



Swedish University of
Agricultural Sciences

**Reproductive patterns in the domestic
dog; a retrospective study with the
Drever breed as model**

Bojana Bobic Gavrilovic
Department of Clinical Sciences
Faculty of Veterinary medicine and Animal science

**Master of Science Programme in Veterinary Medicine
for International Students
Swedish University of Agricultural Sciences**

Uppsala 2007

Report- Master of Science Program in Veterinary Medicine for International Students
Faculty of Veterinary Medicine and Animal Science
Swedish University of Agricultural Sciences
Report No. 68
ISSN 1403-2201

**Reproductive patterns in the domestic
dog; a retrospective study with the
Drever breed as model**

Bojana Bobic Gavrilovic

Department of Clinical Sciences
Faculty of Veterinary medicine and Animal science

**Master of Science Programme in Veterinary Medicine
for International Students
Swedish University of Agricultural Sciences**

Uppsala 2007

The present study is a partial fulfilment of the requirements for the Master of Science (MSc) Degree in Veterinary Medicine for International Students at the Swedish University of Agricultural Sciences (SLU), in the field of Animal Reproduction.

Bojana Bobic Gavrilovic
Division of Reproduction
Department of Clinical Sciences, P.O.Box 7054
Faculty of Veterinary Medicine and Animal Science
Swedish University of Agricultural Sciences (SLU)
SE-750 07 Uppsala Sweden

*To my only son Andrija,
with love*

ABSTRACT

Despite the long history of joint life between man and the dog (*Canis familiaris*), there are still many aspects of canine reproduction that are not well understood. Domestication of the dog from its ancestor the wolf is thought to have occurred at least 14 000 years ago

It is a unique situation that both the ancestor – the wolf, and the domesticated descendant – the dog, are still present world wide, which gives them a very important place in the fields of interest for scientific research. The reproductive pattern of the dog, as a very diverging species among all the mammals, requires specific methodology in research and studies.

To study the differences between seasons of the year regarding the distribution of matings and whelpings, litter size, neonatal deaths and sex ratio, and the effects of age and parity of the bitch at the time of whelping on litter size, as well as the influence of litter size on the length of gestation, and fertility and frequency of whelping problems in the domestic dog, data from the Swedish Kennel Club (SKK) registry for the Drever breed during 1995-2006, 2717 registered litters, was analyzed together with more detailed data from a private, professional kennel of Drevers, with 285 matings and 224 whelpings during the same time period. Significantly more matings took place during winter, and the fewest during summer. Most whelpings took place during the winter and spring seasons. Of the 224 pregnant bitches 78% whelped, 6.25% experienced dystocia, and 5.36% underwent a Ceasarian section. Duration of pregnancy was not different between seasons. The largest litters were born during spring. Litter size was significantly, negatively, correlated with the duration of pregnancy ($r = -0.18$). Each extra pup caused a shortening of the gestation by 0.25 days. Bitches giving birth to their first litter after 5 years of age produced a smaller litter than younger bitches. For all the whelpings, bitches gave birth to 0.33 pups less per litter per additional year of age. Litter size in the private kennel increased from the 1st to the 3rd parity, and then decreased. The number of registered pups, increased from the 1st to the 2nd parity in the SKK data and from the 2nd to the 3rd parity in the private kennel, and then decreased. Mating a bitch only once resulted in a smaller litter size compared to multiple matings. None of the studied factors had any influence on the sex ratio. There were significant differences between males in whelping rate among the mated bitches, but no difference in mean litter size, which indicates a female problem rather than a male one. Available data strongly suggest that the domestic dog still is under a considerable seasonal influence, modified by ambient and management factors.

Key words: seasonality, age, parity, gestation length, litter size, sex ratio, male fertility

Author's address: Bojana Bobic Gavrilovic, Department of Clinical Sciences, Division of Reproduction, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences (SLU), PO Box 7054, SE-750 07 Uppsala, Sweden. On leave from the Velvet Animal Health, Vile Ravijojle 3G, 11040 Beolgrade, Serbia.

Contents

Background, 9

The Drever, 9

Retrospective studies on reproduction in dogs, 10

Dog reproduction – specific pattern, 11

Introduction to the study report, 17

Aims of the study, 17

List of references, 18

Research Report, 20

Bojana Bobic Gavrilovic, Kjell Andersson, Catharina Linde Forsberg

Reproductive Patterns of the domestic dog

Retrospective study with the Drever breed as model

Acknowledgements, 44

BACKGROUND

THE DREVER

The Drever was in this study used as a model for most other breeds of dogs for two main reasons: its reproductive pattern is the one most commonly observed in the dog as a species in this part of the world, and we had the opportunity to access detailed reproductive data from a private, professional kennel for a 12 year-period, of a character that is not registered in the Kennel Club data base. This data was complemented with the registered information available from the Drever registrations in the data base of the Swedish Kennel Club (SKK) during the same time period, and the breed, thus, served as a good model to study the factors affecting reproduction in most of the other breeds of dogs, excepting those that are more strictly seasonal autumn breeds, such as the Basenji.

The Drever is a national hunting breed (scent hound) in Sweden. Although it may not have a big international interest in itself, it serves as a good model for most of the other breeds of dogs in reproduction; and its long history of breeding in Sweden offered a good collection of data and provided information on a large number of individual dogs, and litters of pups to be used for this study, which was a good solution for our research.

In Swedish, a “drev” means the hunting style when a dog is chasing an animal by following its scent. Officially named in 1947, and recognized as a Swedish breed in 1953, the Drever has become one of the most popular hunting breeds in its country of origin. The Swedish Kennel Club recognized this breed in 1949. Outside of the Fédération Cynologique Internationale (FCI) it was officially recognized in Canada in 1956. It belongs to the FCI group 6, breed standard No. 130.

The Drever is one of the short-legged hunting dogs. The breed has become a popular hunting hound especially for roe deer in Sweden, and also in the neighbouring Norway and Finland. The Drever arose, as most of the present breeds, from a mix of a variety of breeds. A very important ancestor is the German Dachsbracke, which also is the ancestor of the Dachshund breeds. The ideal height at the withers of the Drever is 35 cm for the males (32-38 cm) and 33 for the bitches (30-36 cm), which is about 20-25 cm lower than a normal sized Swedish foxhound (Schiller- and Hamiltonstövare). The body weight is usually between 10 to 15 kg. The size of the Drever has its practical reasons, as the hounds are mainly used to hunt roe deer and therefore need to hunt at a slow pace so that the roe deer will move around in circles and not leave the area.

In the northern part of Scandinavia the population of roe deer is much less numerous than further south, so in these areas the Drever should be more persistent in its pursuit of the game than in the more densely populated areas in the mid- and south areas of the country, and it is therefore necessary to have a hound that also tolerates the harsh conditions up north. Steep hills, cold weather, a

varying amount of snow - the hounds have to cope with all this and still perform. The Drever can chase the animal for a long period of time (usually 1-3 hrs or more), it tolerates harsh conditions well, and it is not too fast. Because of its short legs, it is slower than the larger hounds, which makes it ideal for this kind of hunt. The Drever is a slow, steady worker, which also can hunt the hare, the fox, and occasionally other types of deer; this breed even has the courage to pit himself against a wild boar. The Drever is headstrong and tenacious. It has a first-class nose and is a powerful tracker. When kept indoors, as a family member, he tolerates just about anything.

RETROSPECTIVE STUDIES ON REPRODUCTION IN DOGS

The reproductive pattern of the dog, as a very diverging species among all the mammals, requires specific methodology in research and studies. The very extended period between two estrus cycles, and the resulting small number of cycles and, thus, of whelpings during a life time provides less information to researchers striving to develop our knowledge about the rules and patterns that regulate the reproductive processes in this species, and which are different from those of most other species. Compared to the farm animals, kept as production animals, as well as to the experimental, commonly used laboratory animals, dogs are, therefore, more difficult to work with. They are also expensive to keep as research animals, especially in case of research that is connected with some kind of “life time” pattern. In order to find out any guidelines in reproduction of the dog as a species, it is important to have a big sample, a lot of matings, whelpings, and litters. There are also ethical issues that need to be taken into account, in order to justify keeping a large number of animals possibly during their lifetime. On the other hand, cats with a more intensive reproductive pattern could be used more easily, but because of the very pronounced differences in reproductive physiology of those two species, the cat cannot serve as a model for the dog.

To analyze data from privately owned animals already available in various data bases in retrospect is a good way to acquire information about the reproductive patterns in dogs, without having to resort to the use of experimental animals. Some sources of this kind of reproductive data are extensive, such as the SKK registry data base, that contains information about around 15,000 litters resulting in between 50,000 and 60,000 registered pups per year since 30 years, and which thanks to all the technological advances during later years is easily available. In this kind of study, it is not very important if we are looking at events that happened 10 years ago or just some time ago. The reproductive patterns are not changing a lot in such a short time frame; dogs have been living with us humans for more than 15,000 years, and it is a challenging fact that we still don't know all about them.

A source of the data we need could also be the pet owners (with questionnaires, or directly from interviews and phone contacts), the breeders (they usually have some kind of personal diary data base about every breeding dog in the kennel), the

specific breed clubs or the National Kennel Club for all the breeds. Also veterinary clinics and insurance companies could provide a huge database in some instance. This kind of database, on the other side, has some disadvantages too. Some of the data could be erased or lost, and time distance does not give us any possibility to check or change them. This has the consequence that much data may have to be excluded from the study, if we want to have secure and clear results.

The present study provides information from a unique database (at the SKK) and with more detailed information from a large private kennel, which is not ordinarily available for research. The study was done using one dog breed as a model for others, and the registered litters in the Swedish Kennel Club (2717 litters) for a period of 12 years (1995 – 2006) with a deeper analysis of data obtained from a private, professional kennel (284 matings), during the same time period. It gave us the possibility to come up with a number of conclusions about the reproductive pattern in this breed of dog, and also on the effects of human influence such as breeding management and life style on the reproductive performance of this breed of dogs. Food quality and food intake habits, artificial light, heating during winter, keeping the dogs together in the same kennel, choosing the best performing individuals in the breeding program, and breeding them only once or more times during their life time, are all effects that can change the pattern of reproduction in dogs. Comparing a big sample of animals can show us some general rules and environmental factors that can influence the physiological patterns.

The aims of this study were to define differences between seasons of the year regarding the distribution of matings and whelpings, dystocia and Caesarian section events, litter size, pup deaths and sex ratio, and the effects of age and parity of the bitch at the time of whelping on litter size, as well as the influence of litter size on the length of gestation, and differences in fertility among the males in the Drever breed.

DOG REPRODUCTION – SPECIFIC PATTERN

The wolf – the ancestor of the dog

Today when we are surrounded by more than 400 million purebred, mongrel or feral dogs, we know that domestication of this species started at least 15,000 years ago, but some authors have suggested that it happened even more than 40,000 years ago. The ancestor of the domestic dog is nowadays considered to be only the wolf (Vila *et al.*, 1997). It is a unique situation that both the ancestor – the wolf, and the domesticated descendant - the dog, are still present world wide, which gives them a very important place in the fields of interest for scientific research.

Comparing behavioral, physiological and genome differences it is possible to show many events during the process of domestication. Scientific researchers are developing many tools to identify genes and the causes of different disorders, a fact that gives the dog a very important position at present and for the future as a model for research on human diseases.

The reproductive physiology of the wolf as the ancestor of the dog is of interest for us to compare with that of the domestic dog to show the differences and similarities, and any possible links between those two species.

The wolf (*Canis lupus*) is a monoestrous, seasonal breeder, mostly coming in heat in the months of January to March. This season can be extended from December until April depending on the region and the temperature in which the animal lives. The interestrous period of sexual quiescence is very long in the wolf bitch, longer than in most female dogs.

The estrus cycle in the wolf shows some difference in the length of the periods, compared to in the bitch. Proestrus can be longer, 15 – 16 days, with an oestrus period of on average 9 days (Seal *et al.*, 1979). There is no information on ovulation dates in wolves, but it is known that in the coyote (*Canis latrans*), a species that is very closely related, ovulation occurs from 1 – 9 days after the onset of estrus.

The reproductive life of the female wolf is usually shorter than that of the female dog, since there was no wolf bitch that was bred before 22 months of age (Morey, 1994), or after 9 years of age in captivity or after 7 years in the wild (Lentfer & Sanders, 1973). The female wolf also becomes sexually mature much later than does the female dog which becomes fertile (sexually mature) at between 6 – 15 months of age (Wildt *et al.*, 1981) It has not been determined how far back in history this difference first became evident .

Although it has been shown that the fecal androgen metabolite concentrations are low outside of the breeding season in the male red wolf (Walker & al., 2002), during late autumn when the breeding season is approaching the testosterone concentration increases (Asa *et al.*, 1987) and peaks in late February. Short-day photo periods stimulate testis function, to ensure maximal sperm production when the female becomes sexually receptive (Asa *et al.*, 1987; Walker & al., 2002).

Gestation length in the wolf is 62 ± 4 days and the litter size is between 1 and 11 pups, with an average of 6 (Lentfer & Sanders, 1973).

The wolf is monogamous, forming pairs before the breeding season, and usually remaining together for years taking care of the litters together (Malcolm, 1985), in contrast to most dogs. The same monogamous, family style care of the litters could be seen in some other canidae species: maned wolves, coyotes and other (Veado, 2005). One of the most important of the behavioural modifications attributed to domestication in the dog may be the trend towards promiscuous rather than pair-bond mating (Kretchmer and Fox, 1975).

The dog

The dog has passed through many changes during the process of domestication, and when compared to wolves, morphological, behavioral and genetic changes can be seen.

Morphologically we can mention differences in body size, which could be due to an alteration in the selection for social compatibility, including a strong selection on reproductive timing. This has resulted in a lowering of the age at sexual maturation and earlier breeding in dogs, compared to the wolves. Reproduction was planned and managed by the owner, and the pups were raised by man.

The domesticated dog needed to share human's meals, a mixture of meat and plants, in contrast to the wild wolf that is taking the nutrition mainly from meat. From the beginning, dogs were smaller than the wild ancestor the wolf (Morey, 1994). Man selected for the juvenile looks and even juvenile acting in dogs in reproduction to enhance well managed individuals and socialization of the dogs living with humans. Later during domestication and through selection, especially when the breeding of pure breeds of dogs started, the variations in body size and shape became amazing. If we compare the Great Dane with the Chihuahua, the differences are extreme. Also, comparing the domestic dogs with a wolf, there are differences in skull size, brain size, color and quality of the hair coat.

Behaviour changes are the result of the adaptation of the wolf to life with man. It could be that food intake habits made them calm, not to be killed or abandoned (Morey, 1994; Hare *et al.*, 2002). They started barking, which is not common in wolves, to protect the owner – the supplier of food, but also there were acting juvenile, played and acted like pups throughout their life time, which is not usual in mature wolves.

The process of domestication can be described as some kind of a “both ways relationship”, as man's life style has also been radically affected by the animals he started to raise. In a study from 1994 (Morey, 1994), the author suggested that the domestication of animals is an evolutionary process, and the dogs were the pioneers in this event.

The dog's reproductive pattern is very different from that of all other animals, and an understanding of those differences can help in the process of breeding, especially predicting an event in the estrus cycle, or the duration of gestation and the timing of parturition in the bitch. There are certain limits, the temporal changes in reproductive hormones during the cycle of the dog, which can help to predict events (Concannon *et al.*, 1975). Many of the animals have failed to reproduce or have problems with infertility, and this puts a pressure on veterinarians to find solutions, and on the other side, many countries in the world are fighting with stray roaming dogs without any control of reproduction, and more knowledge about their reproductive pattern could be a main key for solving this problem too. Using dogs in other scientific research and experiments, as a model for humans or other animals, requires more knowledge about their specific reproductive pattern too.

Dogs are considered as nonseasonal, mono-estrous breeders.

The bitch

The bitch becomes fertile (sexually mature) at between 6 – 15 months of age, depending on breed and body size (when having reached 85% of the adult body weight). Some of the large breeds can become fertile later (18 – 20 months). The first estrus cycle can be very quiet and with less shown signs, because at this age the level of the reproductive hormones is lower than in the adult animal (Wildt *et al.*, 1981). Fertility lasts until the end of the bitch's life, there is no menopause. It has been shown that fertility can decrease during life time, that the interestrus period can be prolonged with age (Bouchard *et al.*, 1991), and that litter size can decrease in relation to ageing (Andersen, 1965).

The estrus period of the domestic bitch is divided in 4 phases:

- Proestrus
- Estrus
- Metestrus (some prefer to call this stage diestrus)
- Anestrus

Proestrus is the first stage of the cycle of the bitch. It lasts on average 9 days (from 3 – 27 days). It is recognized by the beginning of a vaginal haemorrhage, the males show an interest in the bitch, but it is not ready to mate and refuses the males. It is difficult to predict the beginning of proestrus. The main changes are seen as a swelling and oedema of the vulva. Bleeding starts on day one of proestrus and continues into estrus, Even if the bitch has a vaginal bleeding in proestrus like in the estrus, she refuses mating until estrus. This is a phase of growing follicular activity. The observed haemorrhage from the vulva begins as an intrauterine haemorrhage that flows through the patent cervix into the vagina. Diapedesis and subepithelial capillary rupture in the endometrium in response to increasing follicular estrogen production, synthesized by follicles which begin to develop in anestrus supported by the pituitary hormones. In this phase of estrogen dominance, the vaginal mucosa becomes thick, because the basal germinative cells layers start to grow and replicate, and later during other phases, the keratin production is responsible for the specific vaginal smear, an important diagnostic method to predict estrus, ovulation, and mating or the time to perform artificial insemination.

Estrus, is the phase that begins when the bitch accepts the male. The duration of this phase is usually 9 days, like for the proestrus, (on average) but it can be shorter (3-6 days), or much longer (18- 28 days), and the changes such as the vulvar edema and swelling are less marked. Usually the bitch is consistent in estrus length from cycle to cycle, but this is not always the case. It is very difficult to predict any new event.

The only event which can be used to predict ovulation is the LH peak, and ovulation occurs 24 – 48 hours later, but some follicles can continue to ovulate up to 72 hours later (Wildt *et al.*, 1978). Reduced hemorrhage will continue for some time, and may persist until the end of heat, same as attraction for male can persist. Estrogen starts to decrease, and the final maturation of the follicles is a few days before the first ovulation With maturation of the follicles begins a fast luteinisation

and synthesis of progesterone. Bitch oocytes after ovulation are immature, and maturation takes 2 – 5 days, after which they become fertile (Holst & Plemister, 1971; Tsutsui, 1989). This event takes place in the lower parts of the oviducts. When maturation has taken place, and at the best time for fertilization and thus for mating or AI, the progesterone level is around 30 – 70 nmol/l. Because of the long period of readiness to mate and survival of oocytes a bitch can give birth to litters of pups resulting from matings with different male dogs. Also, it is known that dog spermatozoa can survive in the uterus for 4 – 6 (up to 11) days after single mating (Doak *et al.*, 1967) with storage of sperm within the female reproductive tract, attached to epithelial cells of the endometrium (England *et al.*, 2006).

Progesterone continuously increases during the following 1 – 3 weeks. Increase of progesterone and decrease of estrogen give two important effects: First, the behavioral changes with a readiness to mate, and secondly, a feedback to the pituitary and hypothalamic axis, and synthesis of FSH and LH hormones. This is a key event in the estrus period of the bitch. The dog is unique among all other animal species with this preovulatory production of progesterone and the level of this important reproductive hormone can help us in predicting all the events in this stage of the reproductive scheme.

An accurate prediction of the ovulation day could be done by measuring the LH concentration in peripheral plasma (LH assays are available) but as the event lasts only for one or two days, it would necessitate daily sampling and be expensive in the clinical practice. This means that measuring of the progesterone concentration and taking vaginal smears are used most commonly as methods for determination of the most fertile days during the heat.

The progesterone concentration before the LH peak is basal (<0.5nmol/l) and increases shortly before the LH peak. At the time of ovulation, this level is 12 – 24nmol/l and at the optimal time for breeding or performing artificial insemination (AI) it is between 30 and 70nmol/l. It then continues to increase for another week to a maximum of around of 150nmol/l, and then slowly decreases during the 2-3 month period of metestrus.

Metestrus comes after the estrus period and there is a dispute about its duration. It starts with the bitch refusing the male and lasts for 2-3 months (63 days if the bitch is pregnant). During metestrus the peripheral plasma levels of progesterone is raised, but decreases to basal levels by the time of parturition in the pregnant bitch, or within 2-3 months in the non-pregnant bitch.

Anestrus is the phase of transition between one cycle and the next. It can last for 1 –9 months, and it is characterized by the absence of all signs of reproductive activity. The interval between two estrus periods can be 2 months longer if the bitch has given birth to a litter of pups (Linde-Forsberg & Wallén, 1992).

The male dog

Puberty in the male dog occurs at between 6 – 12 (up to 18) months of age, depending on breed and body size (Olar *et al.*, 1983; Rijsselaere *et al.*, 2007), and some environmental factors, body condition and nutrition. At this age, in dogs, begins sperm production, sperm cell maturation (in the epididymis) and storage of spermatozoa in the tail of the epididymis. Usually, male dogs become sexually mature 2 – 3 months after they have reached 85% of adult body size.

The testes, two in number, produce the spermatozoa and a small amount of the fluid that constitutes the seminal fluid. The rest of the fluid of the ejaculate is secreted by the epididymis and mainly by the prostate gland.

The ejaculate of the dog has three fractions. The first fraction, 0.5 – 7ml of clear fluid, and the last fraction which can be up to 30 – 40ml in large breeds, are secreted by the prostate gland and contain but few spermatozoa. The second fraction, rich in spermatozoa is usually 0.5 – 3ml of differently intense and colored fluid, depending on the sperm concentration. The color is mainly milky white. The total number of spermatozoa in the ejaculate can be between 100 to 5000 x 10⁶, and the motility should be 70% or higher (Oettlè, 1993). The percentage of normal spermatozoa seems to be less important than the total number of motile and fertile cells in the ejaculate (Linde-Forsberg & Forsberg, 1989).

Daily sperm production has been found to be 12 – 17 x 10⁶ spermatozoa per gram of testis parenchyma. And this could be linked to the relation between body weight, or breed of the dog and the sperm producing ability (Olar *et al.*, 1983). It has been shown that the sperm concentration can be influenced by season (Taha *et al.*, 1981b; Martins *et al.*, 2006) but the libido, volume of the ejaculate, and percentage of dead and abnormal spermatozoa was not influenced by season. The same authors, in other reports have shown that the age of the male dog is negatively correlated with the percentage of normal spermatozoa (Taha *et al.*, 1981a; Rijsselaere *et al.*, 2007)

Pregnancy

Gestation length in the dog is the period from the first natural mating or artificial insemination to the day of parturition. On average it lasts 62 -64 days (Concannon *et al.*, 1983). The range of the gestation length seems to be from 56 to 72 days, and it depends on many factors. Even after a single mating, the length can range from 56 to 68 days (Holst & Phemister, 1974).

Breed (Okkens *et al.*, 2001), age of the bitch (Andersen, 1965), and parity at the time of mating, litter size and season are reported to be factors that can effect gestation length (Okkens *et al.*, 2001; Kutzler *et al.*, 2003a; Kutzler *et al.*, 2003b; Tsutsui *et al.*, 2006).

Average litter size for most of the breeds is 3 – 7 pups per litter, but the breeds with smallest and largest litters extends this range from 1 to an extreme, with more than 15 pups. Litter size depends on the breed of the dog, and is linked with body size (Tedor & Reif, 1978; Okkens *et al.*, 2001).

INTRODUCTION TO THE STUDY REPORT

There are many aspects of the reproductive pattern of the domestic dog that have not been explained or proven yet. Many studies have been published on this topic over the years, that have answered some of the questions, making steps in the progress, but also have raised new ones, and several of those questions are still open:

-Are there differences between seasons regarding the distribution of matings and whelpings, effects of the season on the duration of gestation, or litter size, sex ratio and pup deaths?

-To what extent is litter size affected by the age and parity of the bitch at the time of whelping?

-Does litter size affect the duration of gestation, and if so how much?

-Are there any differences in fertility of the male dogs that can affect whelping rate in the bitches they mate or their litter size?

To make it easier to understand the reproductive patterns of dogs, we will try to answer some of those questions. This knowledge of applied science is going to be useful to all people connected with the breeding of dogs. Making an advance breeding plan, it will be possible for breeders to use breeding dogs with higher success rate, without effects on the dogs' health. Additionally it would make it easier for veterinarians to predict all the events related to the reproduction and particularly parturition in this species. Furthermore, after reading the present study report, still more questions and possibilities remain open for future research

AIMS OF THE STUDY

The aims of this study were to define differences between seasons of the year regarding the distribution of matings and whelpings, litter size, pup deaths and sex ratio, and the effects of age and parity of the bitch at the time of whelping on litter size, as well as the influence of litter size on the length of gestation, and also the fertility and frequency of whelping problems, and differences in fertility among the males in the Drever breed

LIST OF REFERENCES

- Andersen, A. 1965. Reproductive ability of female Beagles in relation to advancing age. *Exp Geront* 1, 189-192.
- Asa, C.S., Seal, U.S., Letellier, M., Plotka, E.D. & Peterson, E.K. 1987. Pinealectomy or superior cervical ganglionectomy do not alter reproduction in the wolf (*Canis lupus*). *Biol Reprod* 37, 14-21.
- Bouchard, G. et al. 1991. Seasonality and variability of the interestrus interval in the bitch. *Theriogenology* 36, 41-50.
- Concannon, P., Whaley, S., Lein, D. & Wissler, R. 1983. Canine gestation length: variation related to time of mating and fertile life of sperm. *Am J Vet Res* 44, 1819-1821.
- Concannon, P.W., Hansel, W. & Visek, W.J. 1975. The ovarian cycle of the bitch: plasma estrogen, LH and progesterone. *Biol Reprod* 13, 112-121.
- Doak, R.L., Hall, A. & Dale, H.E. 1967. Longevity of spermatozoa in the reproductive tract of the bitch. *J Reprod Fertil* 13, 51-58.
- England, G.C., Burgess, C.M., Freeman, S.L., Smith, S.C. & Pacey, A.A. 2006. Relationship between the fertile period and sperm transport in the bitch. *Theriogenology* 66, 1410-1418.
- Hare, B., Brown, M., Williamson, C. & Tomasello, M. 2002. The domestication of social cognition in dogs. *Science* 298, 1634-1636.
- Holst, P.A. & Phemister, R.D. 1971. The prenatal development of the dog: preimplantation events. *Biol Reprod* 5, 194-206.
- Holst, P.A. & Phemister, R.D. 1974. Onset of diestrus in the Beagle bitch: definition and significance. *Am J Vet Res* 35, 401-406.
- Kutzler, M.A., Mohammed, H.O., Lamb, S.V. & Meyers-Wallen, V.N. 2003a. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. *Theriogenology* 60, 1187-1196.
- Kutzler, M.A., Yeager, A.E., Mohammed, H.O. & Meyers-Wallen, V.N. 2003b. Accuracy of canine parturition date prediction using fetal measurements obtained by ultrasonography. *Theriogenology* 60, 1309-1317.
- Lentfer, J.W. & Sanders, D.K. 1973. Notes on the captive wolf (*Canis lupus*) colony, Barrow, Alaska. *Can J Zool* 51, 623-627.
- Linde-Forsberg & Forsberg. 1989. Fertility in dogs in relation to semen quality and the time and site of insemination with fresh and frozen semen. *Journal of Reproduction and Fertility, Supplement* 39, 299-310.
- Linde-Forsberg & Wallén. 1992. Effects of whelping and season of the year on interestrus interval in dogs. *Journal of Small Animal Practice* 25, 77-82.
- Malcolm, J.R. 1985. Paternal care in canids. *American Zoologist* 25, 853-856.
- Martins, M.I., de Souza, F.F., Oba, E. & Lopes, M.D. 2006. The effect of season on serum testosterone concentrations in dogs. *Theriogenology* 66, 1603-1605.
- Morey, D. 1994. The early evolution of the domestic dog. *American Scientist* 82, 336-347.
- Oettlè, E.E. 1993. Sperm morphology and fertility in the dog. *J Reprod Fertil Suppl* 47, 257-260.
- Okkens, A.C., Teunissen J.M., Van Osch W., Van Den Brom W.E., Dieleman S.J. & Kooistra H.S. 2001. Influence of litter size and breed on the duration of gestation in dogs. *J Reprod Fertil Suppl* 57, 193-197.
- Olar, T.T., Amann, R.P. & Pickett, B.W. 1983. Relationships among testicular size, daily production and output of spermatozoa, and extragonadal spermatozoal reserves of the dog. *Biol Reprod* 29, 1114-1120.

- Rijsselaere, T., Maes, D., Hoflack, G., de Kruif, A. & Van Soom, A. 2007. Effect of body weight, age and breeding history on canine sperm quality parameters measured by the Hamilton-Thorne analyser. *Reprod Domest Anim* 42, 143-148.
- Seal, U.S., Plotka, E.D., Packard, J.M. & Mech, L.D. 1979. Endocrine correlates of reproduction in the wolf. I. Serum progesterone, estradiol and LH during the estrous cycle. *Biol Reprod* 21, 1057-1066.
- Taha, M.A., Noakes, D.E. & Allen, W.E. 1981a. Some aspects of reproductive function in the male Beagle at puberty. *J Small Anim Pract* 22, 663-667.
- Taha, M.B., Noakes, D.E. & Allen, W.E. 1981b. The effect of season of the year on the characteristics and composition of dog semen. *J Small Anim Pract* 22, 177-184.
- Tedor, J.B. & Reif, J.S. 1978. Natal patterns among registered dogs in the United States. *J Am Vet Med Assoc* 172, 1179-1185.
- Tsutsui, T. 1989. Gamete physiology and timing of ovulation and fertilization in dogs. *J Reprod Fertil Suppl* 39, 269-275.
- Tsutsui, T., Hori, T., Kirihara, N., Kawakami, E. & Concannon, P.W. 2006. Relation between mating or ovulation and the duration of gestation in dogs. *Theriogenology* 66, 1706-1708.
- Walker, S.L. & al., e. 2002. Reproductive endocrine patterns in captive female and male red wolves (*Canis rufus*) assessed by fecal and serum hormone analysis. *Zoo Biology* 21, 321-335.
- Veado, B.V. 2005. Paternal behaviour of Maned wolf *Chrysocyon brachyurus* at Fundação Zoo-Botânica de Belo Horizonte. *International Zoo Yearbook* 39, 198-205.
- Vila, C. et al. 1997. Multiple and ancient origins of the domestic dog. *Science* 276, 1687-1689.
- Wildt, D.E., Chakraborty, P.K., Panko, W.B. & Seager, S.W. 1978. Relationship of reproductive behavior, serum luteinizing hormone and time of ovulation in the bitch. *Biol Reprod* 18, 561-570.
- Wildt, D.E., Seager, S.W. & Chakraborty, P.K. 1981. Behavioral, ovarian and endocrine relationships in the pubertal bitch. *J Anim Sci* 53, 182-191.

Research Report

Reproductive patterns in the domestic dog - a retrospective study, with the Drever breed as model

Bobic Gavrilovic B¹, Andersson K², & Linde Forsberg C¹

¹Department of Clinical Sciences, Division of Reproduction, ²Department of Animal Breeding and Genetics, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract

The aim of this study was to examine the differences, between seasons of the year, in the distribution of matings and whelpings, litter size, neonatal deaths, and sex ratio in domestic dogs. Furthermore, we wanted to examine the effects of age and parity of the bitch at the time of whelping on litter size, as well as the effect of litter size on gestational length. A final aim was to investigate the fertility and frequency of whelping problems in a private kennel of Drever dogs. Data from the Swedish Kennel Club (SKK) registry for the Drever breed during 1995–2006, comprising a total of 2,717 litters, was analyzed together with more detailed data from a private, professional kennel of Drevers, with a total of 285 matings and 224 whelpings, during the same time period. The most matings took place during winter, and the fewest during summer; consequently, most whelpings occurred during the winter and spring seasons. Of the 285 mated bitches, 78.6% whelped, 6.25% experienced dystocia, and 5.36% underwent Cesarean section. The pup death rate was 7.6%. The largest litters were born during spring. Litter size was negatively correlated with duration of pregnancy ($r = -0.18$). Each pup more than average caused a shortening of the gestation by 0.25 days, and each pup less a corresponding lengthening. Bitches giving birth to their first litter after 4 years of age produced a smaller litter than younger bitches. Litter size decreased after 5 years of age in all bitches. The number of born pups at the private kennel increased from the first to the third parity, then decreased. The number of registered pups increased from the first to the second parity in the SKK data and

from the second to the third parity in the data from the private kennel, then decreased. Mating a bitch only once resulted in a smaller litter size. None of the studied factors had any effect on the sex ratio of the pups. There were significant differences between males in whelping rate among the mated bitches, but no difference in mean litter size, which indicates a female problem rather than a male one. Available data strongly suggest that the domestic dog is still under considerable seasonal influence, modified by ambient and management factors.

Key words: seasonality, age, parity, gestational length, dystocia, Cesarean section, litter size, sex ratio, male fertility

1. Introduction

Despite a long history of cohabitation between humans and dogs (*Canis familiaris*), there are still many aspects of canine reproduction that are not well understood. Domestication of the dog from its ancestor, the wolf, is thought to have occurred at least 14,000 years ago [35], although recent research using molecular genetic tools indicates that it may have started as early as around 40,000 years ago. Changes that have occurred over time have been profound, from the diversity of body size and shape (from that of the wolf to the 1.5 kg Chihuahua and the 100 kg or more giant breeds of dogs) and of behavior, food intake, etc to the reproductive pattern [31]. The wolf is a seasonal breeder, the female having only one estrous period per year, from January to March, during which the male also produces spermatozoa. By contrast, domestication has led the domestic dog to undergo cycles twice or sometimes even three times per year, while the males are fertile throughout the year [27]. The reproductive pattern of the canids is different from that of other mammalian species and a better understanding of these differences would be of value to enable us to determine such factors as the regulation of onset of estrus, and the factors that influence the recognition of pregnancy, the duration of gestation, the timing of parturition in the bitch, and the litter size. The dog is also used as a model for research in humans and other species. A better understanding of the differences in reproductive function would, therefore, also be of importance in comparative studies.

Domestic dogs are traditionally considered to be nonseasonal breeders. A major difference between the dog and the noncanid mammalian species is that in dogs, each estrous period is followed by a long interestrous interval (anestrus, or sexual quiescence), and their reproductive pattern is, therefore, described as monocyclic [14]. Although a number of studies have been published on the effects of factors such as breed, age, parity, and litter size on the length of the interestrous period and duration of gestation in the bitch [e.g., 5,8,10,26] and on effects of season of the year on the reproductive pattern [e.g., 5–7,20,24,28,30,32], results have been contradictory.

Due to the peculiar reproductive pattern of the dog, and the great individual variation, especially in cyclicity among bitches, but also in reproductive function among males, research aiming to study factors that influence the reproductive functions in this species needs to encompass a large number of individuals, and in order to avoid bias from variations in climate between years it should also preferably cover a period of several calendar years. Breed differences in fertility have also been described [18,19]. The present study, however, focuses on reproduction in one breed over several years.

Information from kennel club registries is usually only available for matings that were successful, resulting in the birth of a litter of pups. Litters are usually registered at 4–8 weeks of age. Only rarely are a breeder's more detailed kennel records available for research purposes. In addition, dog breeding in Scandinavia is usually a hobby, and few breeders have more than a few animals at any one time. For this study, however, we had access to the kennel records for a 12-year period of a private, professional breeder of Drevers, a Swedish hunting dog. We also obtained all the data recorded at the Swedish Kennel Club (SKK) for this breed over the same time period. In this study, the Drever was used as a model for most other breeds of dogs, as it has the same pattern of producing more litters during the winter and spring than during the other seasons. This was previously shown to be the case for the vast majority of the dog breeds in Sweden [37], the exceptions being some of the breeds that are well known to be seasonal breeders (autumn breeders), in particular the Basenji [13], and to a certain degree also some of the primitive Spitz breeds such as the Chow-Chow [32,37].

The aims of this study were to define differences between seasons of the year regarding the distribution of matings and whelpings, frequency of dystocia and Cesarean section, litter size, pup deaths, and sex ratio of pups. Also, we wanted to study the effects of age and parity of the bitch at the time of whelping on litter size, as well as the effect of litter size on the length of gestation, and differences in fertility among the males of the Drever breed.

2. Materials & methods

2.1 Animals

The Drever is a short-legged scent hound, with an ideal height at withers of 35 cm for the male and 33 cm for the bitch, and with a body weight of between 10 and 15 kg. This retrospective study is based on the records of the data for all litters of the Drever breed that were registered at the SKK during 1995–2006 (SKK data) (www.skk.se). In addition, more detailed information about all matings and whelpings, including the incidence of dystocia, at one private, professional kennel of Drevers (private kennel data) during the same 12-year period was also analyzed. All the acquired data from the private kennel was verified in the SKK database.

The SKK data set contained information on the registered names and SKK registration numbers of each male and female dog, the dates of their birth, the dates of birth of each litter, and the number of registered male and female pups. It did not, however, provide any information about the dates of the matings, problems during parturition, or the number of born and dead or euthanized pups, as this information is not routinely registered in the SKK database. Therefore, neither the whelping rates nor gestational length, cases of dystocia, or actual litter sizes at the time of birth could be calculated for these litters.

The private kennel data set consisted of the registered names and SKK registration numbers of each male and female dog, the dates of their birth, the date of mating(s) (one or more), the date of whelping(s), cases of dystocia and of Cesarean section, the number of pups born in each litter, the number of dead or euthanized pups, and the number and sex ratio of pups registered with the SKK. Only those sets of data for which all the information was available were included in this study.

The data from the private kennel is included in the SKK data set, which represents the total data for the Drever breed in Sweden, except in the part of this study that deals with a comparison of the data from the private kennel and the mean from all other breeders of Drevlers that had registered litters with the SKK during the time period 1995–2006.

2.2 Statistical analyses

The data was analyzed using SAS (SAS, Inc., Cary, NC, USA). The General Linear Model (GLM) procedure was used to analyze and test the significance of different effects in the models, and chi-squared test was used for comparing differences between seasons in number of matings and whelpings. The results are given as overall means \pm standard deviation (SD) or least squares means (LSM). $P < 0.05$ was set as the level of significance. The basic statistical models included year and season of whelping, and parity number or age of the bitch at the time of whelping.

2.2.1. Effects of the year and season of the year

The effect of the year and season during the period 1995–2006 on the number of whelpings and on the number of registered pups was analyzed in both data sets, and for the private kennel the number of matings, the duration of pregnancy, litter size at the time of birth, and number of dead pups were also analyzed.

The months were grouped into four seasons: winter (December – February), spring (March – May), summer (June – August), and autumn (September – November).

In both sets of data, the sex ratio of all registered pups at the time of registration was also analyzed

2.2.2. Effects of the age of the bitch

The effects of the age of the bitch on litter size and number of registered pups were analyzed in both sets of data. The effect of age of the bitch at the time of the first whelping was calculated separately in both data sets. This data was then also included in the calculations of the effects of the age of the bitch on the number of registered pups in all the whelpings.

For the statistical calculations, the age of the bitch at the time of having its first litter was categorized into the following groups:

bitches up to 2 years of age, bitches from 2 to 3 years of age, and so forth.

In case of the data for all the whelpings, the age of the bitch was categorized in the same way. In the model for all litters, parity was not included due to confounding with age.

2.2.3. Effects of the parity of the bitch

The effect of parity on the number of registered pups was studied in the SKK data. In the data from the private kennel, the effect of parity on the duration of pregnancy, litter size, pup death rate, and number of registered pups was studied. The whelpings were grouped into parities 1–5. The parity 5 group also included the few whelpings in higher parities

2.2.4. Effects of number of days between matings in each estrus cycle

In order to study the effects of the number of days between the first and the last mating in each estrous cycle on whelping rate, duration of pregnancy, and litter size in the private kennel, the data was categorized into six groups, as follows:

- 0 – only one mating
- 1 – one day between the first and the last mating
- 2 – two days between the first and the last mating
- 3 – three days between the first and the last mating
- 4 – four days between the first and the last mating
- 5 – five or more days between the first and the last mating

In this analysis, year, season, age or parity at whelping, and number of days between the first and the last mating were included in the model. The effect of one vs. several matings was also analyzed (group 0 compared with groups 1–5 together).

2.2.5. Gestation length

At the private kennel, to calculate the duration of pregnancy, the date of the last mating was used as day 1, and the registered date of birth was used as the last day of pregnancy. In this analysis, year, season, parity, and litter size at birth were included as covariates in the model.

2.2.6 Comparison of the private kennel and the Swedish Kennel Club data

All the data that were provided in both data sets were compared to find similarities and differences between the private kennel data as a model (sample) of the SKK data. Differences in the effect of year and season, as well as the effects of age and parity of the bitch at the time of whelping, on the number of registered pups were studied. The differences in the frequencies of whelpings during different seasons were also studied.

For the comparisons of the results in the private, professional kennel data with those of all other Drever breeders in the SKK data set, the data from the studied kennel was excluded from the SKK data. The effect of year, season, and parity on the number of registered pups was analyzed.

2.2.7. Effects of the male dogs

Only the male dogs that had performed ten matings or more at the private kennel during the 12-year period were used to study male effects on whelping rate and litter size. The model when analyzing litter size included year and season of whelping, parity, and the male dog.

3. Results

3.1. Animals

The descriptive data for the SKK and the private kennel are given in Table 1. Feeding and housing varied for the dogs in the SKK data, but were uniform at the private kennel. These dogs were kenneled in separate pens, 1.5 x 1.5 m to 1.5 x 3 m, with free access to individual runs of 1.8 x 11 m, and with 1–2 hours' daily exercise in a 70 x 70 m run. They were fed Priima dog food (Priima Hundfoder, Örby, Sweden) twice daily, and had access to water ad libitum. Most of the bitches had two estrous periods per year.

Table 1
Descriptive data for the Swedish Kennel Club (SKK) and the private kennel for the Swedish Drever breed during 1995-2006. (Means \pm SD) (range)

	SKK data	Private kennel
No. of female dogs	1665	113
No. of male dogs	668	31
No. of matings	Not available	285
No. of whelpings	2717	224
Whelpings per year (range)	226.4 (159-310)	18.7 (13-26)
Whelping rate (%)	Not available	78.60
Dystocia (%)	Not available	6.25
Caesarean section (%)	Not available	5.36
No. of first whelpings	1416	71
Total no. of pups born	Not available	1525
No. of pups per litter (range)	Not available	6.81 \pm 2.11 (1 – 12)
No. of dead pups per litter (range)	Not available	0.90 \pm 1.23 (0 – 7)
No. of registered pups per litter (range)	5.00 \pm 2.31 (1 - 12)	5.91 \pm 2.11 (1 – 10)
Duration of pregnancy (days) (range)	Not available	61.48 \pm 2.24 (56 – 72)
Sex ratio at the time of registration	0.51 \pm 0.27	0.50 \pm 0.23

3.2.1. Effects of the year and season of the year

An effect of year on the number of registered pups was found in the SKK data ($P < 0.001$). This difference was not observed at the private kennel. There were significant differences in the distribution of matings between seasons at the private kennel ($P < 0.001$), with the most matings taking place during the winter and the fewest during summer (Figure 1). There were also significant differences between seasons in the distribution of whelpings. These differences were more pronounced in the SKK data ($P < 0.001$) than at the private kennel ($P = 0.015$) (Figure 2). At the private kennel, there were significant differences in litter size and number of registered pups between seasons (Figure 3), with the largest litters being born during the spring and, consequently, more pups registered from those litters. By contrast, no such difference was observed for the number of registered pups in the SKK data. No differences were found in duration of pregnancy between seasons at the private kennel.

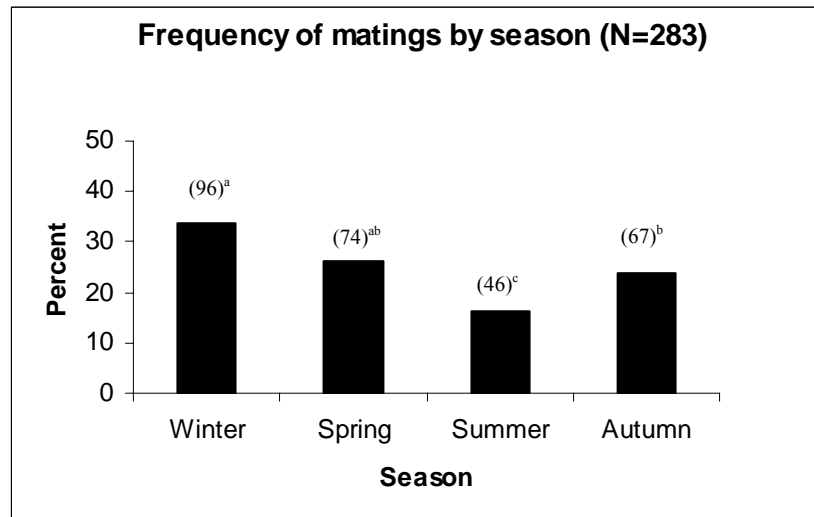


Figure 1. The distribution of matings by season in the private kennel. (Number of matings within brackets). Different superscript letters denotes significant differences

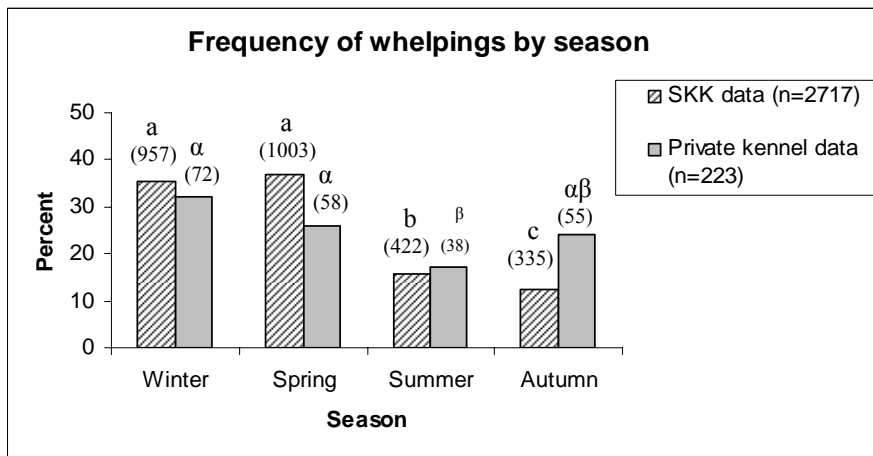


Figure 2. The distribution of whelpings in the Swedish Kennel Club (SKK) data set and in the private kennel. (Number of whelpings within brackets). Different letters denotes significant differences

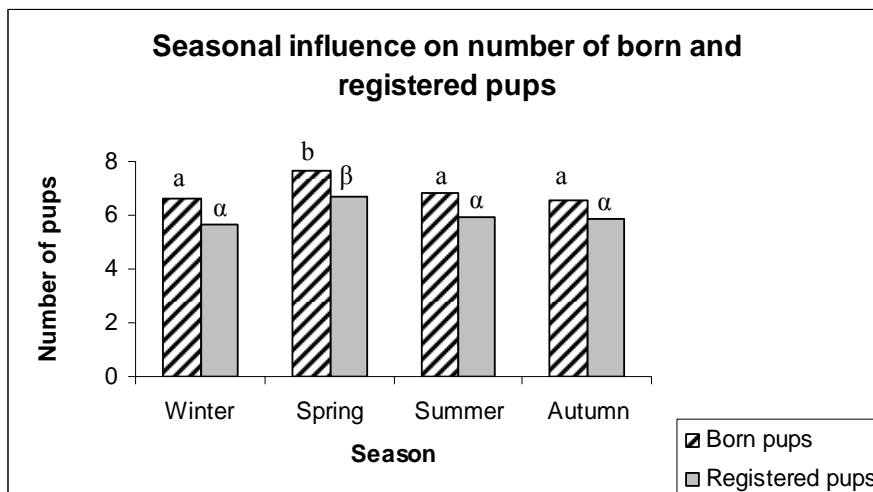


Figure 3. Number of born and of registered pups per litter (LSMean) by season in the private kennel. Different letters denotes significant differences.

The sex ratio among registered pups in both sets of data was similar, the percentage of male pups being 50.5% in the SKK data and 49.6% in the data from the private kennel (Table 1). No significant effect of year, season, or age or parity of the bitch at the time of whelping was found on the sex ratio at the time of registration.

The frequencies of dystocia, Cesarean section and pup deaths in the private kennel were too few to study differences in the basic statistical model.

3.2.2 Effects of the age of the bitch

The numbers of first whelpings and the total number of whelpings in both data sets, grouped by the age of the bitch at the time of whelping, are given in Tables 2a and 2b. Significant effects of the age of the bitch at the time of both the first whelping and all whelpings on the number of registered pups were found in the SKK data. A significantly lower number of registered pups was observed among bitches that were 4 years of age or older when they whelped for the first time. The mean number of registered pups per first born litter for bitches up to 4 years of age was 5.19, compared with 4.70 registered pups per litter for bitches that were >4 years old (Table 2a). Among all the whelpings, a significant decrease in number of registered pups per litter was observed after 5 years of age in the SKK data (Table 2b). The mean number of registered pups per litter in 4–5-year-old bitches was 5.18 and this decreased to 4.24 pups per litter in bitches that were >7 years old. No such differences were observed at the private kennel.

Table 2a. Number of whelpings and registered pups for the first whelping, grouped by the age of the bitch at the time of whelping in the Swedish Kennel Club (SKK) data base and in the private kennel there also including the litter size (LSMean)

Age of the bitch at the time of giving birth to the first litter (yrs)	SKK data		Private kennel data		
	No.	No. of reg. pups	No.	No. of born pups	No. of reg pups
≤ 2yrs	161	5.11 ^a	28	6.24	5.18
> 2-3 yrs	311	5.18 ^a	28	6.30	5.25
> 3-4 yrs	354	5.19 ^a	14	5.44	4.30
> 4-5 yrs	294	4.70 ^b	0	-	-
> 5-6 yrs	181	4.00 ^c	0	-	-
> 6->7yrs	115	3.62 ^c	0	-	-

Different superscript letters denotes significant differences

Table 2b. Number of whelpings and number of registered pups for all the whelpings, grouped by the age of the bitch at the time of whelping in the Swedish Kennel Club (SKK) database and in the private kennel there also including the litter size. (LSMean)

Age of the bitch at the time of giving birth, for all litters (yrs)	No. of whelpings	SKK data		Private kennel data	
		No. of pups	No. of reg. whelpings	No. of born pups	No. of reg pups
≤2	167	5.09 ^a	29	6.04	5.04
>2-3	381	5.38 ^a	43	6.74	6.08
>3-4	519	5.38 ^a	48	6.86	5.69
>4-5	527	5.18 ^b	33	6.93	6.16
>5-6	463	4.86 ^c	35	7.38	6.61
>6-7	323	4.48 ^c	31	6.82	6.03
>7->8	337	4.24 ^d		(included in 7)	

Different superscript letters denotes significant differences

3.2.3. Effects of the parity of the bitch

Numbers of whelpings and litter size and the number of registered pups, grouped by parity, are given in Table 3. Parity was found to have a significant effect on the number of registered pups in both sets of data, and at the private kennel also on the litter size at birth. Litter size at the time of birth, and the number of registered pups increased slightly from the first to the third parity at the private kennel (P=0.004), and the number of registered pups increased with the second parity in the SKK data (P<0.001), and then decreased

Table 3. Number of whelpings, litter size and number of registered pups grouped by parity of the bitch for the Swedish Kennel Club (SKK) data and the private kennel. (LSMean)

Parity no.	SKK data		Private kennel		
	No. of whelpings	No. of reg. pups	No. of whelpings	No. of born pups	No. of reg. pups
1	1416	4.82 ^a	71	6.09 ^a	5.22 ^a
2	669	5.34 ^b	61	7.16 ^b	6.19 ^{ab}
3	326	5.20 ^b	37	7.31 ^b	6.41 ^b
4	189	5.05 ^{ab}	32	7.02 ^b	6.26 ^{ab}
≥5	117	4.80 ^{ab}	20	7.02 ^b	6.06 ^{ab}

Different superscript letters denotes significant differences

3.2.4. Effects of number of days between first and last mating per estrus cycle

At the private kennel, a variety of mating schedules was practiced, from only one mating to multiple matings with different numbers of days between the first and the last mating (Table 4). No effect was found of the number of days between the first and the last mating on the litter size or on the number of registered pups. Mating only once, however, resulted in a 0.52 pups smaller mean litter size at birth and 0.68 fewer registered pups compared with all the other mating schedules analyzed together ($P < 0.05$). The duration of pregnancy was significantly affected when the time from the first to the last mating was 5 days or more.

Table 4. Number of whelpings grouped by number of days between the first and the last mating number of born and registered pups per litter and duration of pregnancy in the private kennel (LSMean)

No. of days between the first and last mating	No. of whelpings	No of born pups	No of reg. pups	Duration of pregnancy (days)
0	36	6.33	5.31	61.0 ^a
1	19	7.24	6.81	60.6 ^a
2	77	6.90	6.09	61.2 ^a
3	39	7.00	6.03	61.5 ^a
4	28	6.81	5.84	61.9 ^a
≥ 5	22	6.50	5.50	63.1 ^b

Different superscript letters denotes significant differences

3.2.5. Gestation length

At the private kennel, the litter size at birth was found to have a significant negative correlation with the duration of pregnancy ($P < 0.001$) ($r = -0.18$). With the number of born pups included in the model as a regression, each extra pup above the mean number caused a shortening of the gestation period by 0.25 days, and each pups less increased it by 0.25 days. The duration of pregnancy was also found to be significantly affected by the number of days between the first and the last mating in each estrous cycle ($P < 0.001$). Neither year, nor season, age, nor parity of the bitch was found to influence the duration of gestation.

3.2.6. Comparison of the private kennel and the Swedish Kennel Club data

A comparison between the SKK and the private kennel data demonstrated some differences. In the SKK data, an effect of the age of the bitch at the time of whelping on the number of registered pups could be seen ($P < 0.001$), while no such effect was observed in the private kennel data. In the SKK data, the mean number of registered pups per litter was independent of season, but at the private kennel, a difference between seasons in the mean number of born and registered pups per litter was found. Parity had a significant effect on number of registered pups in both data sets, but was more pronounced in the SKK data. Although there were significant differences in the frequency of whelpings between seasons at the private kennel ($P = 0.015$), this difference was less pronounced than in the SKK data ($P < 0.001$).

Comparing the results in the private kennel with the results of all the other breeders that had registered litters of Drovers in the SKK database (excluding the results from the private kennel), a significant difference was found in the mean number of registered pups per litter ($P < 0.001$), with a larger mean litter size at the private kennel (5.87 pups compared with 4.93 pups for all the other breeders). The number of registered pups increased at the second parity in both data sets.

3.2.7. Effects of the male dogs

Whelping rates among the mated bitches and litter sizes for the ten male dogs at the private kennel that performed more than ten matings during the 12-year study period are given in Table 5 and Figure 4. There were significant differences in resulting whelping rates between the males ($P=0.003$). Three of the males (No. 1, 3, and 6) had a lower than normal whelping rate [9,11,24], but no difference in mean litter size compared with the other seven dogs.

Table 5. Number of matings and whelpings, whelping rates among the mated bitches (%) and litter sizes for the 10 male dogs in the private kennel with more than 10 matings during 1995-2006 (LSMean)

Male dog	No. of matings	No. of successful matings	Whelping rate (%)	Litter size
1	10	5	50.0 ^b	7.40
2	10	9	90.0 ^a	7.25
3	11	7	63.6 ^b	6.35
4	12	12	100.0 ^a	6.63
5	13	13	100.0 ^a	6.75
6	17	8	47.1 ^b	7.70
7	25	21	84.0 ^a	6.40
8	35	28	82.4 ^a	7.31
9	36	30	83.3 ^a	6.12
10	39	32	82.1 ^a	6.09

Different superscript letters denotes significant differences

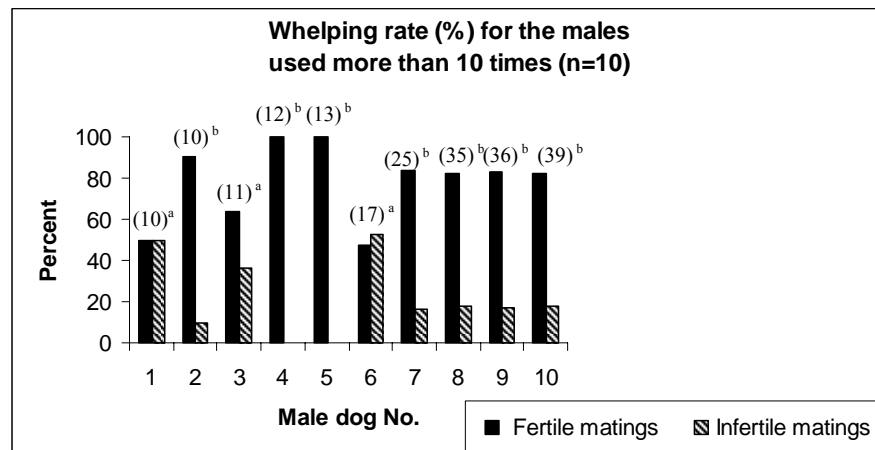


Figure 4. Whelping rate among the bitches mated to the ten male dogs that mated more than 10 bitches each during the 12 year-period of study in the private kennel. (Number of matings within brackets) Different superscript letters denotes significant differences.

4. Discussion

The domestic dog is monoestrous, with a long interestrous interval of sexual quiescence. The bitch usually undergoes two estrous cycles per year, and since bitches may cycle at any time of the year, they have been considered to be nonseasonal, in contrast to their wild ancestor, the wolf. The present study provides data concerning the reproductive pattern of the Swedish dog breed the Drever, here used as a model for the majority of other breeds of dogs, with the exception of those that are known as being strictly seasonal [13], and/or mainly autumn breeders [32,37].

The difference in number of registered pups between the studied years mirrors the changes in popularity of this breed over time, which was reflected in the SKK data, but is not evident from the data of the professional kennel. The reason for this discrepancy is most likely that the private, professional breeder has a constant demand for all the pups he produces, while smaller and newer hobby breeders will suffer from a decrease in popularity for a breed. This is probably also the reason for the somewhat less pronounced variation in frequency of whelpings by season at the private kennel.

The results of the present study demonstrate that the frequencies of matings and whelpings vary significantly between seasons. In both data sets, more litters were produced in winter and spring. This may to a degree reflect a higher demand for pups that are born early in the year and can be raised and housetrained during the summer, and begin the training to hunt during the autumn and winter. However, although most bitches cycle twice a year, there are several studies [7,20,28,32] demonstrating that more bitches come into estrus during the winter and spring than during autumn, and especially compared with summer. This seasonal pattern is similar to that of the dog's ancestor, the wolf, with a reproductive period from January through March in this part of the world, and is probably a vestige of the more strict seasonality of the species.

Previous studies of the effects of season on the cycle and reproductive pattern of the bitch [5–7,20,24,28,30,32] show conflicting results. Bouchard et al. [5], studying 67 colony bitches during 210 estrous cycles over 4 years, found that the overall probability that an estrus would occur at any month of the year was the same for each month. Also, Sokolowski et al. [30], studying 57 bitches in seven breeds, found no influence of season on estrous frequencies by month of the year. In Kenya, south of the equator, a higher frequency of bitches were in estrus during October and a lower frequency in April. However, the authors of that study could not confirm seasonality and only suggest that the peak and nadir of estrous events were in those two months, depending on locality and on the month in which some bitches attained puberty, which is connected with other factors, such as nutrition, breed, and/or poor estrous observations [24]. By contrast, Christie and Bell [7], studying 1,561 cycles in 449 privately owned bitches of 40 breeds of dogs in the UK, found a significantly higher estrous frequency in the period from February to May, compared with June to September ($P < 0.001$) and October to January

($P < 0.01$). Similarly, Tedor and Reif [32], studying 87,880 litter registrations in the USA, observed a distinct, repetitive seasonal distribution of births, with a primary peak in the spring and early summer. Most births in their study occurred in May, and the authors ascribed this pattern to human intervention as well as to genetic factors and environmental conditions. This conclusion is in line with the findings of Linde-Forsberg and Wallén [20]. Compiling data from 319 interestrous intervals in 36 Beagles, 36 German Shepherd dogs, and 20 Labrador Retrievers in Sweden, these authors found that there was an influence of season in the Beagles which were kept as a colony and housed outdoors without heating or supplementary light. The authors noted a peak of cycles in the Beagles in May, but no obvious seasonal influence in the other two breeds that were kept as family dogs. Another factor that was found to influence the estrous frequency was whether the bitches had given birth to and nursed a litter of pups in the preceding cycle, something that prolonged the interestrous interval by 4–8 weeks [5,7,20,24]. In Thailand, the conditions of the rainy season, during September, have been reported to have a positive effect on conception and led to the highest whelping rate [6]. Litter size, too, has been said to be influenced by climate factors such as temperature and humidity [6], which is in agreement with the results of the present study, proving significant differences between seasons in litter size and number of registered pups, with the lowest numbers in summer.

No differences in litter size were found between the months of the year in German Shepherd bitches in Africa over a 15-year period [24,25], while in the present study such differences were found during the 12-year period at the private kennel, but not in the SKK data. These differences most likely reflect a more directed and professional breeding effort by the private kennel, compared with average breeders. In Mexico, an increase in the incidence of bitches in estrus was seen in December [28]. Also, it was observed that temperature and humidity can markedly increase fetal losses during summer [27], which is in agreement with our findings. The study concluded that stray dogs in Yucatan are not seasonal breeders, but their reproductive pattern is modified by environmental factors, such as temperature, humidity, and, probably, photoperiod. Others have suggested that high temperature and substantial rainfall in the tropical zone may reduce serum testosterone concentrations in dogs, which can influence fertility in male dogs under such conditions [22]. The study, however, comprised a relatively small number of dogs, which made it difficult to detect significant seasonal differences. Hossein et al. [12] flushed Fallopian tubes to collect in vivo-matured canine oocytes for assisted reproductive technology (ART) by laparotomy from 124 bitches, and found a seasonal influence on number of corpora lutea, being highest in the spring (March – May), with no difference between the other three seasons. The authors also retrieved significantly more oocytes per bitch during the spring compared with winter (December – February). Supporting these findings is the observation that more artificial inseminations (AIs) are being performed during winter and spring than during summer [17]. In the same study, AIs that were done during summer resulted in a lower conception rate [17]. In the present study, the largest litter size and number of registered pups were observed in spring at the private kennel. Such variation was not found in the SKK data, but was probably

disguised by the variety in husbandry and breeding management between the different smaller kennels.

Consequently, available data strongly suggest that the domestic dog is still under considerable seasonal influence, with a tendency to cycle during what would be the most favorable time of the year in the wild, providing the best climate and the best supply of food for raising a litter. Daylight, climate, environment, and management factors are modulating this tendency of seasonality.

The present study also found effects of age and parity of the bitch at the time of whelping, on litter size. At the private kennel, the data for first litters could not be analyzed because of too little variation in the bitch's age at whelping the first litter, but in the SKK data, a large variation was found (and the 1,416 first litters could be compared with all 2,717 whelpings). Previous studies have found an association between the reproductive ability and age of the bitch, and the length of the anestrus period, which increased in the older bitches. Also, a decrease in the conception rate and litter size has been reported with increasing age [1,2,24,25,34], which is in accordance with our findings. Effects of parity on number of registered pups per litter were found in both sets of data, with slight differences between them. The main reason for these differences could be a tendency to breed the bitches at an earlier age, and more often, during their most fertile period of life at the private, professional kennel, compared with most other kennels in Sweden. Some authors suggest that parity can have an effect on litter size, but not before the fifth whelping, and that reproductive efficiency declines in bitches after 6 years of age [25]. This is the first study to report on differences in litter size, depending on the age and parity of the bitch at the time of whelping before the fifth parity.

The number of days between the first and the last mating per cycle in the present study had no influence on litter size. Mating only once, however, resulted in a smaller litter size, compared with all other mating schedules. This is in accordance with the findings of Linde-Forsberg and Forsberg [19] that the pregnancy rate was significantly higher in bitches inseminated twice with fresh semen than in those inseminated once, and Thomassen et al. [33], that two inseminations yielded a higher mean litter size (6.0 ± 0.2) than one (5.1 ± 0.2). In a previous publication [29], effects of day of mating and gestational length on reproductive efficiency were examined. Surprisingly, it was found that mating 7 days after the luteinizing hormone (LH) peak resulted in a significantly longer gestation period compared with matings performed earlier, i.e., 3 or 5 days after the LH peak. However, few animals were used in each treatment group, and the authors did not consider the possible effect of the variation in litter size between groups on gestational length. The litters from matings on day 7 were the smallest, which may have resulted in a longer gestation.

In the present study, litter size was found to influence the duration of pregnancy, with a significantly shorter gestation period in the larger litters. This finding is in agreement with previous studies showing that a litter size of four or fewer than four pups prolongs gestation by 1 day compared with larger litters [10], and that

duration of pregnancy was longer for bitches pregnant with one or two pups compared with larger litters [33]. In one study of 152 bitches of 39 breeds [38], bitches were divided into three groups by litter size within breed (small, average, and large). Results showed that the group with small litter size had a significantly ($p < 0.001$) longer gestation period than the groups of bitches whelping a litter of average or large size. Okkens et al. [26], studying 113 bitches of six breeds, also found a negative correlation between litter size and gestational length ($r = -0.73$; $P = 0.03$) for litters comprising ≤ 13 pups, but suggested that the breed was the major determinant of the duration of pregnancy. In our study in the Drever breed, we could in fact demonstrate a within-breed difference in duration of pregnancy of -0.25 days per extra pup above average, and a corresponding 0.25 days longer pregnancy for each pup less. In contrast to these findings and to our findings in the Drever breed, however, several previous studies on a variety of other breeds of dogs found no influence of litter size on gestational length [15,16]. The different results may be due to the considerably higher number of observations in our study, but there may possibly also be differences between breeds. A previous study of the influence of parity on the duration of gestation found that only bitches with nine pregnancies were more likely to have a longer gestation [10]. The study included only five bitches with this parity, and the authors suggested that the small sample size made this estimate unreliable. In the present study with a large number of observations, no effect of parity on gestational length was found.

The percentage of male pups of 50.5% (SKK data) and 49.6% (private kennel) in the present study is in agreement with the average ratio of 50.6% male pups previously reported by Tedor and Reif [32], although these authors observed considerable differences between breeds. We found no effects of year, season, or age or parity of the bitch at the time of whelping on the sex ratio of the pups, which is in accordance with the findings of Tedor and Reif [32].

A comparison between the larger SKK data and the data of the private kennel showed many similarities, and proved that the private kennel data can serve as a model (sample) of all Drever data in the country for the parameters that were not available in the SKK registry, i.e., matings, litter size at birth, and, possibly, dystocia. However, there were some differences between the data sets, notably a more consistent way of breeding from the bitches in the professional kennel, in the age when bitches are mated for the first time, in the interval at which they are bred during their most fertile years, in selecting for the best producers, and, probably, in having a better surveillance of the heat periods and matings. In addition, the breeder at the private kennel uses a fresh, frozen food originally developed for farmed mink and modified for dogs, and claims that when he introduced this many years ago, the litter size increased by one pup on average also in the other breeds of this kennel. A comparison of the SKK data and the private kennel for the Jämthund breed during the same 12-year period showed that the mean litter size for this breed in the SKK database (3,040 litters) was 6.32 registered pups, compared with 7.25 pups (64 litters) in the private, professional kennel. The findings in these two breeds are therefore consistent, and the larger mean litter sizes in the private, professional kennel consequently appear to be the result of better management, and selection of breeding stock.

The mean duration of pregnancy, of 61.48 ± 2.24 days, calculated from the last day of mating, shows that the bitches in the private kennel in general were mated on the optimal days during estrus, 2-3 days after ovulation [8,14,15,21]. The longer pregnancy resulting from mating the bitches over 5 or more days shows that these matings started too early during the estrous period. The resulting whelping rate of 78.6% is close to the expected normal range of 80–95% [9,11,24]. The incidence of dystocia (6.25%) and of Cesarean sections (5.36%) in the present study are at the estimated average level for dogs in general [36], and within the 5–10% found in two previous studies in the Drever breed (K Andersson, personal communication). They are low compared with the 16% reported in the Swedish insurance company, AGRIA's, database on ~200.000 bitches of different breeds [3]. The pup death rate in the private kennel was 7.6%, which is low compared with previous reports of 11.6–11.9% among Swedish breeders [18,19] and 10–30% reported by Mosier [23]. The pup death rate was also too low to detect any effects of year, season, or parity of the bitch.

The three male dogs that achieved a lower than normal whelping rate [9,11,24] among the mated bitches had no difference in mean litter size compared with the other seven males with normal fertility. This indicates that the low fertility of these males may have been due either to a variation in their semen quality over time or, more likely, to female factors, for instance low fertility of the bitch or management factors, such as mating on the wrong day of the cycle. Beuing et al. [4], analyzing large data sets from 14 breeds in Germany (for some breeds, taking up to 25 years for collecting the data), found that the genetic male effect on the conception rate was negligible and the permanent, nongenetic effect low, and that the influence of the male partner on litter size was near zero in nearly all breeds. This is in accordance with the present study, although we used a comparatively small sample size.

In conclusion, the results of our study support those earlier publications that claim a remaining seasonal pattern of reproduction in the domestic dog. Furthermore, they suggest that this pattern is modified by a number of environmental and management factors that are not yet well understood. The superior results in the private kennel show the importance of good management. Age and parity of the bitch affects litter size, and litter size influences gestational length. If kennel clubs would register more details about the breedings, much valuable information could be obtained concerning canine reproductive physiology and pathology in the future.

Acknowledgements

We would like to express our sincere gratitude to Lennart and Solvy Davidsson and Kjell Lennartsson of the Härkilas Kennel, for giving us free access to their professional kennel files

References:

- [1] Andersen AC. Reproductive Fitness of the female Beagle. *JAVMA* 1962;141: 1451-4
- [2] Andersen AC. Reproductive Ability of female Beagles in relation to advancing age. *Exp Geront* 1965;1: 189 - 92.
- [3] Bergström A, Nödttvedt A, Lagerstedt AS, Egenvall A. Incidence and breed predilection for dystocia and risk factors for cesarean section in a Swedish population of insured dogs. *Vet Surg* 2006;35: 786-91.
- [4] Beuing R, Janssen N, Brand H. Analysis of fertility in canine populations in respect to genetic and environmental influences. *Proceedings 5th Biannual EVSSAR Congress, Budapest, Hungary 2006*: 60-4.
- [5] Bouchard G, Youngquist RS, Vaillancourt D, Krause GF, Guay P, Paradis M. Seasonality and variability of the interestrus interval in the bitch. *Theriogenology* 1991;36: 41-50.
- [6] Chatdarong K, Tummaruk P, Sirivaidyapong S, Raksil S. Seasonal and breed effects on reproductive parameters in bitches in the tropics: a retrospective study. *J Small Anim Pract* 2007;48: 444-8.
- [7] Christie DW, Bell ET. Some observations on the seasonal incidence and frequency of oestrus in breeding bitches in Britain. *J Small Anim Pract* 1971;12: 159-67.
- [8] Concannon P, Whaley S, Lein D, Wissler R. Canine gestation length: variation related to time of mating and fertile life of sperm. *Am J Vet Res* 1983;44: 1819-21.
- [9] Daurio CP, Gilman MR, Pulliam JD, Seward RL. Reproductive evaluation of male Beagles and the safety of ivermectin. *Am J Vet Res* 1987;48: 1755-60.
- [10] Eilts BE, Davidson AP, Hosgood G, Paccamonti DL, Baker DG. Factors affecting gestation duration in the bitch. *Theriogenology* 2005;64: 242-51.
- [11] England G, Allen W. Seminal characteristics and fertility in dogs *Vet Rec* 1989;125: 399.
- [12] Hossein MS, Kim MK, Jang G, Fibrianto HY, Oh HJ, Kim HJ, Kang SK, Lee BC. Influence of season and parity on the recovery of in vivo canine oocytes by flushing fallopian tubes. *Anim Reprod Sci* 2007;99: 330-41.
- [13] Fuller JL. Photoperiodic control of estrus in the Basenji. *JHered* 1956;47: 179-80.
- [14] Jöchle W, Andersen AC. The estrous cycle in the dog: a review. *Theriogenology* 1977;7: 113-40.
- [15] Kutzler MA, Mohammed HO, Lamb SV, Meyers-Wallen VN. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. *Theriogenology* 2003;60: 1187-96.
- [16] Kutzler MA, Yeager AE, Mohammed HO, Meyers-Wallen VN. Accuracy of canine parturition date prediction using fetal measurements obtained by ultrasonography. *Theriogenology* 2003;60: 1309-17.
- [17] Linde Forsberg C. Artificial insemination in the dog: what can be learnt from results in the field? *WSAVA Congress Proceedings, Bangkok 2003*: 550 - 2.

- [18] Linde-Forsberg C, Forsberg M, Fertility in the dog in relation to semen quality and the time and site of insemination with fresh and frozen semen. *Journal of Reproduction and Fertility, Supplement* 1989;39: 299-310.
- [19] Linde-Forsberg C, Forsberg M. Results of 527 controlled artificial inseminations in dogs. *Journal of Reproduction and Fertility, Supplement* 1993;47: 313-23.
- [20] Linde-Forsberg C, Wallén A. Effect of whelping and season of the year on the interoestrus intervals in dogs. *Journal of Small Animal Practice* 1992;33: 67-70.
- [21] Linde-Forsberg C, Ström Holst B, Govette G. Comparison of fertility data from vaginal vs intrauterine insemination of frozen-thawed dog semen: a retrospective study. *Theriogenology* 1999;52: 11-23
- [22] Martins MI, de Souza FF, Oba E, Lopes MD. The effect of season on serum testosterone concentrations in dogs. *Theriogenology* 2006;66: 1603-5.
- [23] Mosier JE. Introduction to canine pediatrics. *Vet Clin North Am* 1978;8: 3-5.
- [24] Mutembei HM, Mutiga ER, Tsuma VT. A retrospective study on some reproductive parameters of German shepherd bitches in Kenya. *J S Afr Vet Assoc* 2000;71: 115-7.
- [25] Mutembei HM, Mutiga ER, Tsuma VT. An epidemiological survey demonstrating decline in reproductive efficiency with age and non-seasonality of reproductive parameters in German shepherd bitches in Kenya. *J S Afr Vet Assoc* 2002;73: 36-7.
- [26] Okkens AC, Teunissen JM, Van Osch W, Van Den Brom WE, Dieleman SJ, Kooistra HS. Influence of litter size and breed on the duration of gestation in dogs. *J Reprod Fertl Suppl* 2001;57: 193-7.
- [27] Ortega-Pacheco A, Rodriguez-Buenfil JC, Segura-Correa JC, Montes de Oca-Gonzalez AR, Jimenez-Coello M. Prevalence of fetal resorption in stray dogs in Yucatan, Mexico. *J Small Anim Pract* 2006;47: 266-9.
- [28] Ortega-Pacheco A, Segura-Correa JC, Jimenez-Coello M, Linde Forsberg C. Reproductive patterns and reproductive pathologies of stray bitches in the tropics. *Theriogenology* 2007;67: 382-90.
- [29] Shimatsu Y, Yuzawa H, Aruga K, Nakura M. Effect of time for mating and gestation length on reproductive efficiency in dogs. *Reprod Domest Anim* 2007;42: 664-5.
- [30] Sokolowski JH, Stover DG, VanRavenswaay F. Seasonal incidence of estrus and interestrus interval for bitches of seven breeds. *J Am Vet Med Assoc* 1977;171: 271-3.
- [31] Sundqvist AK, Björnerfeldt S, Leonard JA, Hailer F, Hedhammar Å, Ellegren H, Vila C. Unequal contribution of sexes in the origin of dog breeds. *Genetics* 2006;172: 1121-8.
- [32] Tedor JB, Reif JS. Natal patterns among registered dogs in the United States. *J Am Vet Med Assoc* 1978;172: 1179-85.
- [33] Thomassen R, Sanson G, Krogenaes A, Fougner JA, Berg KA, Farstad W. Artificial insemination with frozen semen in dogs: a retrospective study of 10 years using a non-surgical approach. *Theriogenology* 2006;66: 1645-50.

- [34] Tsutsui T, Hori T, Kirihara N, Kawakami E, Concannon PW. Relation between mating or ovulation and the duration of gestation in dogs. *Theriogenology* 2006;66: 1706-8.
- [35] Vila C, Savolainen P, Maldonado JE, Amorim IR, Rice JE, Honeycutt RL, Crandall KA, Lundeberg J, Wayne RK. Multiple and ancient origins of the domestic dog. *Science* 1997;276: 1687-9.
- [36] Walett Darvelid A, Linde Forsberg C. Dystocia in the bitch: A retrospective study of 182 cases. *Journal of Small Animal Practice* 1994; 35: 402-7
- [37] Wikström C, Linde Forsberg C. Fertility and fertility problems in the Chow-Chow, mainly an autumn breeder. *Proceedings 5th Biannual EVSSAR Congress Budapest, Hungary* 2006: 294.
- [38] Vitasek R., Bartoskova A. Influence of body weight and litter size on the duration of gestation in the bitch. *Proceedings 5th Biannual EVSSAR Congress Budapest, Hungary*, 2006: 301.

Acknowledgements

This study was carried out at the Department of Clinical Sciences, Division of Reproduction, Faculty of Veterinary Medicine and Animal Science, Sweden, University of Agricultural Sciences, Uppsala, Sweden.

I gratefully acknowledge scholarship from Swedish Institute without which it was impossible to do this study.

Prof. Björn Ekesten, Head of the Department of Clinical Sciences, I convey my deepest gratitude and regards for offering me a great opportunity to work on my study and allowing me to use the Department's facilities.

Associate Prof. Lennart Söderquist, Head of Division of Reproduction, I convey my gratitude and regards for providing me all sorts of facilities to carry out my research in your division. Many times you were a good friend helping with difficulties in Swedish terms, translating them for me.

Prof. Catharina Linde Forsberg, my Scientific Adviser, you have helped me during whole period of studying. It takes a lot of patience to show me many tips, good ways of thinking and directions for continuing, to encourage me with lot of affection and sympathy. Without you I would never found my way out from the very first idea in the study and raw data Writing a paper and thesis would never be done without touch of your "magical pen". Thank you for offering me opportunity to stay at your house during the summer, enjoying the beautiful landscapes around Uppsala and feeling the real style of Swedish life.

I convey my thanks and regards to Prof Kjell Andersson, from Department of Animal Breeding and Genetics, for providing me great data base from Swedish Kennel Club and for his excellent statistical assistance. Without you I could only stay captive with numbers forever, with no results.

I express my gratitude to Associate Prof. Karin Östensson, Director of the Swedish International Programme on Animal Reproduction, for encourage me to apply for this studies and giving me opportunity to participate in the program.

Marie Sundberg, our best guardian for life in Sweden. You have helped so many times, to find an accommodation, to open a bank account, to choose a food shop...With all that assistance it would be much more difficult. Sometimes I was dreadful providing too many questions, but you have had always few minutes and a smile for all of us.

Associate Prof Eva Axnér I convey my thanks for teaching me a lot of techniques in semen laboratory, and allowing me to participate in clinical work in Small Animals Reproductive laboratory with her. She was very patient for all my questions during last year.

I wish to thank to my dear friends, students from Division Jatesada , Fernando, Paweena, Fikre, Yahoong and Yoseph for helping me all the time during my studies. Without them and their unselfish help in solving problems with computers and statistics, this studying would be “mission impossible”. To my office friend and “brother” Branislav, I wish to thank for many cheering up moments and encourages in every single day in last period.

For all the personal in the Division, I convey my thanks to give me opportunity to become a “part of the family” and enjoy many nice hours and happenings, and to better understand a life style and habitude in Sweden.

I convey my thanks to all my dear friends world wide, for supporting and visiting me virtually every day, they have been my family and best company during long days and nights in Sweden.

Thanks to my dear parents and my sister Milica for supporting me endlessly, for taking care of my son all this time and touching me with love with many packages’ and letters of encouragement.

Lastly, thank to my husband for supporting all my ambitions, for his love and sacrifice living alone and taking care of our son, as good as we could do it together.

To my son, my only treasure, my great inspiration and love, I have biggest thanks for being brave to live without his mother so many months.

