extraordinarily complex series of inter-related intrinsic and extrinsic events (Ojeda and Skinner 2006). For example, body weight, a major parameter affecting the onset of puberty, is a reflection of growth – it, in turn, being dependent on genotype and nutrition – so that to distinguish each relative contribution to the final attainment of sexual maturity is difficult. Other conditions affecting attainment of puberty – such as gender, body condition score, season of the year and social cues – are also discussed below within the context of the canine species.

A given age of the experimental animals is also frequently used as an inclusion criterion in most reports on prepubertal domestic dogs, but the question is whether or not the use of chronological age in canine prepubertal reproductive studies is truly valid. Unless the study involves a homogenous population of one single breed, the answer is that the criterion is not. Among domestic animals, dogs are unique in their huge intraspecies size differences – for example, from a body weight of less than 1.5 kg to one of more than 100 kg (i.e. a difference of nearly 100-fold), for example from an adult Chihuahua to a full-grown English Mastiff.

That biological processes occur differently in different body sizes – although they all are the same species – should always be born in mind, and in this respect, growth and reproduction are not exceptions. Thus, dogs do not grow and develop at the exact same basic rates – that is small-sized dogs grow much more rapidly than large or giant-sized dogs. A reciprocal effect of the growth rate on the timing of the onset of puberty in humans has been described (Bourguignon 1991) that seems to be valid in other species, too. In heifers, for example, the growth rate considerably influences the age for the onset of puberty (Funston et al. 2012).

Small and miniature breeds of dogs, which have steep growth curves, normally reach puberty at the age of 5–6 months (Johnston et al. 2001). Conversely, large and giant breed dogs are still ‘large-sized puppies’ at that age, attaining puberty only after 18 months, and this difference clearly evidences that, although of the same species, they definitively cannot be mixed in a trial

unless other parameters are taken into account (cf. below).

On the basis of these considerations, the logical conclusion is to avoid designing prepubertal or pubertal studies in which the only parameter applied to select dogs is the age, especially if different breeds or mature body sizes are used. In the instance of mixed-breed animals, the adult body size of the parents or the percentage of expected adult body weight (cf. below) would appear to be mandatory for consideration.

Physical growth: body weight

Before focusing on the question of body weight, the reason why this parameter, and not the adult height, will be discussed in this section should be clarified. The height at the withers in adult canines seems to be far more a consequence than an influencing condition of puberty. Although most mammals are assumed to experience growth-plate closure and cessation of bone growth soon after puberty (Kilborn et al. 2002), to the best of the author’s knowledge, only two studies have been reported showing this sequence in the canine species. An early report on beagle dogs describing the appearance of the first spermatozoa and the fusion of growth plates at the respective mean age of 8.5 months and 10 months (Mialot et al. 1985) seems to confirm this principle in dogs as well. In the other study in greyhounds, this same time points were described to occur at an average age of 7.4 and 8 months, respectively (Kilborn et al. 2002). The cessation of growth is secondary to epiphyseal closure – through the action of the sex steroids, and primarily estradiol in both sexes (Lee and Witchel 1997; Frank 2003.). Sex-steroid stimuli hasten skeletal maturity, thus determining adult height.

Conversely, in both animals and humans, body weight clearly influences the onset of puberty (Baker 1985) and is the most consequential parameter determining sexual maturity (Spitzer 1986). The attainment of a threshold or critical body weight is essential for puberty to occur: the status of somatic size is reported to the hypothalamus by some yet-to-be-determined metabolic cue that, in turn, acts as a signal to initiate puberty (Ojeda and Skinner 2006).

Abundant work has also shown that puberty is more closely related to body weight (assuming a normal dietary intake) than age in domestic animals. Thus, dairy cattle usually reach puberty when their body weight is 30–40% of the adult weight; beef cattle, 45–55%; and sheep, 40–60%. It is furthermore notable, that smaller breeds reach puberty earlier than large ones (Hafez and Hafez 1980). Although similar information is unfortunately missing in dogs, domestic cats normally achieve puberty at approximately 75% of the adult body weight (Stamou and Boscios 2001). As neither cats nor dogs have been genetically selected for an early onset of puberty, a similar percentage could be assumed to be valid for the canine species. As a provisional figure, a mean of 70–72% and 81% of the mature body weight could be calculated for the onset of puberty in tables from two different studies carried out on Labrador and beagle dogs (Taha et al. 1981; Wildt et al.

1981), thus supporting the use of this percentage until further more general data are available in canines. As mentioned before, it seems necessary to transform the prepubertal stages in which research interventions (e.g. medical treatments, gonadectomy) are performed if the final aim is to compare reproductive end points – for example puberty prevention or postponement, short- or long-term side effects in different breeds or adult body sizes in mongrels. For example, small and large breeds could be included and analysed together in a trial if they were entered in an experiment at 50% of their mature weight.

Body condition score

Shortly after the formulation of this so-called critical-weight hypothesis for mammalian puberty (Ojeda and Skinner 2006), the concept of critical body composition complemented that notion (Frisch 1997), as the balance between energy intake and expenditure is another major influence modulating the timing of sexual maturation. For example, puberty was argued to be most closely correlated with the attainment of a particular proportion of body fat in monkeys (Plant and Witchel 2006). To obtain not only a threshold of body weight but also a minimum percentage of fat has been proven to be essential for the onset and maintenance of oestrous cycles in different species that have been studied (Senger 2012). This critical amount of body fat clearly implies the requisite of a particular body composition. Although the exact percentage of fat content to attain puberty has not been reported in dogs, the inclusion (and mention) of animals within the normal body-composition score will further contribute to the homogenization of experimental animals into a uniform population.

Gender

A profound sex difference exists in the timing of puberty in many species (Kilborn et al. 2002; Foster and Jackson 2006). Males and females are known to undergo development at different rates, both *in utero* and postnatally up until postpuberty (Aiken and Ozanne 2013), and in this regard, dogs are no exception (Kilborn et al. 2002). As might be expected, sexual maturity is reached earlier in females than in males (Johnston et al. 2001), a situation that is preserved over a range of species from mice to humans (Aiken and Ozanne 2013). Gender differences are not a usual conflicting element, as the age at puberty is treated separately for the two sexes in almost all canine articles and textbooks.

Genetics

A consensus also appears to have arisen that heterosis of crossbred animals facilitates an earlier puberty than in the purebred counterparts. Age at puberty varies among breeds and even strains and families (Hafez and Hafez 1980; Taha et al. 1981). That in cattle, both age and weight at puberty have moderate to high heritability is most significant (Laster et al. 1979). Although similar

information is lacking in dogs, breed has obviously a significant influence on the age at which puberty is attained. Considering what has been said, the use of littermates of the same breed appears as a good and feasible approach to avoid the hazard of experimental bias in prepubertal and pubertal studies.

Photoperiod

Conducting research on reproduction without considering the effects of season even in non-seasonal breeders in certain parts of the world is fraught with pitfalls (Ginther 2013). Although domestic dogs have been traditionally classified as a non-seasonal species (Johnston et al. 2001), that categorization constitutes just one more example of a defective biological classification made in an attempt to organize the different sexual-cycling patterns among species.

Previous research has demonstrated that an extended daylight phase within the photoperiod accelerates pubescence in dairy heifers (Rius et al. 2005). In the example of dogs, what can only be stated for certain is that domestic canids have lost much of the seasonality that the wild counterparts conserve. Thus, the effect of photoperiod on bitches living in the outdoors all year round is widely accepted. Further supporting this, Linde-Forsberg and Wallén (1992) found that heat periods were unevenly distributed throughout the months of the year in beagles bitches kept as a colony and housed outdoors without supplementary light (Linde-Forsberg and Wallén 1992). In this respect, if experimental canines are completely or partially kept outdoors, the qualitative and quantitative description of their light exposure is essential information to be included in a publication. The exact geographical latitude and, preferably, the number of hours of light should be provided to the reader. A statement involving simply the months of the year is usually confusing and difficult to interpret for an international readership and is certainly not descriptive of the lighting circumstance *per se*. Furthermore, photoperiod is a dynamic biphasic occurrence (i.e. with an ascending and a descending limb), and the clear description of its evolution throughout the study period should greatly facilitate comparisons among trials.

Social cues and other conditions

The presence or the absence of mature animals of the opposite and/or same sex during the prepubertal period has not been studied in dogs. Although, until proven, otherwise, the presence of peer canines could be assumed to hasten the onset of puberty, and therefore, prepubertal experimental animals should be isolated from the adult dogs that might be present to avoid confounding factors.

Other less known environmental and nutritional conditions could also have an effect on the physiological development in puberty so as to make the process extremely variable. Thus, the researcher should be aware of these potential pitfalls in advance and the possibility of avoiding those influences in the design of experiments.

Discussion and Conclusion

This article discusses the most frequent pitfalls and bias that are encountered in canine pubertal studies suggesting how to approach planning of this kind of trials. Upon making a comparison with other better studied species, it is concluded that we still have an insufficient understanding of the various parameters involved in the sexual maturation of domestic dogs and possess a limited knowledge of breed-specific differences as well as within-breed variations. Furthermore, the influences triggering the pubertal process act in a complex, interlaced way, making the establishment of a causal relationship difficult unless the experiments are carefully designed. Thus, acknowledging this complex situation, we, canine theriogenologists, should be cautious during the planning stage of experiments. Until further information becomes available, a better standardization of definitions, a detailed and complete description of the end points and variables involved in that process is essential to diminish experimental bias in trials.

This paper can also guide practitioners who are looking for an answer to the usual clinical questions (e.g. when the best time is to have the dog gonadectomized, how it is possible to postpone puberty, if a giant bitch should be treated for primary anestrus) by proposing a critical reading of the reports already published.

Finally, this article also is directed at stimulating research that would point out the principal conditions (e.g. the percentage of mature body weight, among others) to be considered in prepubertal and pubertal studies. Publications having a lack of definitions, incomplete descriptions of experimental variables or end points have seldom met what might be referred to as the incorporation criteria constituting adequacy for inclusion in systematic reviews and thus have remained apart. Such isolated studies, whose variables are poorly or even not at all described, present results with severely limited applicability. Only trials that could be easily classified in groups and compared by a comprehensive meta-analysis will contribute to the improvement of our general knowledge on the reproductive physiology of this unique species.

Conflict of interest

The author of this paper has no financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Author contribution

CG has written all the manuscript.

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Submitted: 27 May 2014; Accepted: 31 Jul 2014

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